SERGEI ALEXANDROVICH STROEV

# The Role of Endogenous Protein Antioxidants in Neuronal Adaptation to Hypobaric Hypoxia

ACADEMIC DISSERTATION
To be presented, with the permission of
the Board of the School of Medicine of the University of Tampere,
for public discussion in the Auditorium of
School of Health Sciences, T Building, Medisiinarinkatu 3,
Tampere, on March 13th, 2014, at 12 o'clock.

UNIVERSITY OF TAMPERE

SERGEI ALEXANDROVICH STROEV

# The Role of Endogenous Protein Antioxidants in Neuronal Adaptation to Hypobaric Hypoxia

*Acta Universitatis Tamperensis 1898*
*Tampere University Press*
*Tampere 2013*

ACADEMIC DISSERTATION
University of Tampere, School of Medicine
Finland
I.P. Pavlov Institute of Physiology of RAS
Russia

*Supervised by*
Docent Markku Tapio Miettinen
University of Tampere
Finland

*Reviewed by*
Docent Irma Elina Holopainen
University of Turku
Finland
Docent Jouni Saulo Ilmari Sirviö
University of Eastern Finland
Finland



Cover design by
Mikko Reinikka

Acta Universitatis Tamperensis 1898
ISBN 978-951-44-9353-9 (print)
ISSN-L 1455-1616
ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 1380
ISBN 978-951-44-9354-6 (pdf)
ISSN 1456-954X
http://tampub.uta.fi

Suomen Yliopistopaino Oy – Juvenes Print
Tampere 2013

**To God, my Lord, Who has created me, has endowed me with mind and free will, and has settled me in this wonderful world that He has made.**

**To my mother Galina A. Stroeva, who gave birth to me;**

**to my grandmother Vera A. Sotnikova**

**and my grandfather Afanasiy M. Sotnikov;**

**and to all my ancestors.**

---

As an epigraph to the study of the phenomenon of preconditioning:

> *Aus der Kriegsschule des Lebens. — Was mich nicht umbringt, macht mich stärker.*
> *[Out of life's school of war: what does not destroy me, makes me stronger]*.
>
> **Friedrich Wilhelm Nietzsche**
> (Sprüche und Pfeile, 8; Götzen-Dämmerung oder Wie man mit dem Hammer philosophirt, 1889).

# Abstract

Severe hypoxia induces substantial morphological and functional injuries in brain neurons up to and including death. The overproduction of reactive oxygen species is one of the key molecular mechanisms of these injuries. The mechanisms of adaptation to hypoxia include the up-regulation of the expression and enzymatic activity of endogenous antioxidant systems. Preconditioning by mild hypoxia substantially increases the resistance of cells to subsequent acute severe hypoxia. In the present work, the effects of non- and preconditioned severe hypobaric hypoxia as well as preconditioning itself on the expression of 4 endogenous protein antioxidants – copper-zinc superoxide dismutase (Cu, Zn-SOD), manganese superoxide dismutase (Mn-SOD), thioredoxin-1 (Trx-1) and thioredoxin-2 (Trx-2) – were studied in neurons of different hippocampal areas and in some cases also in neurons of neocortex of rats. In addition, the effects of non- and preconditioned severe hypobaric hypoxia on Cu, Zn-SOD enzyme activity were studied in certain structures of the brain.

In most cases, severe hypoxia alone increased the expression of antioxidants. Preconditioning by 3 sessions of mild hypoxia further significantly raised expression levels after a subsequent severe hypoxia during the early reoxygenation period which is critical for the induction of apoptosis. However, the 3 sessions of mild hypoxia itself in most cases did not increase the expression of antioxidants at 24 h after the last session, and in some cases, on the contrary, significantly decreased expression levels. Thus, the increase in the expression of antioxidants in neurons of preconditioned rats after subsequent severe hypoxia was caused not by an accumulation of antioxidant proteins during or after preconditioning itself but by modification of the response to severe hypoxia. Investigations of antioxidant expression at different time-points during the preconditioning showed that in some cases the changes were characterised by more or less regular alternation of increasing and decreasing levels. It is likely that this alternation in antioxidant levels causes the neuroprotective effect of preconditioning during and after subsequent severe hypoxia.

# Tiivistelmä

**Endogeenisten antioksidatiivisten proteiinien rooli hermosolujen adaptaatiossa hapen puutteelle.**

Vaikea hapenpuute aiheuttaa merkittäviä morfologisia ja toiminnallisia vaurioita aivojen neuroneihin niiden kuolemaan asti. Reaktiivisten happiradikaalien ylituotanto on yksi tärkeimmistä molekyylitason mekanismeista näiden vaurioiden aiheuttajana. Yksi hypoksiaan sopeutumisen mekanismi on endogeenisten antioksidanttijärjestelmien ekspression ja entsyymiaktiivisuuden nousu. Esikäsittely (ennaltaehdollistaminen) lievällä hypoksialla lisää merkittävästi solujen vastustuskykyä myöhemmin tapahtuvalle akuutille vakavalle hypoksialle. Tässä työssä tutkittiin vakavan hypobarisen hypoksian vaikutuksia esikäsiteltyjen ja esikäsittelemättömien koe-eläinten sekä pelkän esikäsittelyn vaikutuksia neljän endogeenisen antioksidanttiproteiinin – kupari-sinkki superoksididismutaasin (Cu, Zn-SOD), mangaani-superoksididismutaasi (Mn-SOD), thioredoksiini-1 (Trx-1) ja thioredoksiini-2 (Trx-2) – ilmentymiseen neuroneissa hippokampuksen eri osissa ja joissakin tapauksissa myös neokorteksin alueilla. Lisäksi tutkittiin vakavan hypobarisen hypoksian vaikutuksia Cu, Zn-SOD-entsyymin aktiivisuuteen esikäsiteltyjen ja esikäsittelemättömien rottien joissakin aivojen rakenteissa.

Useimmissa tapauksissa vaikea hypoksia yksin lisää antioksidanttien ilmentymistä. Esikäsittely kolmella lievällä hypoksialla lisäsi merkittävästi antioksidanttien ilmentymistä myöhemmän vakavan hypoksian jälkeisessä reoxygenation alkuvaiheessa, joka on kriittinen ajankohta apoptoosin induktiolle. Kuitenkin kolme toistetua lievää hypoksiaa ei itsessään useimmissa tapauksissa lisännyt antioksidanttien ilmentymistä 24 tuntia viimeisestä käsittelystä, mutta joissakin tapauksissa merkittävästi vähensi sitä. Näin ollen nousu esikäsiteltyjen rottien neuronien antioksidanteissa myöhemmän vakavan hypoksian jälkeen ei johtunut antioksidanttiproteiinien kertymisestä esikäsittelyjen aikana tai sen jälkeen, vaan muuttuneesta vasteesta vakavaan hypoksiaan. Tutkimus antioksidanttien ilmentymisestä eri aikapisteissä lievän hypoksian jälkeen osoitti, että joissain tapauksissa muutokset olivat tunnusomaisesti enemmän tai vähemmän säännöllinen vuorottelu niiden tasojen nousuissa tai laskuissa. On todennäköistä, että tämä vuorottelu antioksidanttien tasoissa voi saada aikaan hermosoluja suojaavan vaikutuksen myöhemmin seuraavan vaikean hypoksian aikana ja sen jälkeen.

# Аннотация

**Роль эндогенных белковых антиоксидантов в адаптации нейронов мозга к гипобарической гипоксии.**

Тяжёлая гипоксия, как известно, вызывает существенные структурные и функциональные нарушения нейронов мозга, вплоть до их гибели. Одним из важнейших молекулярных механизмов этих нарушений является гиперпродукция активных форм кислорода. Соответственно, механизм адаптации к гипоксии включает повышение экспрессии и ферментативной активности эндогенных антиоксидантных систем. Прекондиционирование сеансами умеренной гипоксии значительно повышает устойчивость клеток к последующей острой тяжёлой гипоксии. В настоящей работе был исследован эффект непрекондиционированной и прекондиционированной тяжёлой гипобарической гипоксии, а также прекондиционирования самого по себе, на экспрессию четырёх эндогенных белковых антиоксидантов – Cu, Zn-супероксиддисмутазы (Cu, Zn-СОД), Mn-супероксиддисмутазы (Mn-СОД), тиоредоксина-1 (Trx-1) и тиоредоксина-2 (Trx-2) – в нейронах различных областей гиппокампа и, в некоторых случаях, новой коры крыс. Кроме того, в некоторых структурах мозга был изучен эффект непрекондиционированной и прекондиционированной тяжёлой гипобарической гипоксии на ферментативную активность Cu, Zn-СОД.

В большинстве случаев сама по себе тяжёлая гипоксия повышала экспрессию антиоксидантов. Прекондиционирование 3-кратной умеренной гипоксией достоверно усиливало это повышение вслед за последующей тяжёлой гипоксией, причём на ранних сроках реоксигенации, критических для запуска апоптотической программы. Однако сама по себе 3-кратная умеренная гипоксия (до начала последующей тяжёлой) к 24 часам после окончания последнего сеанса в большинстве случаев не повышала экспрессию антиоксидантов, а в ряде случаев даже достоверно снижала её.

Таким образом, повышение экспрессии антиоксидантов в нейронах прекондиционированных крыс после последующей тяжёлой гипоксии связано не с накоплением антиоксидантных белков во время или непосредственно после самого по себе прекондиционирования, а с модификацией ответа на тяжёлую гипоксию. Исследование экспрессии антиоксидантных белков на различных временны́х точках в ходе прекондиционирования показало, что в ряде случаев её изменения характеризуются более или менее регулярным чередованием повышений и снижений. Вероятно, именно эти колебания уровня экспрессии антиоксидантов в нервных клетках могут играть важную роль в индуцируемых прекондиционированием механизмах формирования толерантности мозга к последующей тяжёлой гипоксии.

# Table of contents

# List of abbreviations

ANT – adenine nucleotide translocator

AP-1 – activator protein-1 (a transcription factor)

ASK1 – apoptosis signal-regulating kinase 1

ATP – adenosine triphosphate

BH – Bcl-2 homology domain

CA1, CA2, CA3 – *cornu Ammonis* areas 1, 2 and 3

CAD – caspase-activated DNase

cAMP – cyclic adenosine monophosphate

CARD – caspase recruitment domain

C/EBP – CCAAT/enhancer binding protein

CREB – cAMP-responsive element-binding protein

Cu, Zn-SOD – cytosolic copper-zinc superoxide dismutase

DG – *dentate gyrus* (of the hippocampus)

DNA – deoxyribonucleic acid

DPI – diphosphoinositide

eIF2α – eukaryotic initiator factor 2 alpha

ER – endoplasmic reticulum

HSP – heat shock protein

IL-1β – interleukin-1beta

$IP_3$ – inositol triphosphate

LPO – lipid peroxidation

MAP – mitogen-activated protein

Mn-SOD – mitochondrial manganese superoxide dismutase

mRNA – messenger (matrix, information) ribonucleic acid

NAD – nicotinamide adenosine dinucleotide (oxidised)

NADH – nicotinamide adenosine dinucleotide (reduced)

NADPH – nicotinamide adenosine dinucleotide phosphate (reduced)

NF-1 – nuclear transcriptional factor-1

NF-$\varkappa$B – nuclear factor-kappa B

NGF – nerve growth factor

NMDA – N-methyl-D-aspartate

PDGF – platelet-derived growth factor

PERK – protein kinase-like eIF2α-kinase of endoplasmic reticulum

$pO_2$ – partial pressure of oxygen

PKC – protein kinase *C*

RNA – ribonucleic acid

ROS – reactive oxygen species

SOD – superoxide dismutase

TNF-α – tumour necrosis factor alpha

TPI – triphosphoinositide

Trxs – thioredoxins

Trx-1 – cytosolic thioredoxin-1

Trx-2 – mitochondrial thioredoxin-2

VDAC – voltage-dependent anion channel

# List of original publications

This thesis is based on the following publications, which are referred in the text by their Roman numerals I-X.

I. Stroev S.A., Tjulkova E.I., Gluschenko T.S., Rybnikova E.A., Samoilov M.O., Pelto-Huikko M. The augmentation of brain thioredoxin-1 expression after severe hypobaric hypoxia by the preconditioning in rats. // Neuroscience Letters. 2004 a. 370 (2-3): 224-229.

II. Stroev S.A., Gluschenko T.S., Tjulkova E.I., Spyrou G., Rybnikova E.A., Samoilov M.O., Pelto-Huikko M. Preconditioning enhances the expression of mitochondrial antioxidant thioredoxin-2 in the forebrain of rats exposed to severe hypobaric hypoxia. // Journal of Neuroscience Research. 2004 b. 78 (4): 563-569.

III. Stroev S.A., Gluschenko T.S., Tjulkova E.I., Rybnikova E.A., Samoilov M.O., Pelto-Huikko M. The effect of preconditioning on the Cu, Zn superoxide dismutase expression and enzyme activity in rat brain at the early period after severe hypobaric hypoxia. // Neuroscience Research. 2005. 53 (1): 39-47.

IV. Stroev S.A., Tjulkova E.I., Tugoy I.A., Gluschenko T.S., Samoilov M.O., Pelto-Huikko M. Effects of preconditioning by mild hypobaric hypoxia on the expression of manganese superoxide dismutase in the rat hippocampus. // Neurochemical Journal. 2007. 1 (4): 312–317.

V. Stroev S.A., Tyul'kova E.I., Glushchenko T.S., Tugoi I.A., Samoilov M.O., Pelto-Huikko M. Thioredoxin-1 expression levels in rat hippocampal neurons in moderate hypobaric hypoxia. // Neuroscience and Behavioral Physiology. 2009. 39 (1): 1-5.

VI. Stroev S.A., Tyul'kova E.I., Pelto-Huikko M.T., Samoilov M.O. Threefold exposure to moderate hypobaric hypoxia decreases the expression of Cu, Zn-superoxide dismutase in some regions of rat hippocampus. // Bulletin of Experimental Biology and Medicine. 2011. 151 (3): 301-304.

VII. Stroev S.A., Tjulkova E.I., Samoilov M.O., Pelto-Huikko M.T. One- and three-time mild hypobaric hypoxia modifies expression of mitochondrial thioredoxin-2 in

hippocampus of rat. // Acta Neurobiologiae Experimentalis (Warszawa). 2011. 71 (2): 244-255.

VIII. Stroev S.A., Tyulkova E.I., Samoilov M.O., Pelto-Huikko M.T. Changes in the expression of Mn-superoxide dismutase in the rat hippocampus after one and three episodes of moderate hypobaric hypoxia. // Neuroscience and Behavioral Physiology. 2012. 42 (8): 792-796.

IX. Stroev S.A., Tyul'kova E.I., Samoilov M.O., Pelto-Huikko M.T. A comparison of the effects of single and triple exposures to moderate hypobaric hypoxia on the expression of Cu, Zn-superoxide dismutase in the rat hippocampus. // Neurochemical Journal. 2012. 6 (3): 213-217.

X. Stroev S.A., Tyulkova E.I., Samoilov M.O., Pelto-Huikko M.T. Comparison of the effects of one and three sessions of moderate hypobaric hypoxia on thioredoxin-1 expression in the rat hippocampus. // Neuroscience and Behavioral Physiology. 2013. 43 (4): 497-501.



The thesis also contains some data previously published only in Russian:

Stroev S.A., Gluschenko T.S., Tjulkova E.I., Rybnikova E.A., Samoilov M.O., Pelto-Huikko M. Induction of manganese superoxide dismutase expression after severe hypobaric hypoxia in the hippocampus of preconditioned and non-preconditioned rats. Nejrokhimia 2005. 22 (4): 292-298. (In Russian, summary in English).

# 1. Introduction

Ischaemic stroke is one of the leading causes of death and is the leading cause of disability (Woo *et al.*, 2006). One of the most important mechanisms of brain cell injury during hypoxia/ischaemia and subsequent reoxygenation is the oxidative stress that is caused by overproduction of reactive oxygen species (ROS) and other free radicals as well as by disturbances in the intracellular redox equilibrium (Siesjö, 1978; Samoilov, 1985; Thompson, Hess, 1986; Kloner *et al.*, 1989; Coyle, Puttfarcken, 1993; Choi, 1995; Chan, 1996; Clemens, 2000; Candelario-Jalil *et al.*, 2001; Mohri *et al.*, 2001; Sugawara, Chan, 2003; Kelly *et al.*, 2008; Kim *et al.*, 2009; Zhao *et al.*, 2009; Jung *et al.*, 2010; Mammucari, Rizzuto, 2010; Raffaello, Rizzuto, 2011). In the first hours after a hypoxia episode, oxidative stress causes the release of cytochrome *c* from the mitochondria into the cytosol where it activates a cascade of caspases and induces cell death programmes (Fujimura *et al.*, 2000).

The molecular mechanisms of the protective response to hypoxia/ischaemia have been actively investigated for more than half a century starting at least from the pioneering studies of Dr Zoya Ivanovna Barbashova and coauthors (Barbashova, 1956, 1960; Barbashova, Ginetsinskiy, 1956). The mechanisms of neuronal adaptation to hypoxia and subsequent reoxygenation include the activation of enzymatic and nonenzymatic cellular antioxidant systems. Endogenous antioxidants restore the redox balance, bind and inactivate free radicals. Thus, they prevent lipid peroxidation, oxidative damage of enzymes and nucleic acids, and the apoptosis induction.

Endogenous protein antioxidants – the thioredoxins (Trxs) and superoxide dismutases (SODs) – have well-known cytosolic forms (Trx-1; Cu, Zn-SOD) and specific mitochondrial forms (Trx-2; Mn-SOD). Mitochondria are one of the main sources of free radicals in the cell and the key organelles in the induction of apoptosis (Ueda *et al.*, 2002; Dodson *et al.*, 2013), so the ratio of pro- and antioxidant processes in the mitochondria is particularly important for the development of both pathological and adaptive responses (Cao *et al.*, 2007; Antico Arciuch *et al.*, 2012; Yin *et al.*, 2012).

Modulation of endogenous cellular defense mechanisms via the stress response signaling represents an innovative approach to therapeutic intervention in many diseases (Calabrese *et al.*, 2009). It has been found that preconditioning by mild hypoxia (which does not cause irreversible damage) increases the structural and functional resistance of cells (including neurons) to subsequent severe (damaging) hypoxia (Kitagawa *et al.*, 1990; Samoilov, 1985, 1999; Corbett, Crooks, 1997; Qi *et al.*, 2001; Romanovskii *et al.*, 2001; Samoilov *et al.*, 2001a, 2003a, b; Wu *et al.*, 2001; Semenov *et al.*, 2002; Rybnikova *et al.*, 2005a; Stetler *et al.*, 2009). One of the key molecular mechanisms of the cytoprotective effect of preconditioning is the activation of endogenous antioxidants, including Trxs and SODs (Kato *et al.*, 1995; Duan *et al.*, 1999; Yamashita *et al.*, 2000; Samoilov *et al.*, 2001a, 2003b; Garnier *et al.*, 2001; Andoh *et al.*, 2002 a, b; Hoshida *et al.*, 2002).

There are many published reports on the molecular mechanism of adaptive and maladaptive hypoxic effects, but most of these studies have been carried out using different models of ischaemia. At the same time, the effects of hypobaric hypoxia were still poorly understood in general and have not been investigated at the level of antioxidant expression.

It is known that the very early period (approximately 2-4 h) after reoxygenation following hypoxia is critical for the induction of the adaptive or pathological responses that lead to cell survival or delayed death (Fujimura *et al.*, 2000). Nevertheless, the expression of antioxidants in those very first hours after hypoxia remained the least understood. It has been studied in only a few papers, and the data on different antioxidants have been obtained in different experimental models (Ohtsuki *et al.*, 1993; Liu *et al.*, 1993a, 1994a; Kato *et al.*, 1995; Takagi *et al.*, 1998a, b; Hattori *et al.*, 2002). Thus, the comparison, systematisation and generalisation of these results are quite difficult and problematic.

Thus, it was important to study the effect of hypobaric hypoxia, especially during the early period (3 h after exposure), on the expression of both cytosolic and mitochondrial antioxidants. It was also an essential objective to study the effect of preconditioning by monitoring the expression dynamics of these antioxidants during the preconditioning procedure, after its completion, and after a subsequent severe hypoxia.

# 2. Review of the literature

## 2.1. Hypoxia: experimental models

Hypoxia can be caused by various factors, e.g., circulatory disturbances (circulatory hypoxia or ischaemia), destruction of oxygen-binding haeme-containing proteins, and a decreased partial pressure of oxygen in the organism's environment.

To date, various ischaemic models have been the most frequently studied. One of these models is the focal ischaemia that form in the brain the core of an infarct and the perifocal "penumbra" region (Liu *et al.*, 1993a, b, 1994a, b; Toyoda, Lee, 1997; Bidmon *et al.*, 1998; Keller *et al.*, 1998; Takagi *et al.*, 1998a, b, 1999; Hattori *et al.*, 2002; Kim *et al.*, 2002; Zhou *et al.*, 2009, 2013; Chung *et al.*, 2011; Ma *et al.*, 2012), chronic hypoxic ischaemia induced by bilateral carotid artery occlusion (Yu *et al.*, 2011), and global ischaemia (temporary, which is followed by reperfusion, and permanent, which is not followed by reperfusion). Global ischaemia is often studied in Mongolian gerbils because features of their circulatory system ensure the establishment of complete cerebral ischaemia (Kitagawa *et al.*, 1990; Kirino *et al.*, 1991; Tomimoto *et al.*, 1993; Kato *et al.*, 1995; Corbett, Crooks, 1997). The second group of experimental models is associated with the use of brain slices (Samoilov, Mokrushin, 1997; Samoilov *et al.*, 2001a; Romanovskii *et al.*, 2001). These models make it possible to study the hypoxia induced by decreased oxygen content in incubation solutions and anoxia induced by complete cessation of the oxygen supply for a specified period with or without subsequent reoxygenation. Another of the *in vitro* hypoxic models uses different neuronal cell cultures (Berggren *et al.*, 1996; Isowa *et al.*, 2000; López-Hernández *et al.*, 2012). The next group is acute or chronical hypoxic models *in vivo*, which can be associated with a decrease in the atmospheric pressure (hypobaric hypoxia) or with a decrease in oxygen content while maintaining normal pressures (normobaric hypoxia) (Kreps *et al.*, 1956; Hochachka, 1998; Rybnikova *et al.*, 2005a; Bianciardi *et al.*, 2006; Zhang *et al.*, 2006; Maiti *et al.*, 2007, 2008a, b; Akhavan *et al.*, 2012; Chen *et al.*, 2012; Portnichenko *et al.*, 2012a; Lukyanova *et al.*, 2013). One more practically important area of research has been the studies of the effects of hypobaric or normobaric hypoxic treatments in female animals during gestation on the

development and functional state of their progeny in subsequent postnatal ontogenesis (Zhuravin *et al.*, 2001; Vataeva *et al.*, 2001, 2004a, 2005; Dubrovskaya, Zhuravin, 2010; Tiul'kova *et al.*, 2010; Tyul'kova, *et al.*, 2011; Stroev *et al.*, 2011).

Each of these forms of hypoxia has its own particular peculiarities. Different models of hypoxia, as well as different intensities and durations of exposure, can determine the diverse direction of the response. Obviously ischaemic influences are not confined only to hypoxia, and at minimum, there are combined effects of hypoxia and hypoglycaemia. It should be noted that even identical processes in different models occur with different dynamics: more or less similar effects can be achieved during anoxia in brain slices *in vitro* in the first minutes, but during hypoxia *in vivo*, such changes require tens of minutes or hours. Nevertheless, there are general mechanisms that are uniform in different models of hypoxia (Samoilov, 1999; Stroev, Samoilov, 2006).

## 2.2. Free radicals and oxidative stress

Hypoxia/ischaemia induces changes in the intracellular redox state, ion conduction and neuron excitability, synaptic and intracellular signal transduction, and the activity of genes and their products. The direction and extent of changes in these processes determine the outcome of the hypoxia, i.e., damage and death or survival of brain nerve cells (Samoilov, 1985, 1999).

One of the major mechanisms of neuronal damage in the brain during severe hypoxia/ischaemia and subsequent reoxygenation is the oxidative stress associated with the overproduction of free radicals and other reactive oxygen species (ROS) (Bulkley, 1987; Chan, 2001; Hayashi *et al.*, 2005; Kelly *et al.*, 2008; Kim *et al.*, 2009; Zhao *et al.*, 2009; Jung *et al.*, 2010). ROS play an important role in many physiological and pathological processes: in mechanisms of signal transduction, in oxidative damage of cells, in the potentiation of xenobiotic action, in reactions to hypoxia/reoxygenation, and in the regulation of cell proliferation, carcinogenesis, aging and other processes (Valko *et al.*, 2007; Covarrubias *et al.*, 2008; Mammucari, Rizzuto, 2010; Morgan, Liu, 2011; Raffaello, Rizzuto, 2011; Winterbourn, 2013).

ROS are generated in aerobic cells in many pathways, for example, by NADPH cytochrome P450 reductase in the endoplasmic reticulum, by hypoxanthine/xanthine

oxidase, by lipoxygenase and cyclooxygenase and by various cytotoxic stresses, including γ-ray and ultraviolet light irradiation (Fujino *et al.*, 2006). ROS are produced also by some dehydrogenases including alfa-ketoglutarate dehydrogenase (α-KGDH) and alfa-glycerophosphate dehydrogenase (α-GPDH) (Tretter, Adam-Vizi, 2005; Adam-Vizi, 2005; de Grey, 2005; Adam-Vizi, Tretter, 2013). ROS can be generated as cellular responses to xenobiotics, cytokines, and bacterial invasion (Ray *et al.*, 2012). However, the main sources of ROS in the cell are NADPH oxidase (Girouard, Iadecola, 2006; Miller *et al.*, 2006; Wang *et al.*, 2006b; Kim *et al.*, 2009; Kleikers *et al.*, 2012) and the electron transport chain in mitochondria (Moro *et al.*, 2005; Bolaños *et al.*, 2009; Koopman *et al.*, 2010; Niizuma *et al.*, 2010; Ralph *et al.*, 2011; Ishii *et al.*, 2013; Quinlan *et al.*, 2013).

Multiple intensifications of oxidative processes during a deficiency of oxygen, the basic and universal oxidant, may seem to be a paradox. However, hypoxic conditions suppress the activity of the mitochondrial cytochrome oxidases, which are sensitive to a decrease in $pO_2$, and the electrons are transferred from coenzyme Q to oxygen. Studies have shown that ROS generation during ischaemia in the mitochondrial, microsomal and nuclear membranes of rat liver cells increases compared with intact animals by 5-10 times (Khavinson *et al.*, 2003).

One of the primary products of the ROS family is likely to be the superoxide anion radical ($O_2^{\cdot-}$). The largest amount of $O_2^{\cdot-}$ is generated as a byproduct of oxygen reduction in the mitochondrial respiratory chain (Koltover, 1996; Lemasters, Nieminen, 1997). $O_2^{\cdot-}$ takes part in further dismutation reactions with the formation of hydrogen peroxide ($H_2O_2$):

$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$

Hydrogen peroxide ($H_2O_2$) can then decompose to form the hydroxyl radical ($OH\cdot$), which is one of the most chemically reactive ROS. The sources of $OH\cdot$ are the Haber-Weiss reaction, oxidation of arachidonic acid, microsomal oxidation and photolysis of water (Zenkov *et al.*, 1993), but the principal source of this radical is the Fenton reaction (Koppenol, 1994):

$O_2^{\cdot-} + H_2O_2 \rightarrow OH^- + OH\cdot + O_2$ (Haber-Weiss reaction)

$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH\cdot + Fe^{3+}$ (Fenton reaction)

$O_2^{\cdot-}$ can reduce $Fe^{3+}$ to $Fe^{2+}$, increasing the $Fe^{2+}$ content of the cell (Halliwell, Gutteridge, 1984). In turn, $Fe^{2+}$ takes part in further Fenton reactions and also increases

lipid peroxidation (LPO), participating in the branching chain reaction (Khavinson *et al.*, 2003). High degree of reduction, the accumulation of NADH and the acidosis are characteristics of the hypoxic state. Under these conditions $O_2^{\cdot-}$ interacts with proton ($H^+$) to form $HO_2^{\cdot}$ – another oxygen free radical that is more reactive than $O_2^{\cdot-}$ and able to initiate LPO. The accumulation of unsaturated fatty acids that takes place during hypoxia forms a substrate for the development of LPO chain reactions. In the hypoxia-induced condition of full reduction of the respiratory chain, oxygen has increased opportunities to interact with ubiquinol, so the restoration of the oxygen supply after severe hypoxia increases the production of $O_2^{\cdot-}$ and does not restore the normal state of the cell (Boldyrev, 1998).

Overproduction of free radicals induces not only chain reactions of membrane lipid peroxidation but also the oxidation of proteins and carbohydrates and damage of nucleic acids (Aruoma, 1998; Boldyrev, 1998; Cooke *et al.*, 2003; Khavinson al., 2003; Evans *et al.*, 2004). Depending on the degree of damage, cells can run programmes of DNA repair or (in the case of irreversible damage) apoptotic death. Thus, oxidative stress and ROS overproduction lead to significant functional and structural damage of the cell until its death by necrosis or apoptosis.

## 2.3. The role of oxidative stress in the induction of nerve cell damage

Induction of the programme of delayed apoptotic cell death is one of the most important mechanisms of the damaging effects of oxidative stress (Carmody, Cotter, 2001), particularly when induced by hypoxia (Niizuma *et al.*, 2010). Apoptosis is a special type of cell death distinct from necrosis and other types (Kerr, 1965, 1971, 2002; Kerr *et al.*, 1972; Clarke, Clarke, 1996, 2012; Levin *et al.*, 1999; Lockshin, Zakeri, 2001; Kroemer *et al.*, 2005, 2009; Galluzzi *et al.*, 2007, 2012; Green *et al.*, 2009; Ouyang *et al.*, 2012; Nikoletopoulou *et al.*, 2013). To date, two main (Cory, Adams, 2002; Mirkes, 2002; Galluzzi *et al.*, 2012) (Fig. 1) and some additional (Lee et al., 1999; Li et al., 1999; Häcki et al., 2000; Nakagawa et al., 2000; Rudner et al., 2001; Lassus et al., 2002 ; Rao et al., 2002) pathways of apoptosis initiation have been discovered.

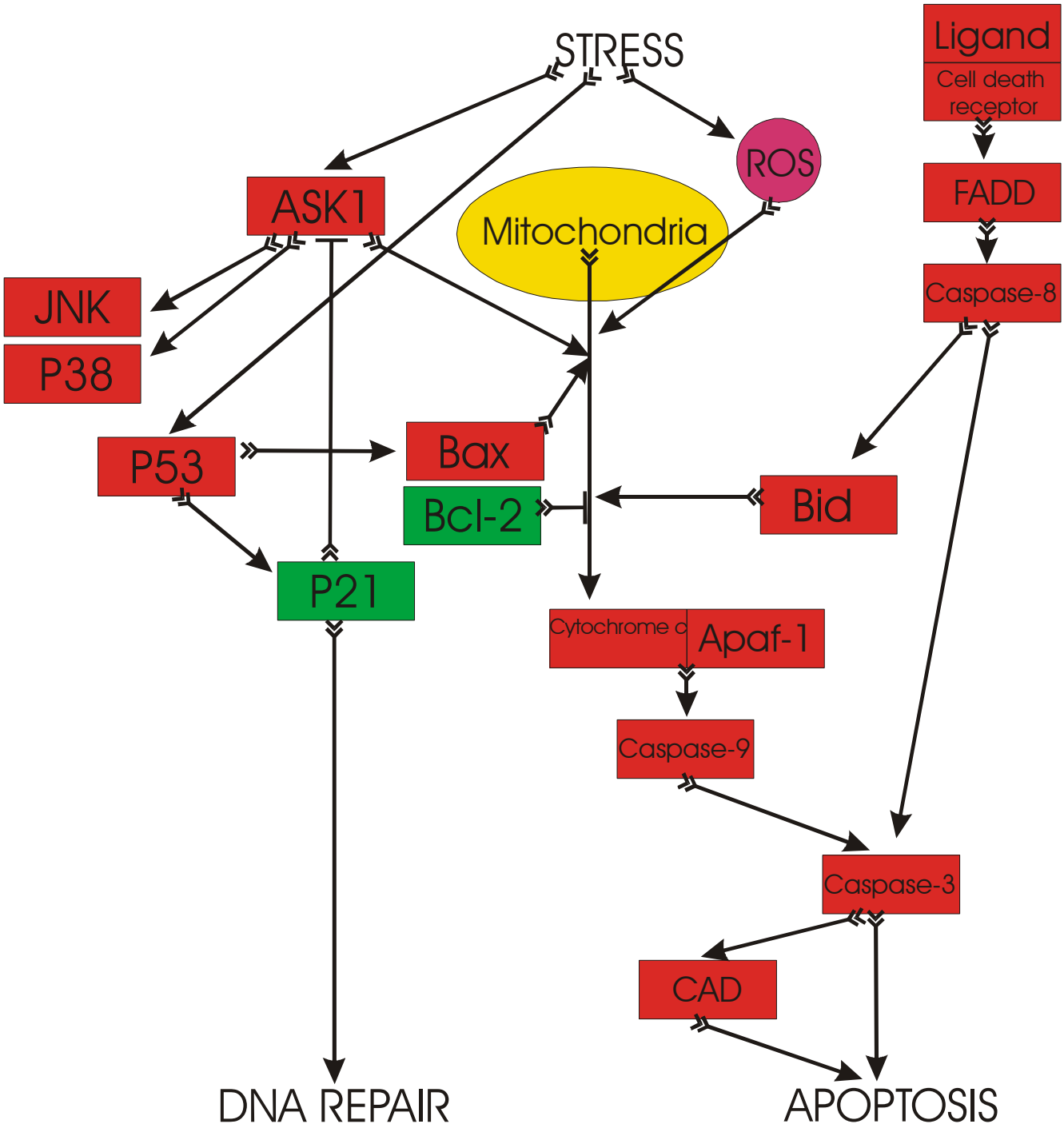



Fig. 1. The main pathways of apoptosis initiation. Abbreviations: Apaf-1 – Apoptotic peptidase-activating factor 1; ASK1 – apoptosis signal-regulating kinase 1; CAD – caspase-activated DNase; FADD – Fas-associated protein with death domain; JNK – c-Jun N-terminal kinase; ROS – reactive oxygen species. The proapoptotic and ambivalent factors are marked in red, and the antiapoptotic factors are marked in green.

The evolutionarily younger extrinsic pathway of apoptosis is mediated by the activation of plasma membrane "death receptors" (Fas, TNF-α receptors and related proteins) as a result of binding with their ligands or with antibodies in experimental models (Itoh *et al.*, 1991; Cory, Adams, 2002; Galluzzi *et al.*, 2012). This binding leads first to changes in the conformation of the adapter proteins (FADD), and as a result, their effector N-terminal domains acquire the ability to interact with and activate caspase-8 (Ulukaya *et al.*, 2011) or caspase-10 (Ola *et al.*, 2011; Galluzzi *et al.*, 2012). Caspases represent a special class of proteases that contain a cysteine residue in their active site and cleave the target protein so that the C-terminal residue is aspartic acid (Ueda *et al.*, 2002). Through limited proteolysis, activated initiator caspase-8 activates the downstream caspase cascade, especially effector caspase-3 (Nagata, 1997). Activated caspases cleave many nuclear and cytoskeletal structural proteins, such as lamins, nuclear mitotic apparatus protein (NuMa), actin, fodrin, and gelsolin (Ueda *et al.*, 2002; Ulukaya *et al.*, 2011). Caspase-3 activates caspase-activated DNase (CAD), removing its intrinsic inhibitor (ICAD) and thereby relieving the inhibitory effect (Sakahira *et al.*, 1998). This disinhibition leads to CAD-mediated DNA fragmentation and cell death by apoptosis.

The second – intrinsic – pathway of apoptosis is considered to be evolutionarily more ancient (Cory, Adams, 2002). It is induced by stress, particularly oxidative, and is mediated by the release of cytochrome *c* from mitochondria into the cytosol. Cytochrome *c* then induces the activation of initiator caspase-9 and, through it, other caspases, including effector caspase-3 (Ueda *et al.*, 2002; Ulukaya *et al.*, 2011; Galluzzi *et al.*, 2012).

The release of cytochrome *c* and other pro-apoptotic factors from mitochondria into the cytosol is regarded as the key moment in the mechanism of cell death induced by oxidative stress (Reed, 1997; Jacotot *et al.*, 1999; Vieira *et al.*, 2000; Ueda *et al.*, 2002; Vieira, Kroemer, 2003; Donovan, Cotter, 2004; Jiang, Wang, 2004; Mohamad *et al.*, 2005; Gogvadze *et al.*, 2006). It is well known that pro- and antiapoptotic proteins of the Bcl-2 family play an important role in regulating the permeabilisation of the mitochondrial membrane and the release of cytochrome *c* (Mignotte, Vayssiere, 1998; Tsujimoto, Shimizu, 2000; Breckenridge, Xue, 2004; Tait, Green, 2010; Estaquier *et al.*, 2012). This family consists of 3 groups. The first group includes 1 proapoptotic protein, Bcl-xS, and 5 antiapoptotic proteins, Bcl-2, Bcl-xL, Bcl-w, A1 and Mcl-1. These proteins include 4 BH domains (BH1, BH2, BH3, BH4) and a transmembrane domain. The second group

includes the Bax-like proapoptotic proteins Bax, Bak and Bok, which include 3 BH domains (BH1, BH2, BH3) and a transmembrane domain. The third group is the BH3-only proteins, which contain a single BH domain that is necessary and sufficient to meet their proapoptotic function. These proteins are Bad, Bid, Bim, Bik, Hrk, Noxa, Bmf and Puma (Harris, Thompson, 2000; Cory, Adams, 2002; Ulukaya *et al.*, 2011).

According to current models, Bax-like proteins are directly involved in the release of cytochrome *c* and, consequently, the induction of apoptosis (Kirkland *et al.*, 2002). The protective function of the antiapoptotic proteins Bcl-2, Bcl-xL and Bcl-w is probably connected with blocking outer mitochondrial membrane permeability and consists of preventing the release of cytochrome *c* and several other proapoptotic products into the cytoplasm. Thus, the antiapoptotic proteins of the Bcl-2 group act as functional antagonists of the proapoptotic proteins of the Bax group (Tait, Green, 2010). The role of the proapoptotic BH3-only proteins is probably to bind and thereby inactivate the Bcl-2-like proteins. BH3-only proteins by themselves in the absence of Bax and Bak are not capable of inducing apoptosis, but by inactivating proteins in the Bcl-2 group, they unlock the activity of Bax and Bak, which leads to the release of cytochrome *c* (Fig. 2).

The proapoptotic factors released from mitochondria into the cytosol can induce both caspase-dependent and caspase-independent mechanisms of cell death (Loeffler, Kroemer, 2000; Yaginuma *et al.*, 2001; Jäättelä, 2002; Belizário *et al.*, 2007). In the caspase-dependent mechanism, the released cytochrome *c* interacts with apoptotic peptidase-activating factor 1 (Apaf-1), which is localised to the cytoplasm. As a result, an oligomeric complex (the so-called "apoptosome") consisting of several molecules of Apaf-1, cytochrome *c* and procaspase-9 is formed (Cain *et al.*, 2002; Twiddy *et al.*, 2004; Spierings *et al.*, 2005; Garrido *et al.*, 2006; Bao, Shi, 2007; Riedl, Salvesen, 2007). In this complex, the procaspase-9 molecules closely approach each other, and autocatalysis is induced as usual for the caspases via limited proteolysis of procaspase-9 to activated caspase-9 (Festjens *et al.*, 2006; Shi, 2006; Bratton *et al.*, 2010; Reubold, Eschenburg, 2012; Yuan, Akey, 2013). Then, initiator caspase-9, by the same limited proteolysis, activates effector caspase-3 and other caspases in the cascade (Li *et al.*, 1997; Pan *et al.*, 1998; Cain, 2003). Activated caspases, similarly to the receptor-dependent apoptosis pathway, cleave a number of structural proteins in the cytoplasm and nucleus and activate CAD, thereby inducing DNA fragmentation (Fig. 2). However, the same cascade of caspase activation can also

have a non-apoptotic role in the signalling of cell proliferation, cell differentiation, cell shape, cell migration and immunity (Weber, Menko, 2005; Garrido *et al.*, 2006; Kuranaga, Miura 2007; Yi, Yuan 2009; Miura, 2012) as well as in the neuroprotection induced by preconditioning (McLaughlin *et al.*, 2003), in long-term potentiation of neurons, neuronal plasticity and in learning and memory processes (Dash *et al.*, 2000; Gulyaeva, 2003; Gulyaeva *et al.*, 2003; Kudryashov *et al.*, 2004; Sherstnev, 2004; Gemma *et al.*, 2005, Stepanichev *et al.*, 2005; Huesmann, Clayton, 2006; Sherstnev *et al.*, 2006; Markina *et al.*, 2007, 2008, 2009; Kudryashova *et al.*, 2009).

Although traditionally the effect of oxidative stress has been associated with the stress-induced cytochrome *c*-dependent way, oxidative stress probably can also induce apoptosis via receptor-dependent mechanisms, in particular, by inducing tumour necrosis factor (TNF-α) expression (Brown *et al.*, 2004; Hughes *et al.*, 2005).

In addition to cytochrome *c*, a number of other proapoptotic molecules are released from mitochondria into the cytosol (Jäättelä *et al.*, 2002; Arnoult *et al.*, 2003; van Gurp *et al.*, 2003; Cregan *et al.*, 2004; Saelens *et al.*, 2004; Ola *et al.*, 2011). Apoptosis-inducing factor (AIF) is involved in chromatin condensation and activating DNase (Susin *et al.*, 1997). Endonuclease G fragments nucleosomal DNA together with CAD. Secondary activators of caspases – the products of the genes Smac/Diablo and Omi/HtrA2 – bind and inhibit the inhibitor of apoptosis proteins (IAP) to disinhibit the activation of caspases (Ola *et al.*, 2011) (Fig. 2). In some cells, procaspases are also released from mitochondria.

Apoptosis signal-regulating kinase 1 (ASK1) plays a special role in the initiation of apoptosis (Fig. 1). It was identified as one of the MAP-kinase kinase kinases (Ichijo *et al.*, 1997). ASK1 is activated by stress (including oxidative) and then activates c-Jun N-terminal kinase (JNK) and p38 MAP-kinase (Ichijo *et al.*, 1997). In this way, ASK1 mediates the apoptotic signal. ASK1 is probably mainly associated with the mitochondrial pathway of apoptosis initiation because its activation induces the release of cytochrome *c* and the activation of caspase-9 and caspase-3 but not caspase-8 (Hatai *et al.*, 2000).

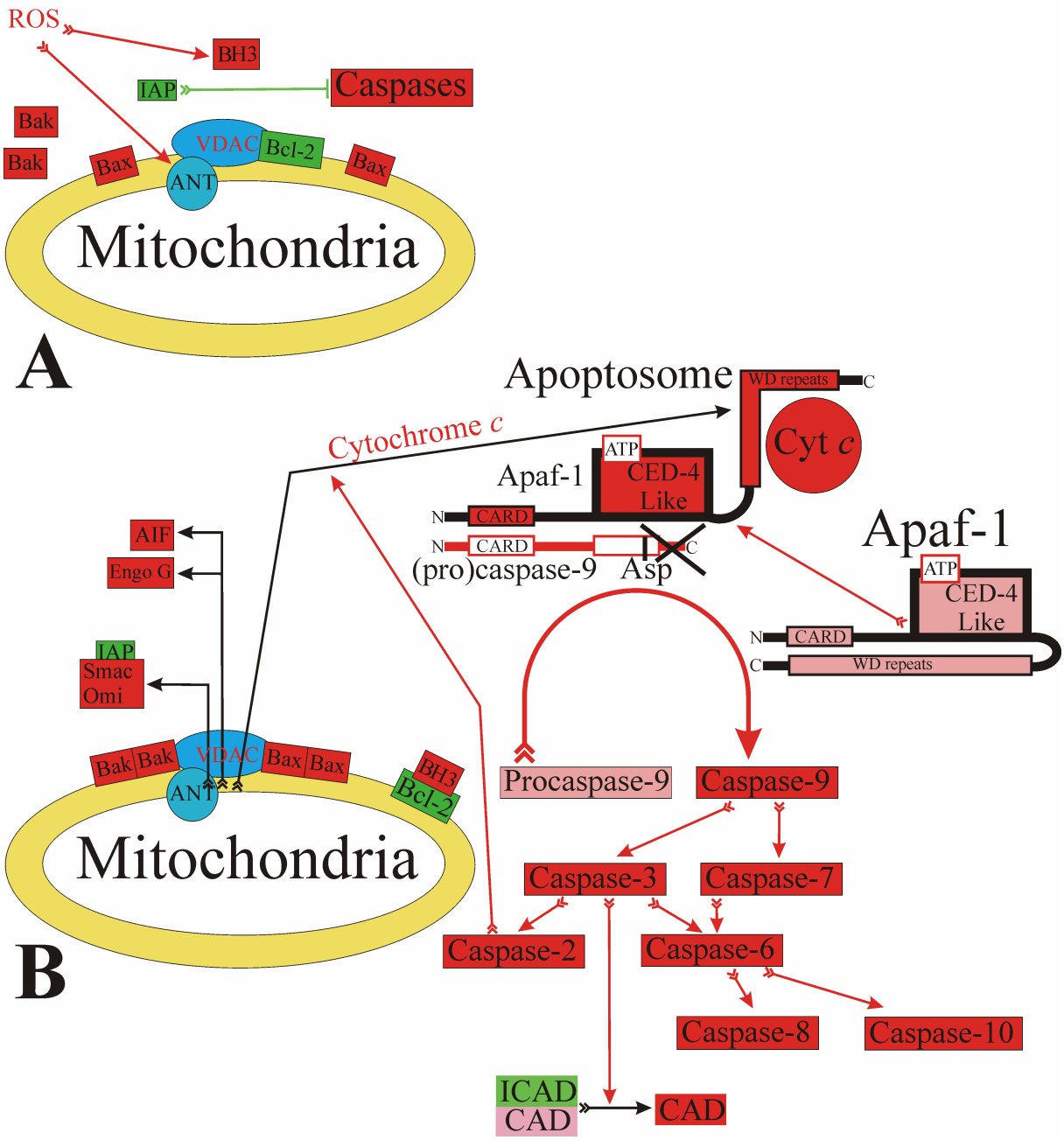



Fig. 2. Apoptosis induced by a mitochondria-dependent mechanism: the release of proapoptotic proteins into the cytosol and the activation of caspases. A – Initiation of the apoptotic signal, B – Implementation of the apoptotic pathway. Abbreviations: AIF – apoptosis-inducing factor; ANT – adenine nucleotide translocator; Apaf-1 – Apoptotic peptidase-activating factor 1; CAD – caspase-activated DNase; CARD – caspase recruitment domain; CED-4-Like – *Caenorhabditis elegans* death factor-4-like domain; Cyt c – cytochrome *c*; EndoG – endonuclease G; IAP – inhibitor of apoptosis proteins; ICAD – inhibitor of caspase-activated DNase; ROS – reactive oxygen species; Smac – secondary activators of caspases; VDAC – voltage-dependent anion-selective channels; WD repeat – a structural motif of approximately 40 amino acids terminating in a tryptophan-aspartic (W-D) dipeptide. The proapoptotic factors are marked in red, and the antiapoptotic factors are marked in green.

An additional mechanism of apoptosis initiation is related to the regulatory protein p53 (Fig. 1), which is also induced by oxidative stress, particularly after ischaemia, and may, depending on the intensity of the stress and the redox status of the cell, act as a proapoptogen that activates Bax (Miyashita, Reed, 1995) and binds to Bcl-xL (Endo *et al.*, 2006a, c), or it may act as an adaptogen that protects the genome from oxidation (Sablina *et al.*, 2005), stops the cell cycle to repair the DNA damaged by oxidative stress (Harper *et al.*, 1993; Hartwell, Kastan, 1994; Amundson *et al.*, 1998) and upregulates the sestrins (Budanov, 2011; Sanchis-Gomar, 2013).

Nevertheless, at least in nervous tissue, cytochrome *c* probably plays a key role in apoptosis induction (Cory, Adams, 2002). Consequently, the protective responses, which are developing at the earliest possible stages following hypoxia/ischaemia before the intensive release of cytochrome *c*, are especially crucial for cell survival.

## 2.4. The role of intracellular antioxidants in molecular mechanisms of neuroprotection

Oxidative stress is a key mechanism of neuronal damage during hypoxia and subsequent reoxygenation, and it is one of the most important direct causes of cell death. Thus, endogenous antioxidants, which remove or at least substantially ameliorate the effect of oxidative stress, represent one of the major neuroprotective systems.

The term "oxidative stress" often means two interrelated but different process. On one hand, it means the synthesis of reactive oxygen species and other free radicals, and on the other hand, it is a violation of the oxidation-reduction (redox) balance, namely, its displacement in the direction of oxidation. These two processes commonly run in parallel and are interdependent but not in all cases. In particular, during severe hypoxia before reoxygenation, the redox state is markedly shifted toward reduction, but free-radical oxidation processes are already occurring at the same time.

Accordingly, the role of antioxidants is twofold: first, they can act as antagonists of free radical oxidation (free radical scavengers, metal chelators, antiperoxidants), and second, some of them can act as reducing agents and regulators of the redox balance. There are a

number of endogenous antioxidants: intra- and extracellular, hydrophilic and hydrophobic, and enzymatic and non-enzymatic. Enzymatic antioxidant systems include SODs, peroxidases (including catalase) and the enzymes of the thioredoxin and glutathione-glutaredoxin systems. Non-enzymatic antioxidants include glutathione, metal chelators (ferritin, hemosiderin, transferrin, ceruloplasmin, lactic and uric acids and a number of peptides), scavengers of free radicals (ascorbate; retinol and other forms of vitamin A and carotenoids; phenols, such as tocopherols and tocotrienols, flavonoids, ubiquinones, vitamin K; uracil and its derivatives; tryptophan, melatonin and phenylalanine; L-carnitine, and carnosine; taurine; vitamin D, glucocorticoids and some other steroids) and others.

In the present work, the focus will be on 4 intracellular endogenous antioxidants: two forms of Trx (cytosolic Trx-1 and mitochondrial Trx-2) and two forms of SOD (cytosolic Cu, Zn-SOD and mitochondrial Mn-SOD).

It should be noted that the brain has all of the conditions for the extensive occurrence of free radical oxidation (Galkina, 2000). First, there is a high level of oxygen consumption; the brain accounts for 20-25% of the total $O_2$ consumed by the organism despite the fact that its mass accounts for only approximately 2% of body weight (Eshchenko, 1999). Moreover, the neurons represent only approximately 5% of brain volume, but they use 25% of the oxygen consumed by the brain. Second, the brain tissue has additional ROS-generating systems related to neurotransmitters, such as glutamate, dopamine, serotonin and catecholamines. Third, the brain is characterised by a high lipid content (approximately 50% of the dry weight of the tissue) with an extremely high level of fatty acids unsaturation.

The amount and activity of some antioxidant enzymes in the brain is much lower compared with such tissues as the liver, kidney or heart (L'vova, Abaeva, 1996; Levadnaya *et al.*, 1998); the content of SOD in the brain is on average 3-7 times lower, and the catalase activity is often 50-70 times lower than in the liver. Hence, we can draw two conclusions. First, the brain tissue is very sensitive to oxidative stress, in particular stress caused by hypoxia/reoxygenation. Second, thiol antioxidants, such as the Trxs, probably have an important role in the protection of nerve cells against hypoxic effects. The latter assumption is in a good agreement with data on the peculiarities of mitochondrial Mn-SOD and Trx-2 gene expression in rat brains after hypoxia (Samoilov *et al.*, 2002).

## 2.4.1. Thioredoxins

**Structure, main function and distribution of Trxs**

Trxs are small multifunctional ubiquitous proteins containing two redox-active cysteine groups in their active centre, which includes the conserved amino acid sequence -Cys-Gly-Pro-Cys- (Holmgren, 1985). These proteins can exist both in reduced dithiol (Trx-(SH)2) and oxidised disulphide (Trx-S2) forms. Trx-(SH)2 is a complete protein disulphide reductase involved in many thiol-dependent reduction reactions through the reversible oxidation of the dithiol of its active centre to disulphide (Holmgren, 1985; Patwari *et al.*, 2006). In this way, Trxs can catalyse a dithiol-disulphide exchange reaction. Oxidised Trx-S2 is usually then reduced by flavoprotein thioredoxin reductase using electrons from NADPH. Trx, thioredoxin reductase (Trx-R) and NADPH form the so-called "thioredoxin system" (Powis, Montfort, 2001a, b; Biaglow, Miller, 2005; Kohárýová, Kollárová, 2008). Trx-Rs of mammals are represented by 3 isoforms (Kipp *et al.*, 2012; Lu, Holmgren, 2012) that are expressed from 3 separate genes (Arnér, 2009); all of the forms are selenium-containing flavoprotein oxidoreductases (Arnér, 2009) and belong to a large class of Se-dependent enzymes (Björnstedt *et al.*, 1997; Ganther, 1999; Schweizer *et al.*, 2004).

The expression of Trx is upregulated by oestrogen (Chiueh *et al.*, 2003). It is also induced by nuclear erythroid 2-related factor 2 (Nrf2), which translocates from the cytoplasm after oxidative stress (Kobayashi, Yamamoto, 2006; Tanaka *et al.*, 2011). In this regard, Trx is considered a downstream protein of the Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid 2-related factor 2 (Nrf2) system (Holtzclaw *et al.*, 2004; Motohashi, Yamamoto, 2004; Tanaka *et al.*, 2011).

The study of Trxs has shown an unexpected diversity in their functions (Holmgren, 1985; Arner and Holmgren, 2000; Hanschmann *et al.*, 2013). Primarily, they are known as hydrogen donors for ribonucleotide reductase, which is an important enzyme involved in the synthesis of deoxyribonucleotides for DNA replication. Trxs serve as a substrate for other reducing enzymes (such as methionine sulphoxide reductase and sulphate reductase), act as protein disulphide oxidoreductases (in particular, in the degradation of insulin), are regulatory factors for a number of enzymes and receptors (so-called thiol reduction controls), constitute a subunit of viral DNA polymerase (for example, in phage

T7) and act as an important participant in the assembly of small viruses (for example, single-stranded DNA-containing phage f1). Trxs are involved in such processes as apoptosis and redox signalling, protein and DNA synthesis, folding and the formation of protein structure, enzymatic reactions, photosynthesis, aging, cellular proliferation, angiogenesis and carcinogenesis (Holmgren, 1985; Powis *et al.*, 1997, 1998, 2000; Yoshida *et al.*, 2003, 2005; Dunn *et al.*, 2010). Trxs are important for the mechanisms of a variety of diseases, protective reactions and medical treatments (Kirkpatrick *et al.*, 1997; Patenaude *et al.*, 2005; Arnér E.S., Holmgren, 2006; World *et al.*, 2006; Kaimul *et al.*, 2007; Lillig, Holmgren, 2007; Ebrahimian, Touyz, 2008; Okuyama *et al.*, 2008; Altschmied, Haendeler, 2009; Tonissen, Di Trapani, 2009; Holmgren, Lu, 2010; Xu *et al.*, 2012). It has been shown that Trxs may also be involved in the antibacterial response (Umasuthan *et al.*, 2012).

Trxs are present in various pro- and eukaryotic cells and are ubiquitous in all of them (Holmgren, 1985, Gleason, Holmgren, 1988). In the cells of bacteria, yeasts, plants and animals, all Trxs have a molecular mass of approximately 12 kDa and evolutionarily originated from a single molecule (Holmgren, 1985). A Trx was isolated for the first time in 1964 from *E. coli* (Laurent *et al.*, 1964). This is the most well-studied Trx.

The 3-dimensional structure of the Trx molecule has been determined crystallographically to a resolution of 2.8 Å (Holmgren *et al.*, 1975). The molecule consists of 108 known amino acid residues, is compact and always has a spherical shape. The molecule contains a nucleus consisting of 5 β-segments flanked by 4 α-helices. In Trx-S2, the disulphide active centre is located in a unique protrusion of the 3-dimensional structure formed by residues 29-37 (Holmgren, 1989). The active centre is formed by a disulphide ring of 14 residues located at the C-terminus of β-strand β-2 and in the first circuit of the long α-helix α-2. Based on the homology of their primary structures, all of the cellular Trxs may have the same folding pattern.

The Trx of mammalian cells has 27% homology with the Trx of *E. coli*. The site of the active centre is the most conserved and includes the sequence Val-Asp-Phe-Xaa-Ala-Xaa-Trp-Cys-Gly-Pro-Cys-(Lys)-(Met)-(Ile)-Xaa-Pro (Holmgren, 1989). Mammalian Trx has been purified from rat Novikoff hepatoma, calf liver, rat liver, rabbit bone marrow, and human placenta (Holmgren, 1985). Human Trx was originally cloned as adult T cell

leukaemia-derived factor (ADF) that is produced by cells transformed by human T lymphotropic virus-1 (HTLV-I) (Tanaka *et al.*, 2000).

Trx-1 and Trx-2, as well as Trx-R1 and Trx-R2, are well represented in the neurons of the various regions of the rat brain (Aon-Bertolino *et al.*, 2011). In addition to the long-known and most-studied cytosolic Trx-1 and mitochondrial Trx-2, the family of mammalian Trxs involves many other members (Nakamura, 2005) (Table 1).

Table 1. The members of the family of mammalian Trxs.

| **Member of Trxs family** | **Abbreviation** | **Reference** |
|---|---|---|
| Larger thioredoxin-like protein | p32TrxL | Hirota *et al.*, 2002 |
| Thioredoxin-like protein-1 | Txl-1 | Miranda-Vizuete *et al.*, 1998; Jiménez *et al.*, 2006 |
| Thioredoxin-like protein-2 | Txl-2 | Sadek *et al.*, 2003 |
| Spermatid-specific thioredoxin-1 | Sptrx-1 | Miranda-Vizuete *et al.*, 2001; Jiménez *et al.*, 2002a, b |
| Spermatid-specific thioredoxin-2 | Sptrx-2 | Sadek *et al.*, 2001; Miranda-Vizuete *et al.*, 2003 |
| Spermatid-specific thioredoxin-3 | Sptrx-3 | Jiménez *et al.*, 2004 |
| Specific Trx of the endoplasmic reticulum | ERp18 | Alanen *et al.*, 2003 |
| Specific Trx of the endoplasmic reticulum | ERdj5 | Cunnea *et al.*, 2003 |
| Phospholipase *C-alpha* | ERp57 | Hirano *et al.*, 1994 |
| Protein disulphide isomerase, the rat analogue of the hamster protein P5 | CaBP1 | Rupp *et al.*, 1994 |
| Protein disulphide isomerases, the rat analogue of the murine protein ERp72 | CaBP2 | Rupp *et al.*, 1994 |
| Thioredoxin-related protein 14 | TRP14 | Jeong *et al.*, 2004a, b, 2009 |

**Protection against oxidative stress and the role of Trx in hypoxia/ischaemia**

Trxs are antioxidant proteins that protect cells against various types of oxidative stress (Hori *et al.*, 1994; Sasada *et al.*, 1996; Holmgren, 2000; Nishinaka *et al.*, 2001a, b; Nordberg, Arnér, 2001; Yamawaki *et al.*, 2003; Patenaude *et al.*, 2005; Go *et al.*, 2007; Yang *et al.*, 2011; Zhou *et al.*, 2012). In particular, in experiments with transgenic mice, it has been shown that overexpression of Trx protects brain cells from damage during focal ischaemia (Takagi *et al.*, 1999; Zhou *et al.*, 2009), and that the addition of Trx to the culture medium significantly reduces the damaging effects of hypoxia/reoxygenation in cell culture (Isowa *et al.*, 2000). The administration of recombinant human Trx-1 can decrease brain damage (Hattori *et al.*, 2004; Ma *et al.*, 2012), attenuate the extensive neuronal loss caused by focal and global cerebral ischaemia in the hippocampus of rats and mice and augment neurogenesis (Bai *et al.*, 2003; Ma *et al.*, 2012; Tian *et al.*, 2013; Zhou *et al.*, 2013). Increased transcription of Trxs may reduce spinal ischaemia/reperfusion damage (Wang, Jiang, 2009). The increased Trx expression induced by electroacupuncture treatments potentiates the disulphide-reducing activities of the thioredoxin system and thus minimises oxidative stress in reperfused rat brains following ischaemia (Siu *et al.*, 2005). In contrast, the inhibition of Trx increases oxidative stress (Yamamoto *et al.*, 2003). Deactivation of Trx-1 and Trx-2 proteins by the covalent binding of gambogic acid to the active cysteine residues in the functional domain leads to the stimulation of ROS toxicity and selective induction of apoptosis in cancer cells (Yang *et al.*, 2012a). Dysfunction in Trxs may be the cause of various neurodegenerative diseases (Conrad *et al.*, 2013).

Different forms of hypoxia/ischaemia may cause increased expression of Trx. For example, in glial cells of the gerbil hippocampus, the induction of Trx has been shown to be a response to temporary global cerebral ischaemia (Tomimoto *et al.*, 1993). A 14-fold increase in Trx mRNA expression has been observed in response to hypoxia in a culture of cancer cells (Berggren *et al.*, 1996). In the peri-infarct regions of mouse brains, Trx begins to increase by 8–24 h and reaches peaks expressions at time-points 24-72 h after reperfusion following a transient middle cerebral artery occlusion (Tanaka *et al.*, 2011). However, extremely intense hypoxic exposure causing complete suppression of molecular defences can suppress the expression of Trx. For example, suppression of the expression of Trx and its RNA has been noted in the ischaemic core at 4 h after a focal cerebral

ischaemia, whereas in the boundary area adjacent to this ischaemic core, the expression is increased, and Trx translocates to the nucleus (Takagi *et al.*, 1998a, b; Hattori *et al.*, 2002). Trx is significantly decreased in the cerebral cortex of rats at 24 h after a middle cerebral artery occlusion (Koh *et al.*, 2010). Severe chronic intermittent hypoxia also decreases Trx mRNA and protein levels in the rat hippocampus (Yang *et al.*, 2012b). In these experiments, the apoptotic index and impairments in spatial learning and memory are correlated with decreased Trx mRNA and protein levels. In contrast, rats that perform better in a Morris water maze show higher levels of Trx mRNA and protein in the hippocampus (Yang *et al.*, 2012b). It has been shown that oxidative stress can mediate the induction of thioredoxin-interacting protein (Txnip) (Kim *et al.*, 2012; Lane *et al.*, 2013), which is the intracellular endogenous inhibitor of Trx and is also known as vitamin D3 up-regulated protein 1 (VDUP1) and thioredoxin-binding protein-2 (TBP-2) (Nishiyama *et al.*, 1999; Junn *et al.*, 2000; Nishinaka *et al.*, 2001a; Yodoi *et al.*, 2002; Nishinaka *et al.*, 2004; Yoshida *et al.*, 2006; Watanabe *et al.*, 2010; Aon-Bertolino *et al.*, 2011; Zhou *et al.*, 2011).

The functions of Trx in molecular mechanisms of neuroprotection under oxidative stress are varied and not yet fully understood. One of its key protective functions is determined by its direct antioxidant properties; Trx serves as a regulator of the redox balance, a powerful singlet oxygen quencher and a scavenger of hydroxyl radicals and others free radicals (Nakamura *et al.*, 1994; Das, Das, 2000; Ueda *et al.*, 2002; Rhee *et al.*, 2005). Taken together, these functions inhibit the release of cytochrome *c* from mitochondria into the cytosol (Andoh *et al.*, 2002a, b), thereby inhibiting apoptosis. However, in severe injuries when cell death is inevitable, Trx can at least switch the cell death from a necrotic to an apoptotic pathway through the regulation of the activity of caspases that are sensitive to the redox state (Ueda *et al.*, 1998, 2002).

The cytoprotective functions of Trx are probably not limited solely to its role as a thiol reducing agent and free radical scavenger. It also acts as an inducer of other endogenous antioxidants, in particular, mitochondrial Mn-SOD (Das *et al.*, 1997; Andoh *et al.*, 2003, 2005) and as an inducer of Bcl-2 (Andoh *et al.*, 2003, 2005). Trx is involved in signal transduction processes (Takagi *et al.*, 1998a, 1999; Patenaude *et al.*, 2005). Trx is able to transnitrosylate or denitrosylate specific proteins (Wu *et al.*, 2011; Sengupta, Holmgren, 2013) and, in particular, to regulate in this way the activity of sirtuins (Radak *al.*, 2013).

Trx facilitates the production of hydrogen sulphide ($H_2S$), which may function as a signalling molecule in neuromodulation in the brain and has a cytoprotective effect (Calabrese *et al.*, 2010; Kimura *et al.*, 2012).

Trxs reduce inter- and intramolecular disulphide bonds in many proteins and thereby regulate their activity. It is known that the redox balance and cysteine oxidation and reduction of signalling proteins, such as protein kinases, phosphatases and transcription factors, play important roles in the regulation of signal transduction (Cross, Templeton, 2006; Kondo *et al.*, 2006).

In particular, Trxs reduce disulphide bonds in many isoforms of oxidised peroxiredoxins (Prxs), which together with glutathione peroxidases (GPXs) represent one of the two major families of the thiol/selenol peroxidases (Dayer *et al.*, 2008) that disrupt organic hydroperoxides, $H_2O_2$, and peroxynitrite (Kalinina *et al.*, 2008; Rhee *et al.*, 2012). Therefore, Trxs are essential for the activity of most peroxiredoxins. Until it was found that some peroxiredoxins can use other electron donors, the family of peroxiredoxins was called thiol-specific antioxidant enzymes (TSA) or thioredoxin peroxidases (TPx) (Kim *et al.*, 1989; Chae *et al.*, 1994; Ichimiya *et al.*, 1997; Zhang *et al.*, 1997; Ghosh *et al.*, 1998; Kang *et al.*, 1998; Lu *et al.*, 1998; Jeong *et al.*, 1999; Rhee *et al.*, 2001) to emphasise the obligatory role of Trxs for their activity. Peroxiredoxins are present in diverse subcellular compartments and are divided into 4 types: 2-Cys Prxs, 1-Cys Prxs, Prx-Q, and atypical 2-Cys Prxs or type II Prx (Dayer *et al.*, 2008). Mammalian cells express 6 isoforms of Prx (PrxI to PrxVI) that belong to the 2-Cys, atypical 2-Cys, and 1-Cys types (Jeong *et al.*, 2006, 2012; Dayer *et al.*, 2008). All 6 isoforms are expressed in the central nervous system and are important for neuroprotection against oxidative stress (Hattori, Oikawa, 2007). Trxs act as electron donors to reduce oxidised peroxidatic cysteine and thus to regenerate the 2-Cys peroxiredoxins (mammalian isoforms PrxI to PrxIV) (Dayer *et al.*, 2008; Rhee, Woo, 2011; Jeong *et al.*, 2012) and mammalian atypical 2-Cys peroxiredoxin (isoform PrxV) (Seo *et al.*, 2000; Knoops *et al.*, 2011) but not the 1-Cys peroxiredoxin (isoform PrxVI) (Kang *et al.*, 1998; Schremmer *et al.*, 2007; Fisher, 2011). For example, the neuronal-specific peroxiredoxin-2 failed to provide additional neuroprotection against ischaemic injury in Trx-knockdown neuron cultures (Gan *et al.*, 2012).

Trx is secreted from cells (Nakamura *et al.*, 2006) and exhibits cytokine-like and chemokine-like activities (Tanaka *et al.*, 2000; Banerjee, 2012). During oxidative stress, Trx

translocates from the cytoplasm to the nucleus (Masutani *et al.*, 1996), interacts with redox factor 1 (APE1/Ref-1) (Nakamura *et al.*, 1997, 2002; Tell *et al.*, 2009) and activates transcription factors (Nishiyama *et al.*, 2001; Hirota *et al.*, 2002), increasing their ability to bind DNA target (Table 2).

Table 2. Transcription factors and receptors that are activated by Trx in the nucleus.

| **Intracellular factors that are activated by Trx** | **Abbreviation** | **Reference** |
|---|---|---|
| Polyoma virus enhancer-binding protein 2/core binding factor | PEBP2/CBF | Akamatsu *et al.*, 1997 |
| Nuclear transcription factor κB | NF-κB | Hirota *et al.*, 1999, 2000 |
| Activator protein 1 | AP-1 | Hirota *et al.*, 1997, 2000 |
| Cyclic adenosine monophosphate (cAMP) response element-binding protein | CREB | Hirota *et al.*, 2000 |
| Hypoxia-inducible factor-1 | HIF-1 | Welsh *et al.*, 2003 |
| Oestrogen receptors | ERs | Hayashi *et al.*, 1997 |
| Glucocorticoids receptors | GRs | Makino *et al.*, 1999 |

Trx also increases the DNA binding of the regulatory protein p53 (Ueno *et al.*, 1999). Protein p53 is activated in cases of oxidative stress (Ueda *et al.*, 2002), and it can activate the inhibitor of the G1 cyclin-dependent kinase $p21^{Cip1/WAF1}$, which stops the cell cycle to repair DNA damaged by oxidative stress (Harper *et al.*, 1993; Ueda *et al.*, 2002). However, p53 can also activate the proapoptotic protein Bax (Banasiak, Haddad, 1998), which leads to the release of cytochrome *c* (Miyashita, Reed, 1995) and induction of apoptosis. Trx increases the p53-dependent expression of p21 and thereby switches the signal from the

initiation of programmed cell death to the initiation of a DNA repair and survival programme (Ueno *et al.*, 1999; Kelley *et al.*, 2012).

Trx inhibits the activity of apoptosis signal-regulating kinase 1 (ASK1) (Saitoh *et al.*, 1998; Geleziunas *et al.*, 2001; Watson *et al.*, 2004; Katagiri *et al.*, 2010; Lu, Holmgren, 2012), which has been identified as a MAP-kinase kinase kinase that activates c-Jun N-terminal kinase (JNK) and p38 MAP-kinase and thereby mediates an apoptosis signal (Ichijo *et al.*, 1997). In contrast, ASK1 activation and cell death are induced by toxic metals and metalloids, such as arsenic, cadmium and mercury, that oxidise Trxs (Hansen *et al.*, 2006b). By stimulating the release of cytochrome *c*, ASK1 induces apoptosis mainly through mitochondria-dependent caspase activation (Hatai *et al.*, 2000). Thus, the negative regulation of ASK1 is another cytoprotective mechanism of Trx. It is known that Trx also suppresses the activation of MAP-kinase p38 induced by tumour necrosis factor alpha (TNF-α) under oxidative stress (Hashimoto *et al.*, 1999; Ichijo *et al.*, 1997).

However, there have been some sporadic and unexpected data about the participation of the thioredoxin system also in proapoptotic mechanisms. It has been shown that the Trx-1 system helps mediate the N-methyl-D-aspartate receptor-dependent denitrosylation of neuronal nitric oxide synthase during the early stages of ischaemia/reperfusion (Qu *et al.*, 2012). In this model, the down-regulation of Trx-R1 attenuates the neuronal apoptosis induced by oxygen-glucose deprivation/reoxygenation (Qu *et al.*, 2012). There are also data that Trx-2 and Trx-R2 together with glutamate receptor 6 and Fas ligand can participate in mediation of ischemia/reperfusion-induced denitrosylation and activation of procaspase-3 (Sun *et al.*, 2013).

**The detection and specificity of Trx-2**

Until 1997, only one form of mammalian Trx was known, and the majority of Trx research describes cytosolic Trx-1. In 1997, a mitochondrial Trx-2 was cloned (Spyrou *et al.*, 1997). This protein contains the cysteine-rich active site that is common to all Trxs, but it lacks the structural cysteine residues present in other mammalian Trxs. At the N-terminus of the Trx-2 molecule, there is a sequence signalling its translocation into the mitochondria. Cleavage of this sequence by a presumed mitochondrial peptidase gives a mature mitochondrial protein with a molecular mass of 12.2 kDa. The specific localisation to the inner mitochondrial membrane and high resistance to oxidation provides evidence

that Trx-2 is the primary line of defence against oxidative stress caused by ROS in the course of mitochondria functioning (Spyrou *et al.*, 1997; Miranda-Vizuete *et al.*, 2000; Ueda *et al.*, 2002; Zhang *et al.*, 2007a). A specific mitochondrial Trx has also been identified and characterised in yeast (Pedrajas *et al.*, 1999).

The known mechanisms of the antiapoptotic protective functions of Trx-2 are the inhibition of ASK1 (Zhang *et al.*, 2004), the decrease of TNF-α-induced ROS generation (Hansen *et al.*, 2006a), the control of mitochondrial outer membrane permeabilisation and Bcl-xL level (Wang *et al.*, 2006a) and blocking of cytochrome *c* release from mitochondria (Chen *et al.*, 2002; Nonn *et al.*, 2003; Wang *et al.*, 2006a), possibly by regulating the interaction between voltage-dependent anion channels (VDACs) and adenine-nucleotide translocator (ANT) (Ueda *et al.*, 2002). It has been shown that Trx-2 interacts with peroxiredoxin-3 *in vivo* and acts as an electron donor for it (Miranda-Vizuete *et al.*, 2000; Zhang *et al.*, 2007a).

Experiments with DT40 cultures of chicken B cells have shown that the suppression of Trx-2 gene expression leads to the release of cytochrome *c* into the cytosol (Tanaka *et al.*, 2002). Homozygous mutant mice not expressing Trx-2 die between 10.5 and 12.5 days of embryonic development as a result of massive apoptosis (Nonn *et al.*, 2003). A dominant-negative active-site mutation (C93S-Trx2) of Trx-2 enhances the sensitivity to cell death induced by tert-butylhydroperoxide or by TNF-α (Zhang *et al.*, 2007a). Transgenic human embryo kidney 293 cells with increased expression of Trx-2 (HEK-Trx2) are more resistant to etoposide, which induces the release of cytochrome *c* from mitochondria (Damdimopoulos *et al.*, 2002). In similar experiments with a line of transgenic human osteosarcoma cells, it has been shown that increased expression of Trx-2 up-regulates resistance to the apoptosis induced by oxidants (Chen *et al.*, 2002). However, there has been at least one study in which neither Trx-2 nor Trx-R2 gain of function modified the redox regulation of mitochondria-dependent apoptosis in mammalian cells (Patenaude *et al.*, 2004).

In a study on the effect of resveratrol, it has been shown that Trx-2 likely plays a role in switching the death signal induced by ischaemia and reperfusion to a survival signal (Das *et al.*, 2008). Intraventricular administration of Trx-2 and peroxiredoxin 3 (Prx-3) significantly reduces the hyperactivity of gerbils after 5 min of transient cerebral ischaemia in a spontaneous motor test and protects CA1 pyramidal neurons from ischaemic damage

(Hwang *et al.*, 2010). In this model, the activation of astrocytes and microglia is decreased in the ischaemic CA1 region following Prx-3/Trx-2 treatment; treatment with Prx-3/Trx-2 also significantly reduces lipid peroxidation and the release of cytochrome *c* from the mitochondria in the ischaemic CA1 region (Hwang *et al.*, 2010). The increased immunoreactivities of Trx-2, Trx-R2 and Prx-3 in the CA1 pyramidal neurons of young gerbils compared with adult gerbils at the fourth day after a 5 min transient cerebral ischaemia correlates with significantly lower levels of delayed neuronal death (Yan *et al.*, 2012).

Trx-2 is involved in the regulation of mitochondrial membrane permeability by its disulphide reductase activity (Rigobello *et al.*, 1998), which may play an important role in the implementation of its antiapoptotic functions (Damdimopoulos *et al.*, 2002; Tanaka *et al.*, 2002; Nonn *et al.*, 2003).

## 2.4.2. Superoxide dismutases

**The functions and diversity of the superoxide dismutases**

Superoxide anion radical ($O_2^{\cdot-}$) is one of the most dangerous ROS whose formation is increased several fold during hypoxia/ischaemia and subsequent reoxygenation/reperfusion (Chan, 1996; Chan at al., 1996; Dawson, Dawson, 1996; Szabo, 1996). SODs are a family of antioxidant proteins that contain metal ions of variable valence. Their function is dismutation of superoxide anion to molecular oxygen and hydrogen peroxide (Marklund, 1984), which is then metabolised by peroxidases to water or by catalases to molecular oxygen and water.

The various known SOD forms are presented in Table 3.

Dismutation of superoxide anion (in case of subsequent successful metabolisation of hydrogen peroxide) contributes to the inhibition of the oxidative stress-induced release of cytochrome *c* and secondary activators of caspase (Smac) from mitochondria (Fujimura *et al.*, 2000; Sugawara *et al.*, 2002), thereby blocking apoptosis (Fujimura *et al.*, 2000). Thus, SODs protect cells of different tissues from damage and death caused by oxidative stress (Kinoshita *et al.*, 1991; Chen *et al.*, 1998; Abunasra *et al.*, 2001; Huang *et al.*, 2012). The high activity level of SODs is a mechanism of adaptation to hypoxia that has been

demonstrated in the subterranean mole rat *Spalax ehrenbergi* in particular (Caballero *et al.*, 2006).

Table 3. The members of the SOD family.

| **SOD family member** | **Abbreviation** | **Form and distribution** | **Reference** |
|---|---|---|---|
| Copper-zinc-containing SOD | Cu, Zn-SOD | Presents in the cytosol and nucleus of almost all types of cells, 32 kDa homodimer | McCord, Fridovich, 1969; Crapo *et al.*, 1992 |
| Manganese-containing SOD | Mn-SOD | Presents in the mitochondrial matrix, 96 kDa homotetramer | Weisiger, Fridovich, 1973; Slot *et al.*, 1986; Lindenau *et al.*, 2000 |
| Extracellular SOD | | Presents in extracellular media, such as lymph, synovial fluid and blood plasma, 130-150 kDa tetramer | Marklund *et al.*, 1982; Ookawara *et al.*, 1997; Fattman *et al.*, 2003 |
| Iron-containing SOD | Fe-SOD | Presents in some plants and prokaryotes | Bannister *et al.*, 1987 |
| Nickel-containing SOD | Ni-SOD | Presents in some species of bacteria | Youn *et al.*, 1996 |

**Mitochondrial Mn-SOD**

Mitochondria as respiration organelles are one of the main sources of free radicals (Ueda *et al.*, 2002), so mitochondrial Mn-SOD plays an especially important role (Miriyala *et al.*, 2011). It has been shown that mutant mice with a deficiency in this enzyme die during the first weeks after birth (Li *et al.*, 1995; Lebovitz *et al.*, 1996). Experiments with mutant mice have shown that Cu, Zn-SOD overexpression does not prevent the early deaths of animals with Mn-SOD deficiency (Copin *et al.*, 2000), but the overexpression of Mn-SOD significantly reduces neuronal death in rats with mutations in Cu, Zn-SOD gene (Flanagan *et al.*, 2002). Although both intracellular enzymes are present under normal conditions (Liu *et al.*, 1993a, 1994a, b), and both of them can be induced by oxidative stress (Bidmon *et al.*, 1998; Takeuchi *et al.*, 2000), Mn-SOD is the more inducible form, whereas Cu, Zn-SOD is a more constitutively expressed form of the enzyme (Kato *et al.*, 1995; Takeuchi *et al.*, 2000).

Activation of Mn-SOD expression protects cells against oxidative stress, particularly stress induced by focal cerebral ischaemia (Bidmon *et al.*, 1998; Keller *et al.*, 1998; Kim *et al.*, 2002). For example, increased expression of this enzyme in transgenic mice and cell lines prevents neuronal apoptosis caused by focal cerebral ischaemia (Keller *et al.*, 1998). In contrast, mice with a deficit of Mn-SOD show aggravated brain damage after ischaemia (Mehta *et al.*, 2011), an increased release of cytochrome *c* after traumatic brain injury, and, consequently, increased cell mortality (by necrosis and apoptosis) in the brain (Lewen *et al.*, 2001) as well as high rates of brain haemorrhage after ischaemia (Maier *et al.*, 2006; Jung *et al.*, 2010) and elevated expression of matrix metalloproteinase 9, which may contribute to blood-brain barrier breakdown (Maier *et al.*, 2004). Mn-SOD blocks cytochrome *c* release into the cytosol and the activation of caspase-9, thereby preventing the development of apoptosis after focal cerebral ischaemia (Fujimura *et al.*, 1999a; Noshita *et al.*, 2001). Increased Mn-SOD expression mediates the induction of the antiapoptotic protein Bcl-2, inhibits the induction of the proapoptotic adapter protein p66 (shc) (Andoh *et al.*, 2002a) and modulates the activity of c-Jun-associated transcription factors (Kiningham, St Clair, 1997).

The biologically active molecules that are involved in regulating the expression of Mn-SOD are presented in Table 4.

Table 4. Molecules that are involved in regulating Mn-SOD expression.

| **Molecules that regulate Mn-SOD expression** | **Abbreviation** | **Reference** |
|---|---|---|
| Reactive oxygen species | ROS | Oberley *et al.*, 1987 |
| Platelet-derived growth factor | PDGF | Maehara *et al.*, 2001 |
| Tumour necrosis factor alpha | TNF-α | Xu *et al.*, 1999; Kiningham *et al.*, 2001; Hoshida *et al.*, 2002 |
| Interleukin-1 beta | IL-1β | Xu *et al.*, 1999; Kiningham *et al.*, 2001; Hoshida *et al.*, 2002 |
| Nuclear factor kappa B | NF-κB | Xu *et al.*, 1999; Kiningham *et al.*, 2001 |
| CCAAT/enhancer binding protein | C/EBP | Xu *et al.*, 1999; Kiningham *et al.*, 2001 |
| Nuclear transcriptional factor-1 | NF-1 | Xu *et al.*, 1999; Kiningham *et al.*, 2001 |
| Adenosine | | Dana *et al.*, 2000 |
| Thioredoxin | Trx | Das *et al.*, 1997 |
| Protein kinase *C* delta | PK *Cδ* | Das *et al.*, 1998 |

**Cytosolic Cu, Zn-SOD**

Cu, Zn-SOD also protects neurons against damage caused by various stress influences (Greenlund at al., 1995; Mikawa at al., 1996; Kamada *et al.*, 2007), including hypoxia/ischaemia (Kinouchi *et al.*, 1998; Bordet *et al.*, 2000; Morita-Fujimura *et al.*, 2001; Hayashi *et al.*, 2004). A similar cytoprotective reaction has been shown in cells of other tissues (Sasaki *et al.*, 1998; Wang *et al.*, 1998).

It is known that the translation of Cu, Zn-SOD is promoted by taurine at least in some types of cells (Higuchi *et al.*, 2012). Melatonin, testosterone, dihydrotestosterone, estradiol, and vitamin D induces a sustained activation over time of Cu, Zn-SOD by means of

mitochondrial cytochrome P450 in intermembrane space of intact mitochondria (Iñarrea *et al.*, 2011).

The mechanisms of the participation of Cu, Zn-SOD in the adaptive responses to hypoxia are diverse. They are not limited to the suppression of cytochrome *c* release only by reducing of superoxide anion, which is one of the inducers of cytochrome *c* release.

Cu, Zn-SOD promotes function of the integrin-linked kinase (ILK) that is phosphatidylinositol-3 kinase (PI3-K)-dependent serine-threonine kinase, which activates the serine-threonine kinase Akt and so takes part in survival signalling pathway upstream of Akt and downstream of PI3-K (Saito *et al.*, 2004a). Overexpression of Cu, Zn-SOD enhances the phosphorylation of Akt and glycogen synthase kinase-3β (Endo *et al.*, 2006b) after transient global cerebral ischemia. Cu, Zn-SOD promotes the expression of phosphorylated proline-rich Akt substrates (Saito *et al.*, 2006). Thus, Cu, Zn-SOD is involved in the activation of the Akt, which inhibits apoptosis by inactivating Bad or caspase-9 or other apoptogenic components (Noshita *et al.*, 2003).

Cu, Zn-SOD overexpression in transgenic animals prevents ischaemia-induced dephosphorylation and translocation of the proapoptotic protein Bad and the formation of the Bad/Bcl-xL complex. In contrast, it contributes to the formation of the Bad/14-3-3 complex. Thus, Cu, Zn-SOD blocks the Bad-dependent signalling pathway of cell death (Saito *et al.*, 2003). Cu, Zn-SOD may prevent the ischaemia-induced activation of extracellular signal-regulating kinase (ERK1/2), which may be involved in the induction of apoptosis (Noshita *et al.*, 2002).

Cu, Zn-SOD also attenuates the ischaemia-induced Omi/HtrA2 pathway of apoptotic neuronal cell death (Saito *et al.*, 2004b), takes part in the regulation of p53 degradation (Saito *et al.*, 2005), inhibits the upregulation of the proapoptotic p53-upregulated modulator of apoptosis (PUMA) (Niizuma *et al.*, 2009), prevents NF-κB activation (Song *et al.*, 2005, 2007), diminishes the activation and phosphorylation of p38 mitogen-activated protein kinase and cytosolic phospholipase A2 (Nito *et al.*, 2008) and reduces the activation of matrix metalloproteinase-9 (Kamada *et al.*, 2007) after transient focal cerebral ischaemia. Overexpression of Cu, Zn-SOD reduces the gene and protein expression levels of monocyte chemoattractant protein 1 and macrophage inflammatory protein-1 alpha (Nishi *et al.*, 2005) in neurons after ischaemia.

Cu, Zn-SOD protects DNA against the fragmentation caused by transient focal cerebral ischaemia via the prevention or at least attenuation of the early decrease in the expression of apurinic/apyrimidinic endonuclease (APE/Ref-1), a multifunctional protein involved in DNA repair (Fujimura *et al.*, 1999b; Narasimhan *et al.*, 2005).

Overexpression of Cu, Zn-SOD reduces the intensity of endoplasmic reticulum damage caused by ischaemia and increases the levels of the phosphorylated forms of eukaryotic initiator factor 2 alpha (eIF2α) and RNA-dependent protein kinase-like eIF2α-kinase of endoplasmic reticulum (PERK), each of which plays a role in the regulation of apoptotic processes (Hayashi *et al.*, 2003, 2004, 2005).

Overexpression of Cu, Zn-SOD induces heat shock protein *hsp70* mRNA expression (Kondo *et al.*, 1996). In addition, it is known that Cu, Zn-SOD may be functionally linked to glutathione peroxidase-1 in exercising its neuroprotective role under conditions caused by ischaemia/reperfusion injury (Crack *et al.*, 2003).

The effect of Cu, Zn-SOD on cell survival has been shown in experiments with activators and inhibitors of both its synthesis and enzymatic activity (Bordet *et al.*, 2000). Brain neurons in transgenic animals that overexpress Cu, Zn-SOD are more resistant to ischaemia (Kinouchi *et al.*, 1998; Chan *et al.*, 1998; Morita-Fujimura *et al.*, 2001; Sugawara *et al.*, 2002). In contrast, disruption of the Cu, Zn-SOD gene increases the sensitivity of neurons to ischaemic effects (Kondo *et al.*, 1997a, b).

**The effect of hypoxia/ischaemia on the activity and expression of SODs**

Hypoxia/ischaemia, depending on the intensity and duration of the incident as well as on the timing after the event, may have a different effect on SODs. In various experiments with various models, hypoxia/ischaemia can increase SOD activity and expression (Sutherland *et al.*, 1991; Nakanishi *et al.*, 1995; Pohle, Rauca, 1997; Bidmon *et al.*, 1998; Candelario-Jalil *et al.*, 2001), have no effect on them (Tokuda *et al.*, 1993) or suppress them (Duan *et al.*, 1999; Liu *et al.*, 1993a, 1994a, b; Islekel *et al.*, 1999; Homi *et al.*, 2002). For example, decreased Cu, Zn-SOD expression has been noted 4 h after a 3-minute global cerebral ischaemia event (Kato *et al.*, 1995).

It is interesting that the direction of change of SOD expression may be different in various areas. For example, the active degeneration of neurons and the rapid decline or even disappearance of immunoreactivity for Cu, Zn-SOD have been observed at 4 h after

focal cerebral ischaemia in the ischaemic core (Liu *et al.*, 1994a). In the same period, in the majority of the neurons in the area immediately adjacent to the core, Cu, Zn-SOD immunoreactivity was also decreased, although histopathological changes were observed only in a few neurons. At 24 h after ischaemia typical pathological changes in the morphology of the neurons in the ischaemic core have been observed in parallel with a reduction in immunoreactivity to Cu, Zn-SOD. However, in the intermediate area between the infarction core and normal tissue where the development of the opposing pro- and antiapoptotic trends reaches a maximum, some neurons show increased expression of both SODs at the third and seventh days following the ischaemia (Liu *et al.*, 1994a). In an analogous study, it has been observed that the survival of neurons is correlated with increased protein and mRNA expression of both SODs in the area outside the zone of intense neuronal damage caused by focal brain injury (Takeuchi *et al.*, 2000). Similar results have been obtained for SOD enzymatic activity. It was significantly increased in the intermediate area between the infarction and normal tissue but not in the ischaemic core (Toyoda, Lee, 1997).

Obviously, the induction of SODs is a normal protective response to injury. In particular, in models of focal ischaemia in the intermediate region between normal and infarcted tissue, SOD induction can act as a protective and adaptive mechanism (Liu *et al.*, 1994a). However, if the intensity of the stress factor exceeds the reserves of the adaptive capacity, then maladaptive pathological processes develop. Such pathological processes can be associated with the inhibition of the expression and activity of SODs, especially in the early period after ischaemic stroke. This SOD suppression, obviously, leads to the development of neuronal damage. However, there are some data that are not entirely consistent with this view (Chan *et al.*, 1993; Fujimura *et al.*, 2001).

The effect of hypoxia on SOD expression and enzymatic activity in the very first hours after exposure, which are the most important for the choice between survival and the initiation of cell death programmes, remains insufficiently investigated. A few older papers have shown changes in the expression of Cu, Zn- and Mn-SOD during the first hours after hypoxia using only ischaemic models (Ohtsuki *et al.*, 1993; Liu *et al.*, 1993a, 1994a; Kato *et al.*, 1995). Thus, the roles of SODs as well as of other endogenous antioxidants in the protective mechanisms in response to hypobaric and normobaric hypoxia require further study.

# 2.5. Hypobaric hypoxia

Hypobaric hypoxia is hypoxia caused by a low pressure in the surrounding atmosphere. The effects of hypobaric hypoxia on the physiological condition of the human induced by elevation to considerable heights have been studied in detail (Hochachka, 1998). In particular, mechanisms of acute short-term adaptation and long-term acclimatisation of individual organisms as well as the evolutionary adaptation of permanently living at high altitudes human populations have been shown. At the level of the physiological response of an organism to hypobaric hypoxia, at least 5 general systems have been found: 1) the carotid body $O_2$ sensors initiate the hypoxic ventilatory response; 2) the pulmonary vasculature $O_2$ sensors initiate regulation of the hypoxic pulmonary vasoconstrictor response and hence adjustments in lung perfusion and in ventilation-perfusion matching; 3) $O_2$ sensors in the vasculature of other tissues activate the expression of vascular endothelial growth factor 1 and thus promote angiogenesis, especially in the heart and most likely the brain; 4) $O_2$ sensors in the kidney and liver activate the expression of erythropoietin, thus initiating the process of up-regulating red blood cell mass; and 5) tissue-specific $O_2$-sensing and signal transduction pathways lead to metabolic reorganisation, presumably by altering the rates of expression of hypoxia-sensitive genes for metabolic enzymes and metabolite transporters (Hochachka, 1998).

The treatment and prevention of damage caused by hypobaric hypoxia is an important problem in medicine. It has been studied in various animal models. Hypobaric hypoxia created in a chamber may be regarded as an imitation of climbing at a given height. Researchers use models that differ in intensity and duration of exposure, such as acute forms that create very severe hypoxia for 2-3 h and chronic forms with many days of intermittent or continuous moderate hypobaric hypoxia.

It has been found that severe hypobaric hypoxia can cause a range of functional and structural damages that are reversible or irreversible depending on the intensity and duration of exposure (Rybnikova *et al.*, 2005a; Maiti *et al.*, 2007, 2008a, b; Shi *et al.*, 2012). Various experimental models have shown that severe hypobaric hypoxia disrupts spatial memory (Shukitt-Hale *et al.*, 1994, 1996; Simonova *et al.*, 2003; Maiti *et al.*, 2008a, b; Barhwal *et al.*, 2009; Shi *et al.*, 2012), behaviour in an open field and the formation of the

passive and active avoidance reflexes (Trofimov *et al.*, 1993a, b; Vataeva *et al.*, 2004b), feeding behaviour (Singh *et al.*, 1997; Westerterp-Plantenga, 1999; Sharma *et al.*, 2002) and sleeping (Trofimov *et al.*, 1993b). It increases anxiety levels (Vataeva *et al.*, 2004c) and can cause retrograde amnesia (Drago *et al.*, 1990; Medico *et al.*, 2002; Vataeva *et al.*, 2004b). Hypobaric hypoxic exposure can significantly disrupt ontogenetic development (Maslova *et al.*, 2001). Glutamatergic brain structures, including the hippocampus, are the most sensitive to the detrimental effects of hypoxia. Several days of intermittent hypobaric hypoxia causes a decrease in the content of neurofilaments, astrocytes and oligodendrocytes in the hippocampus of young (24-day-old) rats, and these changes affect the formation of tissue structures. Structural changes have also been observed in the hippocampus of adult rats exposed to similar hypoxia (Simonova *et al.*, 2003).

Hypobaric hypoxia causes changes in the regulatory system of nitrogen oxide in the cells of various brain structures (Encinas *et al.*, 2003, 2004; Serrano *et al.*, 2003) and the intensification of lipid peroxidation (Zamorskii, Pishak, 2000). It also affects the function and expression of the P2X receptors in pyramidal cells in the hippocampus (Zhao *et al.*, 2012). It has been shown that hypobaric hypoxia induces changes in $Ca^{2+}$-mediated glutamatergic signal transduction (Semenov *et al.*, 2008), the expression of early genes and their products (Samoilov *et al.*, 2001b, 2003a; Rybnikova *et al.*, 2002, 2005a, b), and protein regulators of apoptosis (Rybnikova *et al.*, 2006b; Shi *et al.*, 2012). One can assume that the mechanisms of injury triggered by hypobaric hypoxia are similar to the mechanisms induced by other forms of hypoxic influence, including the well-studied ischaemia. However, it is important to note that the distribution of more sensitive and relatively resistant areas can be different in ischaemic *vs.* hypobaric models (Yamaoka *et al.*, 1993; Abe *et al.*, 2004; Schmidt-Kastner *et al.*, 2004; Sun *et al.*, 2012). Therefore, extrapolating the data obtained in numerous ischaemic studies to other types of hypoxia (including hypobaric) requires caution.

The study of hypobaric hypoxia has important practical significance. On the one hand, this is the type of hypoxia that can cause the chronic mountain sickness in high-altitude residents (Zubieta-Castillo *et al.*, 2006; Zubieta-Calleja *et al.*, 2006, 2011) as well as acute altitude diseases (Imray *et al.*, 2011; Kedzierewicz, Cabane, 2013). It can be a professional risk factor for pilots, mountain climbers and mountain rescuers. On the other hand, the creation of moderate hypobaric hypoxia in a chamber, unlike ischaemia, does not require

the occlusion of blood vessels or any other surgical operations. Therefore, moderate hypobaric hypoxia can be used in barotherapy as a non-drug treatment for the prevention of a number of disorders. Nevertheless, the molecular mechanisms of hypobaric hypoxia are still poorly understood, especially in nervous tissue, and are an important research topic in modern neuroscience. In particular, the effect of hypobaric hypoxia on the antioxidant systems in neurons has remained practically unexplored.

## 2.6. Preconditioning

It is known that an organism is capable of adapting to hypoxia (Lu *et al.*, 1999). Preconditioning is a peculiar training phenomenon wherein the subjection to preliminary moderate hypoxia significantly increases the resistance of the whole organism, as well as of individual organs, tissues and cells, to the effects of subsequent severe hypoxia.

**Discovery of the hypoxic tolerance effect in various experimental models**

The phenomenon of preconditioning was demonstrated by Vladimirov, Sirotinin and Kreps (Vladimirov *et al.*, 1939; Sirotinin, 1939; Kreps *et al.*, 1956), although the term "preconditioning" was introduced much later. In 1990-1991, the phenomenon of ischaemic tolerance in the brain (similar to ischaemic tolerance of the myocardium) was discovered. It was shown that 2 minutes of global cerebral ischaemia could prevent neuronal death in the CA1 area of Mongolian gerbil hippocampus caused by a subsequent 5-minute ischaemic episode (Kitagawa *et al.*, 1990; Kirino *et al.*, 1991). It is important to note that a single preconditioning session in these experiments only partly ameliorated the effects of severe ischaemia, but 2 sessions of preconditioning protected neuronal cells almost completely. The same effect has been shown in the model of a 10-minute global cerebral ischaemia in gerbils in a number of other brain structures – areas CA2, CA3 and CA4 of the hippocampus, as well as in the cortex, striatum, and thalamus. At the same time, a 1-minute ischaemia is too weak and does not cause tolerance to severe lethal ischaemia (Kitagawa *et al.*, 1991). Thus, it has been shown that the influence of preconditioning must be sufficiently intense to induce the protective effect.

A similar result was obtained in electrophysiological and neurochemical experiments with anoxia *in vivo* in the cat sensorimotor cortex (Samoilov *et al.*, 1994). 90 seconds of anoxic preconditioning prevented the prolonged inhibition of the generation of action potentials of cerebral cortex neurons that occurred during the first several tens minutes to hours after a 5-minute anoxia. 15-20 minutes after the end of 90 seconds of preconditioning anoxia, a moderate but persistent hyperactivation of neurons developed. It began to decay only after 55 minutes. This dynamic of neuronal electrical activity has been observed in the context of moderate but sustained activation of calcium-dependent, phosphoinositide and cAMP regulatory systems, which is expressed as significant changes in the $Ca^{2+}$, cAMP and TPI content in the period up to 60-90 min following reoxygenation compared with their pre-anoxic levels. Thus, short-term hyperactivation of neurons and the moderate activation of intracellular regulatory systems are essential for the development of anoxic tolerance. This hyperactivation probably determines the mechanism of tolerance induction. The interval between the preconditioning and severe anoxic exposure must be long enough (at least 60 minutes) for the mobilisation of the evolutionarily prevailing and genetically determined mechanisms of the adaptive responses of the neuronal cells. If the preconditioning anoxia is interrupted before the development of hypoxic "convulsive" hyperactivation (50-60 seconds), the first subsequent changes are similar, but at 30 minutes following reoxygenation, the $Ca^{2+}$, TPI, DPI and cAMP levels are close to their pre-anoxic levels. Therefore, the termination of anoxia at 50-60 s hinders the development of a sustainable preconditioning effect (Samoilov *et al.*, 1994).

The effects of moderate hypoxia have been studied in a series of experiments with cultured slices of rat olfactory cortex *in vitro*. The slices were exposed to both the separate and combined impacts of short (2 min) and long (10 min) anoxic episodes, which were created by replacing oxygen with nitrogen in an artificial environment that mimics cerebrospinal fluid (Samoilov, Mokrushin, 1997). A 2-minute preconditioning session prevented the suppression of focal postsynaptic potentials and hyperactivation of the calcium and phosphoinositide systems induced by a 10-minute anoxic episode (Samoilov *et al.*, 2001a). In the first 20-25 minutes after the 2-minute exposure, a significant increase in the amplitude and duration of the focal excitatory postsynaptic potentials was observed. This increase is a post-anoxic long-term neuronal hyperactivation, similar to the

hyperactivation observed in experiments with cats *in vivo*. The mechanism of this anoxic long-term potentiation could be associated with the restructuring the activity of the calcium and phosphoinositide signal transduction systems (Samoilov *et al.*, 2001a). During reoxygenation following 2 minutes of anoxia, there was a moderate but prolonged increase in the intracellular $Ca^{2+}$ content as well as phase oscillations in the polyphosphoinositide content and in the metabolic rate of their phosphate groups. In the next series of experiments, it was shown that preconditioning by moderate hypobaric hypoxia *in vivo* prevented the inhibition of focal potentials caused by a subsequent severe anoxia in cultured brain slices *in vitro* (Romanovskii *et al.*, 2001).

Some interesting data about preconditioning have been obtained in experiments involving the transfer of neuromodulatory factors. These factors were extracted from preconditioned donor slices and then added to the medium of non-preconditioned recipient slices. As a result, the recipient slices became more resistant to a subsequent severe hypoxia. Neuromodulator factors from perfusates of donor slices that were collected in the late (60-90 min), but not the early (0-20 min), reoxygenation period after a brief anoxia had a distinct preconditioning effect on the generation of focal postsynaptic potentials as well as on the dynamics of the intracellular calcium content and polyphosphoinositide metabolism in the recipient slices that had been subjected to subsequent prolonged anoxia. These neuromodulatory factors are probably peptides because cycloheximide blockade of protein synthesis in the first 60 minutes after the preconditioning led to a loss of the neuroprotective properties of the perfusate that was collected between 60 and 90 minutes. The chemical nature of these factors is still unknown, but the literature suggests that at least neurotrophins and opioid peptides could take part in such preconditioning-induced neuroprotective mechanisms (Samoilov *et al.*, 2001a). Gel electrophoretic analysis of the incubation media of these slices has shown that preconditioning has a pronounced effect on changes in the content of a number of peptides extracted from the cells after prolonged anoxia (Samoilov *et al.*, 2001a).

The effect of hypoxic preconditioning has been shown in a number of studies in different hypoxic models. In morphological studies, it has been shown that 1.5 minutes of preconditioning significantly increases the survival of nerve cells in the CA1 area of the hippocampus in Mongolian gerbils after a subsequent 5-minute global ischaemia (Corbett, Crooks, 1997). A similar result has been obtained in experiments with forebrain ischaemia

in mice: 6-minutes of ischaemic preconditioning significantly reduces the number of dying cells and the degree of DNA fragmentation as a marker of apoptosis after 18 minutes of severe ischaemia (Wu *et al.*, 2001), while the molecular mechanism of preconditioning-induced tolerance has been shown to be associated with the prevention of severe ischaemia-induced activation of caspase-3 (Qi *et al.*, 2001).

It has been shown that the rapid adaptation of mitochondrial function to acute hypoxia in rats acclimatised to the highlands is more intense than in plains animals. This is achieved by a significant increase in the rate of NAD-dependent substrate oxidation, especially lipids, and an improved efficiency in mitochondrial respiration and an increased economy of oxygen utilisation (Portnichenko *et al.*, 2012b).

**The cross-effect of preconditioning**

It is known that preconditioning by moderate hypoxia increases the resistance of nerve cells not only to a subsequent severe hypoxia but also to other types of damaging influences, including ferrous citrate (iron)-induced oxidative stress (Lin *et al.*, 2002), the introduction of kainic acid (Emerson *et al.*, 1999; Costa *et al.*, 2013) and severe emotional stress causing post-stress depression (Rybnikova *et al.*, 2006a, 2007a, b; Baranova *et al.*, 2012). The antidepressant-like effects of moderate hypoxia have also been demonstrated in other models (Zhu *et al.*, 2010). Hypoxic preconditioning can be used for the treatment and prevention of many diseases, such as acute myeloid leukaemia (Liu *et al.*, 2006) and hypertension (Serebrovskaya *et al.*, 2008). On the other hand, it is known that chemical and pharmacological preconditioning using inhibitor of succinate dehydrogenase (Sugino *et al.*, 1999; Aketa *et al.*, 2000), respiratory chain inhibitors (Wiegand *et al.*, 1999), inhibitor of glycine transporters type 1 (Pinto *et al.*, 2012), ethanol (Yuan *et al.*, 2011), carbon monoxide (Queiroga *et al.*, 2012) and the antibiotic minocycline (Sakata *et al.*, 2012) increases resistance to severe hypoxia/ischaemia. Preconditioning using various exercises, including swimming training and treadmill exercise, has beneficial effects on cognitive function, neurogenesis, antioxidant activity, ROS levels and oxidative stress markers in brain (Somani *et al.*, 1995; Somani, Husain, 1997; Radak *et al.*, 2001, 2006, 2007; Lappalainen *et al.*, 2009). It has been shown that tolerance to subsequent hypoxia/ischaemia can be induced by treadmill exercise (Hamakawa *et al.*, 2013), by training at high temperatures (Agarkov, 1960), and by immobilisation stress (Meerson *et*

*al.*, 1994). Moreover, preconditioning by immobilisation affective stress can induce hypoxic tolerance even more effectively than hypoxic preconditioning (Meerson *et al.*, 1993; Golubeva *et al.*, 1995). Thus, preconditioning can be regarded as a universal mechanism of adaptation to stress in general (Lehotský *et al.*, 2009) and to stress caused by different types of hypoxia in particular. This conclusion is consistent with the classical concept of Professor János (Hans) Hugo Bruno Selye about stress as a non-specific general adaptation syndrome (Selye, 1936, 1938, 1955, 1956, 1974).

**The molecular mechanisms of preconditioning**

The molecular mechanisms of preconditioning are very diverse (Kirino *et al.*, 2002; Obrenovitch *et al.*, 2008; Cadet, Krasnova, 2009). They can include, in particular, modulation of the activity and expression of ion channels, which perhaps reduces the energy demand and delays the anoxic depolarisation of neurons (Xia, Haddad, 1999; Stenzel-Poore *et al.*, 2003); inhibition of the opening of mitochondrial permeability transition pores (Zhu *et al.*, 2006); activation of N-methyl-D-aspartate (NMDA) and adenosine receptors; activation of intracellular signalling pathways, such as mitogen activated protein kinases (MAPKs) and other protein kinases; up-regulation of Bcl-2 and heat shock proteins (HSPs); activation of the ubiquitin-proteasome pathway and the autophagic-lysosomal pathway (Liu *et al.*, 2009); and promotion of hippocampal neurogenesis (Zhu *et al.*, 2010).

An important molecular mechanism of the protective action of preconditioning is most likely the induction of heat shock proteins – mainly, *HSP 70* (Kitagawa *et al.*, 1990; Kirino *et al.*, 1991; Liu *et al.*, 1993b; Glazier *et al.*, 1994; Chen *et al.*, 1996). The temporal pattern of the induction of HSPs is in accordance with the development of ischaemic tolerance (Samoilov *et al.*, 2001a). It is important to note that non-preconditioned severe ischaemia also induces the expression of HSPs, but their expression occurs at later stages when the cell death programme is already running. In contrast, preconditioning causes an increase in the expression of HSPs in the early stages of the subsequent severe ischaemia and reperfusion, even before the start of the apoptotic programme (Liu *et al.*, 1993b).

It has been shown that caspase-3 activation can be essential for neuroprotection in preconditioning (McLaughlin *et al.*, 2003). In this case, the cleavage of caspase-3 does not induce delayed cell death but on the contrary increases the cell's resistance to subsequent

excitotoxicity. Hypoxia-inducible factor (HIF), which attenuates the injurious effects of the hypoxia/ischaemia (Dai *et al.*, 2013), is also activated by hypoxic preconditioning (Sharp *et al.*, 2004; Kirova, 2012; Wacker *et al.*, 2012). HIF in its turn can activate the expression of genes of other important neuroprotective factors of the preconditioning response, such as cyclin-dependent kinase inhibitor 1 ($p21^{WAF1/CIP1}$) (Mergenthaler *et al.*, 2013) and erythropoietin (Grimm *et al.*, 2005).

It is obvious that glutamate signal transduction is involved in the molecular mechanisms of preconditioning. In experiments with slices of rat olfactory cortex, it has been found that adding NMDA receptor antagonists (APV, MK-801) to the culture medium 20 min prior to preconditioning by a short anoxic episode and during the first 30 minutes after reoxygenation significantly reduces the effect of preconditioning on the magnitude of the synaptic potentials, the content of intracellular calcium and the polyphosphoinositide metabolism during and after a subsequent prolonged anoxia (Samoilov *et al.*, 2001a). It has been assumed that down-regulation (Douen *et al.*, 2000; Kosugi *et al.*, 2005; Yamada *et al.*, 2006) or up-regulation (Romera *et al.*, 2004; Verma *et al.*, 2010) of the expression and activity of excitatory amino acid transporters (EAATs) may contribute to ischaemic tolerance by modulating the glutamate levels in neurons. In particular, it has been shown that cerebral ischaemic preconditioning (Zhang *et al.*, 2007b) and intermittent hypobaric hypoxia preconditioning (Gong *et al.*, 2012) induce brain ischaemic tolerance by up-regulating glial glutamate transporter-1 (EAAT2).

The next step in these investigations was the discovery of preconditioning-induced up-regulation of SOD enzymatic activity (Toyoda *et al.*, 1997), Trx-1 expression (Andoh *et al.*, 2002 a, b) and mRNA expression of mitochondrial antioxidants (Samoilov *et al.*, 2002). It appears that one of the important molecular mechanisms of the protective effect of hypoxia is to prevent apoptosis in nerve cells through the activation of endogenous antioxidant systems.

**The effects of preconditioning in the specific model used in the present study**

In the present work, we studied the effects of severe (3 h, 180 Torr) and 1 and 3 sessions of mild (2 h, 360 Torr) hypobaric hypoxia and also their combination using 3 sessions of mild hypoxia as preconditioning for a subsequent severe episode (for details, see "Materials and methods"). This model has the advantage that it is well studied at the

morphological, biochemical, endocrinological and behavioural levels, which allows for comparison of the results and offers a comprehensive rather than a fragmented view of the response to hypoxia (Samoilov *et al.*, 2001b, 2003a, b, 2005, 2012a, b; Rybnikova *et al.*, 2002, 2005a, b, 2006b, 2008b, 2009; Vataeva *et al.*, 2004 b, c; Stroev, Samoilov, 2006; Stroev *et al.*, 2008; Semenov *et al.*, 2008, 2009a, b, 2010; Churilova *et al.*, 2009, 2010; Kislin *et al.*, 2010, 2011, 2012; Samoilov, Rybnikova, 2012). The combination of the same severe and 3 sessions of mild hypobaric hypoxia has also been studied in model of postconditioning (Rybnikova *et al.*, 2012a, b, 2013; Samoilov, Rybnikova, 2012; Vorob'ev *et al.*, 2012); however, in the present work, only the preconditioning model will be discussed.

It has been shown that this method of preconditioning significantly reduces the mortality of the animals during subsequent severe hypoxia. While the survival of non-preconditioned animals averages approximately 50%, the survival of preconditioned ones goes up to 85% (Rybnikova *et al.*, 2005a). 3 to 7 days after severe hypoxia, the marked responses of the nerve cells of the "light" (an early degree of chromatolysis, cytoplasmic vacuolisation, ghost cells) and "dark" (hyperchromatosis, shrinkage, glial nodes at sites of dead cells) types have been observed in different areas of the neocortex and hippocampus of survived non-preconditioned rats. These responses probably reflect the development of necrosis and apoptosis, respectively, at the morphological level. Pericellular oedema and, in some neurons, chromatolysis and vacuolisation of cytoplasm have been observed. For example, in the CA1 area of the hippocampus on the seventh day after severe hypoxia, the death of approximately 30% of the cells has been observed. In animals that have been preconditioned by 3 sessions of mild hypoxia, there is a significant decrease in the number of damaged and dead neurons in the studied brain structures (Rybnikova *et al.*, 2005a, b, 2006b).

The protective effects of preconditioning in this model have also been observed at the behavioural level. Preconditioning almost completely prevents the retrograde amnesia caused by severe hypobaric hypoxia. It restores the learning capacity disturbed by severe hypoxia (Vataeva *et al.*, 2004b; Rybnikova *et al.*, 2005b). Anxiety levels, as determined using models of open fields and elevated cruciform labyrinths, are increased by non-preconditioned severe hypoxia, but they are significantly reduced by preconditioned severe hypoxia (Vataeva *et al.*, 2004c). Preconditioning also prevents the development of

post-stress depression-like states (Rybnikova *et al.*, 2006a, 2007a, b, 2008c, d; Baranova *et al.*, 2009, 2010, 2012).

Preconditioning significantly alters the pattern of the expression of early genes and their protein products after severe hypoxia (Samoilov *et al.*, 2001b, 2003a; Rybnikova *et al.*, 2002, 2005a, b). For example, the expression of the protein product of the early gene NGFI-A after severe hypoxia is markedly reduced in non-preconditioned rats, but it is increased in preconditioned ones (Rybnikova *et al.*, 2005a, b). Non-preconditioned severe hypoxia does not alter the expression of the protein c-Fos, but preconditioned severe hypoxia significantly increases it (Rybnikova *et al.*, 2005a). After non-preconditioned hypoxia, the mRNA expression levels of early genes (NGFI-A, JunB) rapidly increase during the early period followed by a subsequent long decline to below control levels. At the same time, after preconditioned severe hypoxia, a slow but sustainable increase occurs in the expression of these genes (Samoilov *et al.*, 2001b; Rybnikova *et al.*, 2002).

Preconditioning shifts the ratio of pro- and antiapoptotic proteins of the Bcl-family in favour of the antiapoptotic ones (Rybnikova *et al.*, 2006b; Samoilov *et al.*, 2005), reduces the severe hypoxia-evoked alterations in glutamatergic $Ca^{2+}$ signalling (Semenov *et al.*, 2008, 2010, 2012), and modifies the expression of glucocorticoid and mineralocorticoid receptors (Rybnikova *et al.*, 2011; Samoilov *et al.*, 2012a) and the transcription factors pCREB and NF-$\varkappa$B (Rybnikova *et al.*, 2008a; Churilova *et al.*, 2010). It has also been shown that the preconditioning used in this experimental model prevents the disturbance of lipid peroxidation processes in the hippocampus and neocortex (Kislin *et al.*, 2010, 2011) and significantly increases the expression of mitochondrial antioxidant genes, such as genes of Mn-SOD and Trx-2, after a subsequent severe hypobaric hypoxia (Samoilov *et al.*, 2002).

The responses of expression of genes and their products during and after different hypoxic impacts are not always identical. Therefore, the pattern of expression of mitochondrial and cytosolic antioxidant proteins in response to severe hypobaric hypoxia in non- and preconditioned animals remained an open question. Resolving this question was one of the purposes of this work. The second purpose was to investigate the expression pattern of antioxidant proteins during and after preconditioning to determine the effect of moderate hypobaric hypoxia (preconditioning) itself before or without subsequent severe hypoxia.

# 3. Aims of the study

Oxidative stress is a key mechanism of neuronal damage during and after severe hypoxia. Accordingly, regulation of the expression and enzyme activity of antioxidants plays an important role in neuroprotection.

The purpose of the present thesis was to investigate the effects of non- and preconditioned severe hypobaric hypoxia as well as 1 and 3 sessions of preconditioning itself on the expression of 4 endogenous protein antioxidants – cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, cytosolic Trx-1 and mitochondrial Trx-2 – in rat brain neurons at 3 h and 24 h after exposure. This research was important for understanding the molecular mechanisms of adaptation to hypoxia and the induction of hypoxic tolerance during preconditioning exercises. It may have practical significance for the treatment and prevention of disorders associated with cerebral ischaemia, acute and chronic hypoxic states, and possibly neurodegeneration of various aetiologies. It has the potential to be of practical use in clinic practice and prophylaxis of diseases in newborn and also for the training of persons (such as pilots, mountain climbers, mountain rescuers, divers, submariners and so on) for which hypoxia is a professional risk factor.

The specific aims of this study were the following:

1. To study the effect of severe hypobaric hypoxia and subsequent reoxygenation on the protein expression of Cu, Zn-SOD, Mn-SOD, Trx-1 and Trx-2 in the hippocampus and frontoparietal neocortex of rats;

2. To study the effect of severe hypobaric hypoxia and subsequent reoxygenation on the enzymatic activity of Cu, Zn-SOD in different brain structures (whole neocortex, the piriform cortex, Ammon's horn of the hippocampus and the striatum);

3. To study the effect of preconditioning by 3 sessions of mild hypoxia on the protein expression of Cu, Zn-SOD, Mn-SOD, Trx-1 and Trx-2 in the hippocampus and frontoparietal neocortex and on the enzymatic activity of Cu, Zn-SOD in different structures of rat brain at 3 h and 24 h after a subsequent severe hypoxia; and

4. To study the effect of 1 and 3 sessions of mild hypobaric hypoxia itself (without a subsequent severe hypoxia) on the protein expression of Cu, Zn-SOD, Mn-SOD, Trx-1 and Trx-2 at 3 h and 24 h after the end of exposure in the hippocampus of rats.

# 4. Materials and methods

## 4.1. Animals

The study was performed on Wistar male rats weighing 200-250 g bred at the vivarium of the I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences (Koltushi, Leningrad region, Russia). The rats were maintained at 21 ± 2ºC on a 12-h diurnal light cycle (lights on at 08:00 A.M.). Food and water were provided *ad libitum*. Adequate measures were taken to minimise the pain or discomfort of the animals used in this study. The experiments were carried out in compliance with protocols and guidelines approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC), under the approval of the Ethical Committee for the Use of Animal Subjects at the I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences. All efforts were made to minimise both the number of animals used and their suffering.

## 4.2. Hypoxia procedures

Rats were subjected to different types of hypobaric hypoxia (1 mild session, 3 mild sessions, severe session, and severe session 24 h after 3 mild sessions). Severe hypoxia was produced in a flowing-type altitude hypobaric chamber by maintaining the pressure at 160-180 Torr (equivalent to 5% of normobaric oxygen or 11000 m altitude) for 3 h at a temperature of 20-25°C. Mild hypoxia was produced in the same chamber by maintaining the pressure at 360 Torr (equivalent to 10% of normobaric oxygen or 5000 m altitude) 1 time for 2 h or 3 times for 2 h once a day at the same temperature. The standard animal treatment protocol is presented in Table 5.

Table 5. The animal treatment protocol used in the experiments.

| Pressure, mmHg | Duration of treatment at this pressure, min | Latency to reach pressure from the start of elevation, min |
|---|---|---|
| 660 | 1 | - |
| 560 | 2 | 1 |
| 460 | 3 | 3 |
| 360 | 5 | 6 |
| 260 | 5 | 11 |
| 240 | 2 | 16 |
| 220 | 2 | 18 |
| 200 | 5 | 20 |
| 180 | - | 25 |

## 4.3. Determination of Cu, Zn-SOD enzyme activity

The rats were divided into 5 groups (6 rats per group): 1) rats subjected to severe hypoxia without preconditioning, which were studied at 3 h after the end of the hypoxia (Fig. 3A); 2) rats subjected to severe hypoxia without preconditioning, which were studied at 24 h after the end of the hypoxia (Fig. 3A); 3) rats subjected to severe hypoxia 24 h after the end of the last of 3 sessions (once per day) of preconditioning by mild hypoxia, which were studied at 3 h after the end of the severe hypoxia (Fig. 3B); 4) rats subjected to severe hypoxia 24 h after the end of the last of 3 sessions (once per day) of preconditioning by mild hypoxia, which were studied at 24 h after the end of the severe hypoxia (Fig. 3B); and 5) control rats placed in the chamber for 3 h with no hypoxia.

4 brain structures were investigated: the neocortex, the piriform cortex, Ammon's horn of the hippocampus and the striatum. The large brain areas (neocortex and hippocampus) gave good results in all of the experiments, but some of the experiments with the smaller brain areas (piriform cortex and striatum) failed. Thus, the number of animals that were

analysed for the various areas and in the different groups was different, but the number was never less than 4 or more than 6 (Table 6).

Table 6. The number of animals analysed for the various areas and groups in the experiments to determine Cu, Zn-SOD enzyme activity.

| | Groups of rats | Neocortex | Ammon's horn | Piriform cortex | Striatum |
|---|---|---|---|---|---|
| 1 | Severe hypoxia + 3 h after reoxygenation | 5 | 5 | 5 | 4 |
| 2 | Severe hypoxia + 24 h after reoxygenation | 5 | 5 | 4 | 5 |
| 3 | 3 preconditioning sessions + 24 h + severe hypoxia + 3 h after reoxygenation | 6 | 6 | 5 | 5 |
| 4 | 3 preconditioning sessions + 24 h + severe hypoxia + 24 h after reoxygenation | 6 | 6 | 6 | 5 |
| 5 | Controls for groups 1-4 | 6 | 6 | 6 | 6 |

The enzyme activity was determined using a colour redox reaction (Chevari *et al.*, 1985). For the enzymatic experiments, the rats were anaesthetised using Nembutal (50 mg/kg intraperitoneally, i.e., approximately, 10-13 mg of Nembutal dissolved in 0.5 ml of water per rat) and decapitated. Their brains were rapidly isolated under cold conditions. Whole neocortex, piriform cortex, whole Ammon's horn of the hippocampus and striatum were excised and homogenised in phosphate buffer (pH 7.8, 5 ml of buffer per 1 g of tissue). The homogenates were centrifuged for 20 min at 17300 g. A chloroform-ethanol mixture was added to the supernatant at a rate of 0.15 ml of chloroform, 0.3 ml

ethanol and 300 mg $KH_2PO_4$ to 1 ml of supernatant. Over 15 min, this mixture was mixed under cold conditions and then centrifuged for 50 min at 20000 g.

The Cu, Zn-SOD enzyme activity was measured in the supernatant using a colour redox reaction with a spectrophotometer (SF16, LOMO, Russia) in a photometric cuvette at a wavelength ($\lambda$) of 540 nm. Reagent 1 was prepared using 18.6 mg of EDTA, 27.6 mg of phenazine methosulphate and 150 mg of nitroblue tetrazolium in 300 ml of phosphate buffer. Then, 0.05 ml of supernatant was added to 2 ml of reagent 1 (in the controls, the 0.05 ml of phosphate buffer was added in place of supernatant). The extinction coefficient was measured ($E_1$). Then, 0.1 ml of NADH was added, and after 10 min, the extinction coefficient was measured again ($E_2$). The Cu, Zn-SOD enzyme activity (EA) was expressed as a percentage of the oxidative process suppression:

$$EA = ((E_{2\ control} - E_{1\ control}) - (E_{2\ sample} - E_{1\ sample})) * 100\% / (E_{2\ control} - E_{1\ control}).$$

Then, the enzyme activity was converted to conventional units (c.u.). One conventional unit corresponded to a 50% suppression of oxidation:

$$N_{c.u.} = EA / 50.$$

The result was converted to the specific enzyme activity (c.u./tissue mass) using the following formula:

$$N_{specific} = (N_{c.u.} * V_{homogenate}) / (0.05 * M_{tissue}).$$

The changes in the Cu, Zn-SOD enzyme activity after hypoxia were expressed as a percentage of the enzyme activity in the control animals.

## 4.4. Immunocytochemical analysis of protein expression

The immunocytochemical analyses of protein expression was performed on different rats from the ones used in the study of Cu, Zn-SOD enzyme activity because it was impossible to use the same brains for both immunocytochemistry and enzymatic activity analysis. However, the animals were of the same breed and sex, were approximately the same age (male Wistar rats weighing 200-250 g) and had been raised in the same nursery and under the same conditions.

The immunoreactivity of all 4 endogenous antioxidant proteins – Cu, Zn-SOD, Mn-SOD, Trx-1 and Trx-2 – was studied on the brain sections obtained from the same animals. All of the immunocytochemical studies were conducted in two large series of experiments.

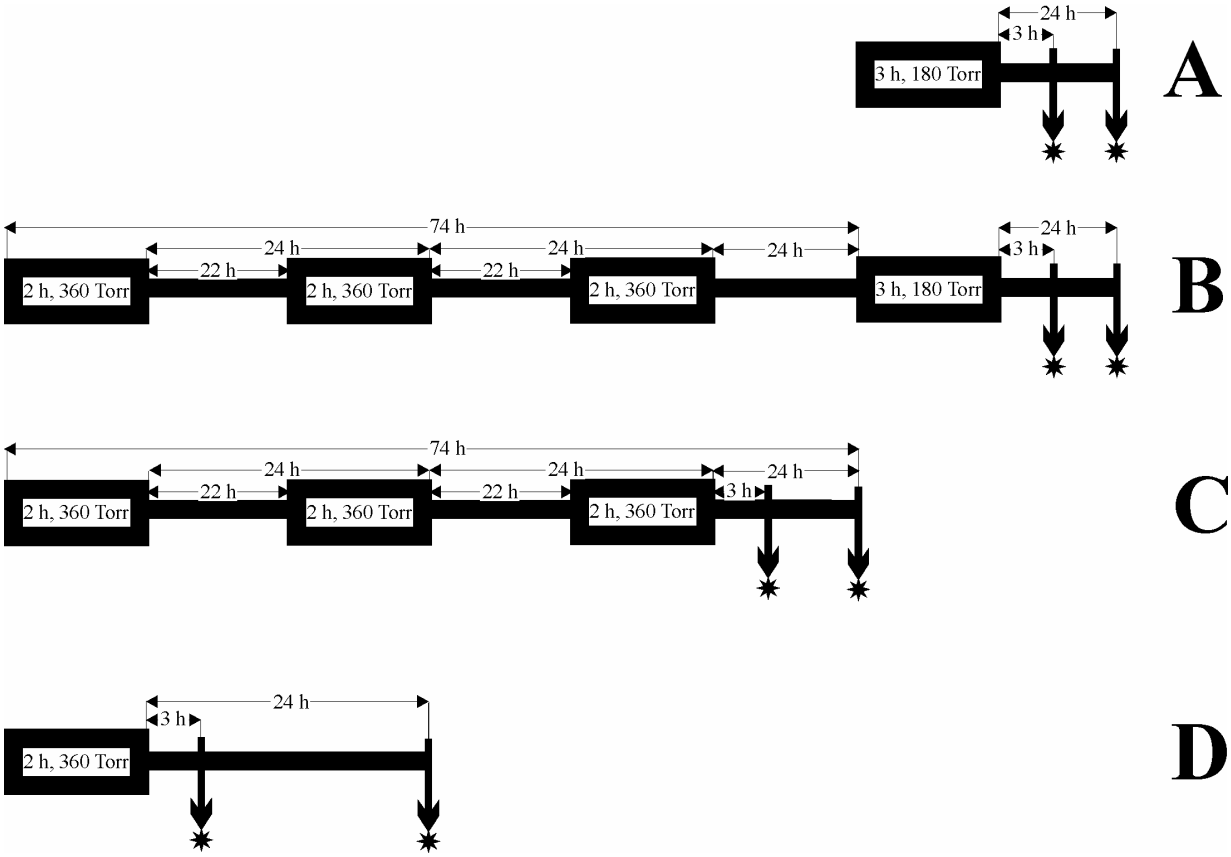



Fig. 3. The time-points of the experimental scheme. A – Non-preconditioned severe hypoxia analysed at 3 h and 24 h after the end of the hypoxic session. B – Preconditioned severe hypoxia analysed at 3 h and 24 h after the end of the severe hypoxia. C – 3 sessions of mild hypoxia that is preconditioning itself without severe hypoxia analysed at 3 h and 24 h after the end of the last session. D – 1 session of mild hypoxia without severe hypoxia analysed at 3 h and 24 h after the end of the session.

In the first series, 5 groups of animals were studied: 1) rats subjected to severe hypoxia without preconditioning, which were studied at 3 h after the end of the hypoxia (Fig. 3A); 2) rats subjected to severe hypoxia without preconditioning, which were studied at 24 h after the end of the hypoxia (Fig. 3A); 3) rats subjected to severe hypoxia 24 h after the end of the last of the 3 sessions (once per day) of preconditioning by mild hypoxia, which

were studied at 3 h after the end of the severe hypoxia (Fig. 3B); 4) rats subjected to severe hypoxia 24 h after the end of the last of the 3 sessions (once per day) of preconditioning by mild hypoxia, which were studied at 24 h after the end of the severe hypoxia (Fig. 3B); and 5) control rats placed in the chamber for 3 h with no hypoxia.

In the second series of experiments, the next 5 groups of animals were similarly studied: 1) rats subjected only to 3 sessions (once per day) of preconditioning by mild hypoxia without a subsequent severe hypoxia, which were studied at 3 h after the end of the last session (Fig. 3C); 2) rats subjected to 3 sessions (once per day) of preconditioning by mild hypoxia without a subsequent severe hypoxia, which were studied at 24 h after the end of the last session (Fig. 3C); 3) rats subjected to 1 session of preconditioning by mild hypoxia without a subsequent severe hypoxia, which were studied at 3 h after the end of the mild hypoxia (Fig. 3D); 4) rats subjected to 1 session of preconditioning by mild hypoxia without a subsequent severe hypoxia, which were studied at 24 h after the end of the mild hypoxia (Fig. 3D); and 5) control rats placed in the chamber for 2 h with no hypoxia.

Thus, the model of hypoxia was completely identical to the model that was used in the study of the Cu, Zn-SOD enzyme activity. However, the cases of 1 session and 3 sessions of mild hypoxia without severe hypoxia were added to the studies of the expression of the antioxidant proteins.

For immunocytochemistry, all of the rats were anaesthetised using Nembutal (50 mg/kg intraperitoneally, i.e., approximately, 10-13 mg of Nembutal dissolved in 0.5 ml of water per rat) and perfused transcardially first with 100 ml of saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.3) for 4-5 min. After perfusion, the brains were excised and subsequently fixed by immersion in the same 4% paraformaldehyde solution in 0.1 M phosphate-buffered saline for 60 min. The samples were cryoprotected with 15% sucrose in PBS and stored at +4°C until the immunocytochemical analysis. The samples were assembled into blocks and were frozen in Tissue-Tek® O.C.T™ Compound (Sakura Finetek). The frozen blocks were immediately sectioned in a cryostat at -20°C.

Originally, there were 6 rats in each studied group. Each block contained, to the extent possible, the brain samples of the rats of each of the 5 groups studied in the first or second large experimental series. Thus, in each of the two large experimental series, there

were 6 analogous blocks, and in most of them, there were 5 brain samples corresponding to the 5 experimental groups. However, some of the blocks were not complete because severe hypoxia caused 50% mortality in the animals, and in some cases, the quantity of the some brain samples was not enough to include them to each block.

From each block, 24 slices were made in the first large experimental series and 28 slices were made in the second large experimental series. Thus, in the first large experimental series, each section on the glass slide was composed of 5 (in some incomplete blocks – 4) slices of brain samples: 2 brains of non-preconditioned rats 3 and 24 h after severe hypoxia, 2 brains of preconditioned rats 3 and 24 h after severe hypoxia, and 1 brain of a control rat. In the second large experimental series, each section on the glass slide was composed of 5 slices of brain samples: 2 brains of rats 3 and 24 h after 1 session of preconditioning without a severe hypoxia, 2 brains of rats 3 and 24 h after 3 sessions of preconditioning without a severe hypoxia, and 1 brain of a control rat. However, some series of the sections were excluded because the immunocytochemical reaction was of insufficient quality. Therefore, the number of animals that were actually analysed varied for each experimental group and each antibody, but the number was never less than 4 or more than 6 (Table 7).

Immunocytochemistry was performed using an avidin and biotinylated horseradish peroxidase macro-molecular complex (ABC) method (a detailed description of the method is given below). Coronal slices (11 μm) of brain tissue (approximately 2.80 mm below the bregma, Paxinos, Watson, 1986) were mounted onto glass slides (Menzel-Gläser, Germany) coated with poly-L-lysine and preincubated for 15 min in a 1% solution of bovine serum albumin (BSA, Boehringer Mannheim GmbH, Germany) in PBS. Then, the slides with the sections of the brains of the rats (24 sections in the first large experimental series and 28 sections in the second large experimental series) were divided into 4 classes corresponding to the 4 studied protein antioxidants (6 sections per class in the first large experimental series and 7 sections per class in the second large experimental series). Thus, the subsequent analysis of the expression of 4 individual antioxidant proteins was conducted using the brain samples of the same rats.

Table 7. The number of animals analysed in the various groups and for the various antioxidants in the experiments to determine antioxidant protein expression.

| | Groups of rats | Trx-1 | Trx-2 | Cu,Zn-SOD | Mn-SOD |
|---|---|---|---|---|---|
| 1 | Severe hypoxia + 3 h after reoxygenation | 5 | 4 | 5 | 4 |
| 2 | Severe hypoxia + 24 h after reoxygenation | 5 | 4 | 5 | 5 |
| 3 | 3 sessions of preconditioning + 24 h + severe hypoxia + 3 h after reoxygenation | 6 | 5 | 6 | 5 |
| 4 | 3 sessions of preconditioning + 24 h + severe hypoxia + 24 h after reoxygenation | 6 | 5 | 6 | 5 |
| 5 | Controls for groups 1-4 | 6 | 5 | 6 | 5 |
| | | | | | |
| 6 | 3 sessions of preconditioning + 3 h after reoxygenation | 6 | 5 | 5 | 6 |
| 7 | 3 sessions of preconditioning + 24 h after reoxygenation | 6 | 5 | 5 | 6 |
| 8 | 1 session of preconditioning + 3 h after reoxygenation | 6 | 5 | 5 | 6 |
| 9 | 1 session of preconditioning + 24 h after reoxygenation | 6 | 5 | 5 | 6 |
| 10 | Controls for groups 6-10 | 6 | 5 | 5 | 6 |

Sections of each of the 4 classes corresponding to the 4 studied protein antioxidants were incubated at +4°C overnight separately with one of the following 4 primary antibodies:

1) affinity-purified rabbit antiserum against bovine Cu, Zn-SOD (White *et al.*, 1994), kindly provided by Dr Ling Yi L. Chang (dil. 1:200 in PBS containing 1% BSA and 0.3% Triton X-100);

2) affinity-purified rabbit antiserum against human Mn-SOD (StressGen Biotechnologies Corp., San Diego, California, USA, dil. 1:2000 in PBS containing 1% BSA and 0.3% Triton X-100);

3) affinity-purified rabbit antiserum against mouse Trx-1 (Takagi *et al.*, 1999), kindly provided by Dr Yumiko Nishinaka and Professor Junji Yodoi (dil. 1:500 in PBS containing 1% BSA and 0.3% Triton X-100); and

4) affinity-purified rabbit antiserum against rat Trx-2 (Spyrou *et al.*, 1997), kindly provided by Professor Giannis Spyrou (dil. 1:250 in PBS containing 1% BSA and 0.3% Triton X-100).

After 3 washes in PBS for 15 min each, the sections of all 4 classes were incubated with biotinylated goat anti-rabbit (Vector Labs, Burlingame, California, USA) antibodies (dil. 1:300) for 30 min. Then after 3 washes in PBS for 15 min each, the sections were incubated with ABC complex for 30 min. After 3 washes in PBS for 15 min each, diaminobenzidine was used as a chromogen to visualise the sites expressing immunoreactivity to the antioxidant proteins.

After 1 wash in PBS for 15 min and 1 wash in distilled water for 15 min, the sections were dehydrated, mounted and assayed using an image analysis system consisting of a computer, a Nikon Microphot-FXA microscope, a SensiCam digital camera (PCO Computer Optics GmbH, Kelheim, Bavaria, Germany) and the Image-Pro Plus (Media Cybernetics, Rockville, Maryland, USA) program.

The expression of the antioxidant proteins was examined in the CA1, CA2, and CA3 hippocampal fields, in the *dentate gyrus* (DG), and in some cases, in the frontoparietal neocortex. The cells that were immunoreactive for the antioxidant proteins were quantified in an area 500 μm in length (in the hippocampus) or in a 300x300 μm square (in the neocortex) using the Morphix program, which was created by us especially for such analyses (Tugoy, Stroev, 2006). 6-7 slices were analysed from each brain for each

antioxidant protein; 1 field of each brain area studied was measured per slice. The intensity of immunostaining was expressed as a conventional value on an optical density scale from 0 (absolute white) to 100 (absolute black). The immunoreactive cells were divided into 2 relative classes: lightly labelled (staining intensity of 1-10 conventional units above the background) and intensely labelled (more than 10 units above the background). Immunoreactivity to the antioxidant proteins was assayed using two criteria: the total number of immunoreactive cells shown as a percentage of the control ($N_+$) and the number of only the intensely labelled cells shown as a percentage of the control (Ni).

## 4.5. Statistical analysis of the data

One-way ANOVA was used for the statistical analyses of all of the data. The level of statistical significance was $p<0.05$. The nonparametric Wilcoxon-Mann-Whitney two-sample U-test was also used to verify the results.

# 5. Results

## 5.1. Cu, Zn-SOD enzyme activity (III)

In all of the structures studied except whole neocortex, at 3 h after severe hypoxia, the Cu, Zn-SOD enzyme activity was markedly down-regulated (by 43% in the hippocampus, by 24% in the striatum and by 39% in the piriform cortex) compared with the control (III) (Fig. 4a). At 24 h after severe hypoxia, the enzyme activity had returned to the control level in all of the structures (III) (Fig. 4b).

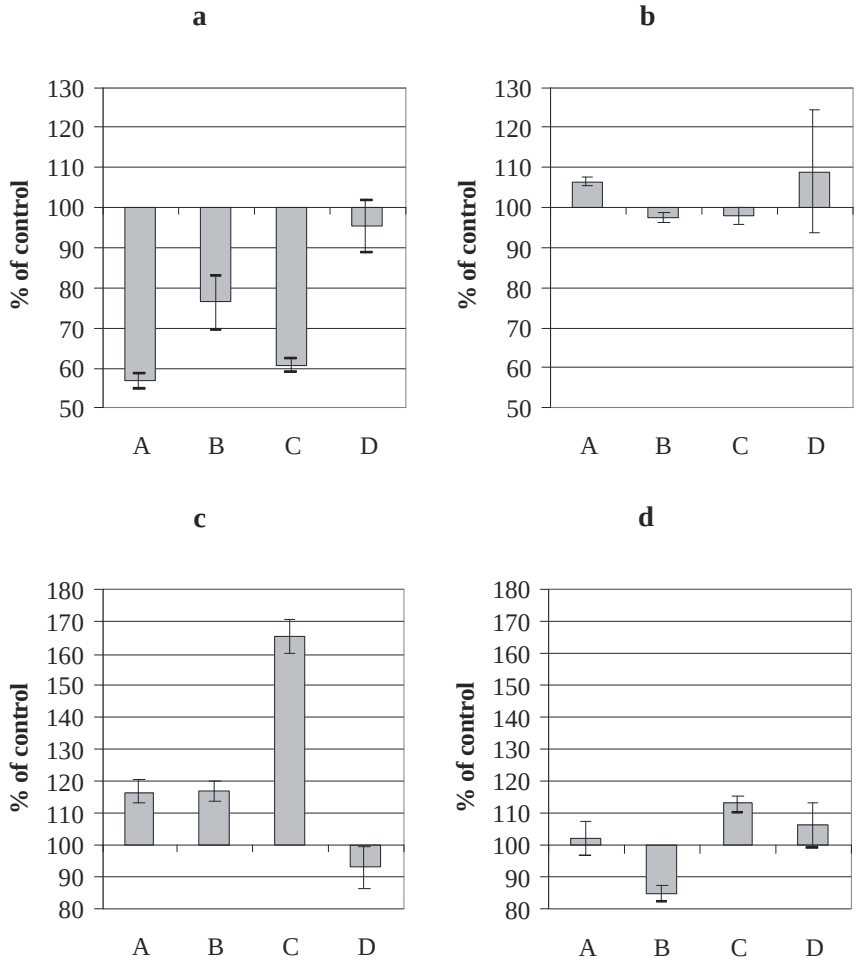



Fig. 4. The enzyme activity of Cu, Zn-SOD at 3 h (**a**) and 24 h (**b**) after non-preconditioned severe hypoxia and at 3 h (**c**) and 24 h (**d**) after preconditioned severe hypoxia in different brain structures. A – hippocampus, B – striatum, C – piriform cortex, D – whole neocortex. On the vertical axis, the changes in the Cu, Zn-SOD activity are expressed as a percentage of the control. This figure was published in “Neuroscience Research” (III).

In contrast, the Cu, Zn-SOD activity in preconditioned rats 3 h after severe hypoxia (Fig. 4c) was significantly increased in all of the structures studied except whole neocortex (by 17% in the hippocampus, 17% in the striatum and 66% in the piriform cortex) (III). At 24 h the enzyme activity had fallen back (Fig. 4d). Only in the piriform cortex the activity remained significantly higher (by 13%) than the controls; in the hippocampus, the activity was similar to the control; and in the striatum, the activity was significantly lower (by 15%) than the control (III).

No changes in the Cu, Zn-SOD enzyme activity were detected in the whole neocortex of either experimental group (III).

## 5.2. Effects of non- and preconditioned severe hypobaric hypoxia on the expression of antioxidants (I-III)

The results of the immunocytochemical study of the effects of non- and preconditioned severe hypobaric hypoxia on the expression of endogenous antioxidant proteins in the CA1, CA2, and CA3 hippocampal fields, DG, and frontoparietal neocortex at 3 h and 24 h after the end of exposure are summarised in Figures 5-8: Trx-1 (I) (Fig. 5), Trx-2 (II) (Fig. 6), Mn-SOD (Stroev *et al.*, 2005) (Fig. 7) and Cu, Zn-SOD (III) (Fig. 8). These results are described in more detail below.

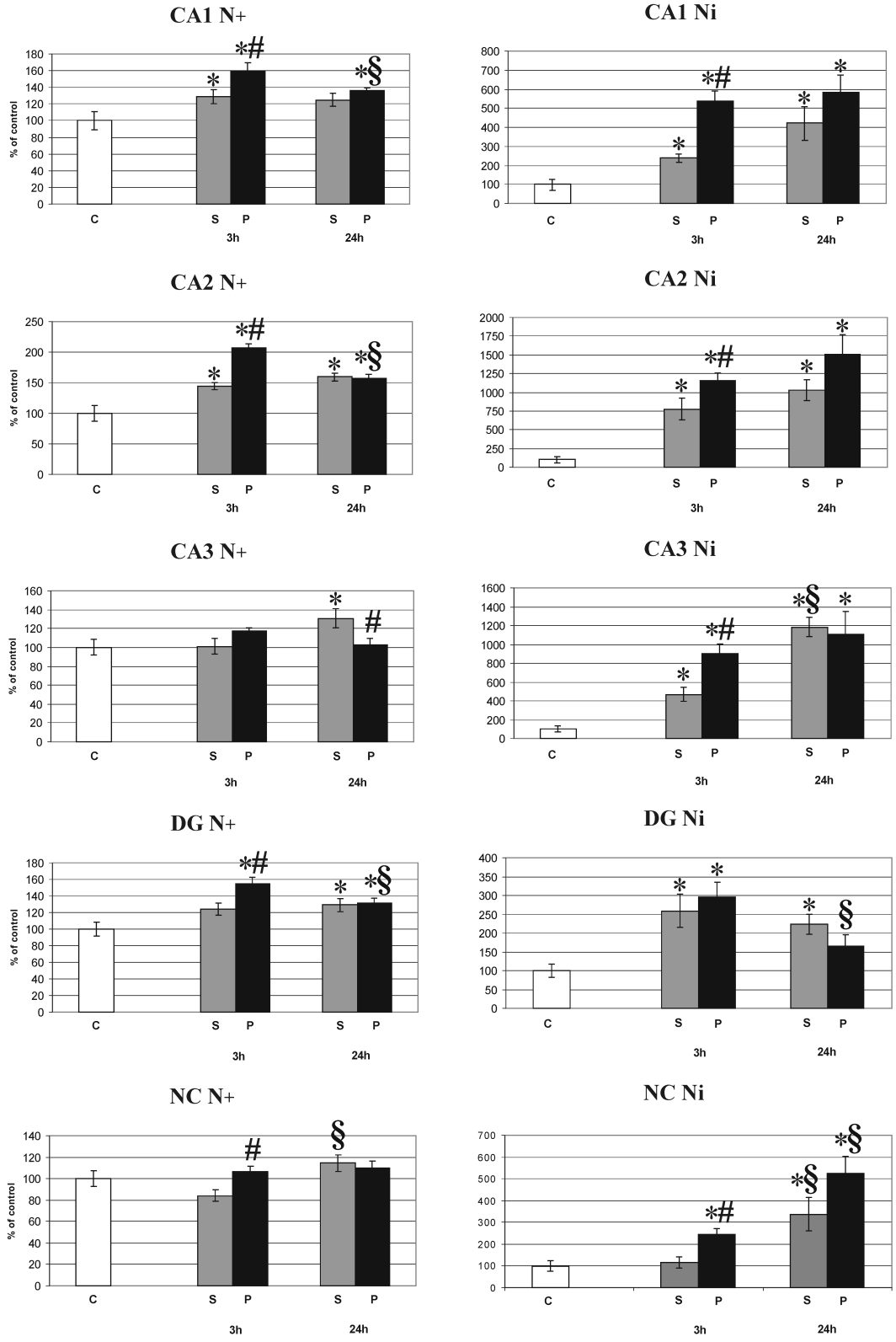



Fig. 5. Changes in the total number of Trx-1-immunoreactive cells ($N_+$) and the number of intensely expressing Trx-1 cells (Ni) in the various formations of the brain in non- (S) and preconditioned (P) rats at 3 h and 24 h after severe hypoxia as a percentage of the control (C). The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – in preconditioned rats compared with non-preconditioned rats; and § – at the 24 h time-point compared with the 3 h time-point. These data were published in “Neuroscience Letters” (I).

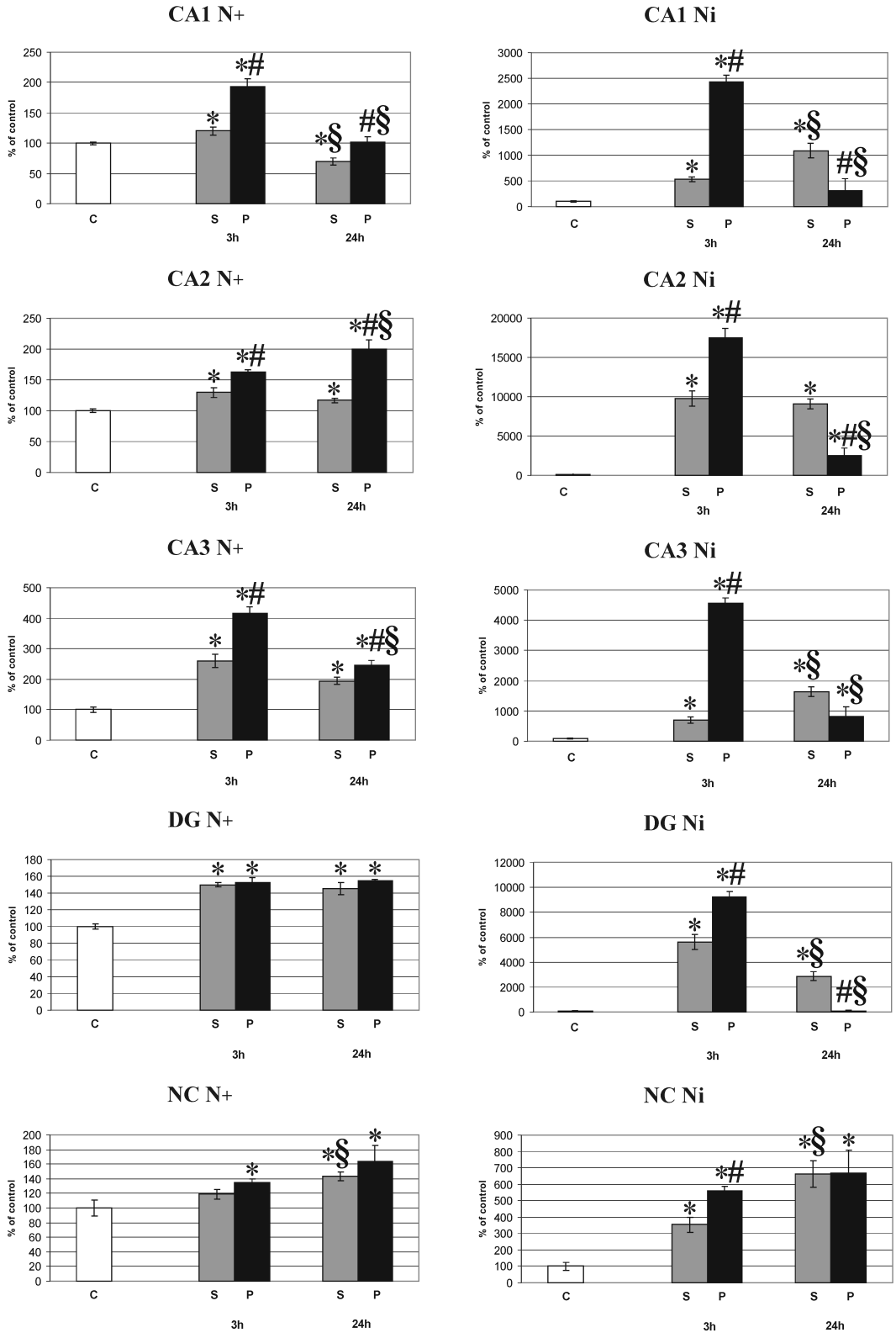



Fig. 6. Changes in the total number of Trx-2-immunoreactive cells ($N_+$) and the number of intensely expressing Trx-2 cells (Ni) in the various formations of the brain in non- (S) and preconditioned (P) rats at 3 h and 24 h after severe hypoxia as a percentage of the control (C). The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – in preconditioned rats compared with non-preconditioned rats; and § – at the 24 h time-point compared with the 3 h time-point. These data were published in “Journal of Neuroscience Research” (II).

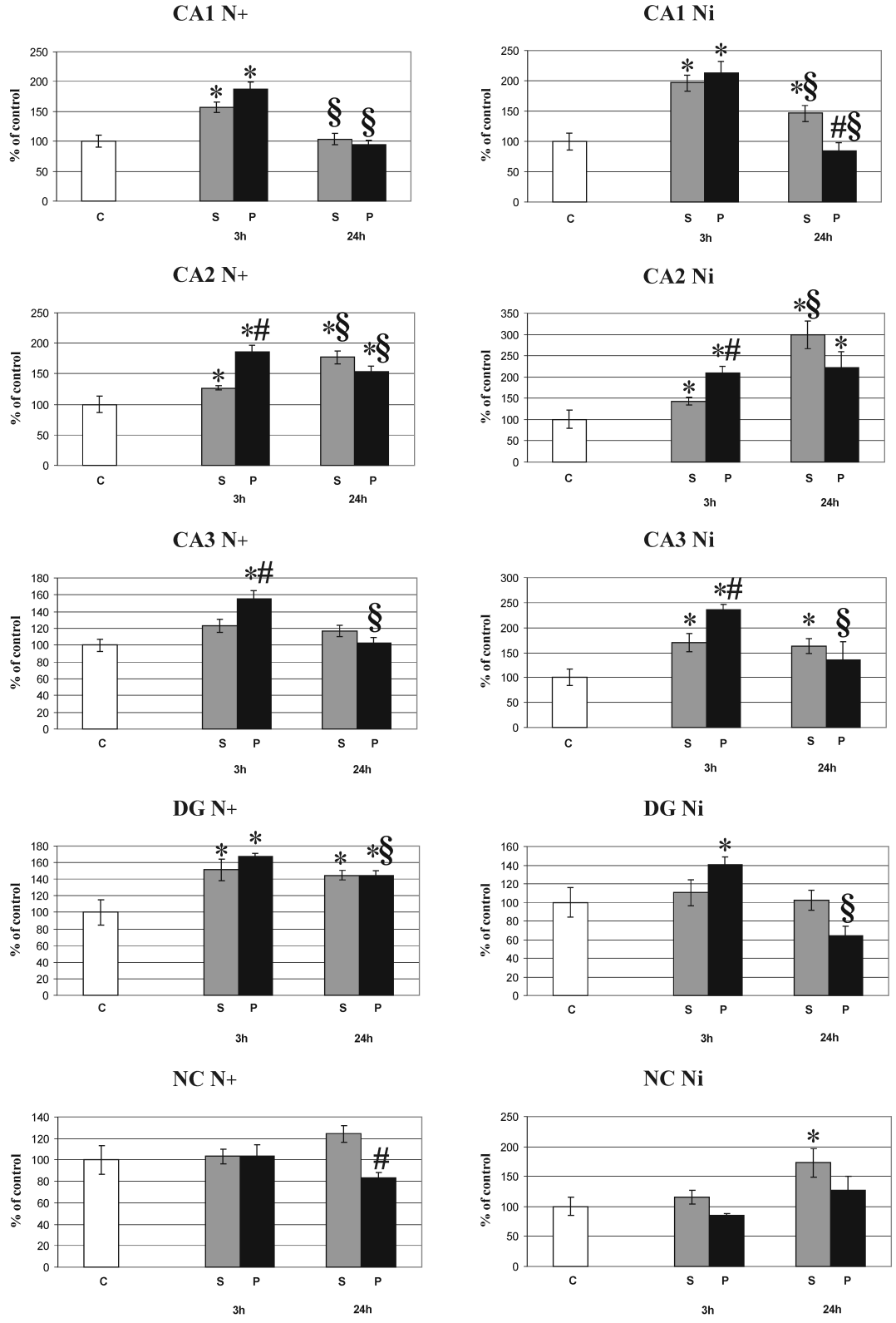



Fig. 7. Changes in the total number of Mn-SOD-immunoreactive cells ($N_+$) and the number of intensely expressing Mn-SOD cells (Ni) in the various formations of the brain in non- (S) and preconditioned (P) rats at 3 h and 24 h after severe hypoxia as a percentage of the control (C). The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – in preconditioned rats compared with non-preconditioned rats; and § – at the 24 h time-point compared with the 3 h time-point. These data were published in “Nejrokhimia” (Stroev *et al.*, 2005).

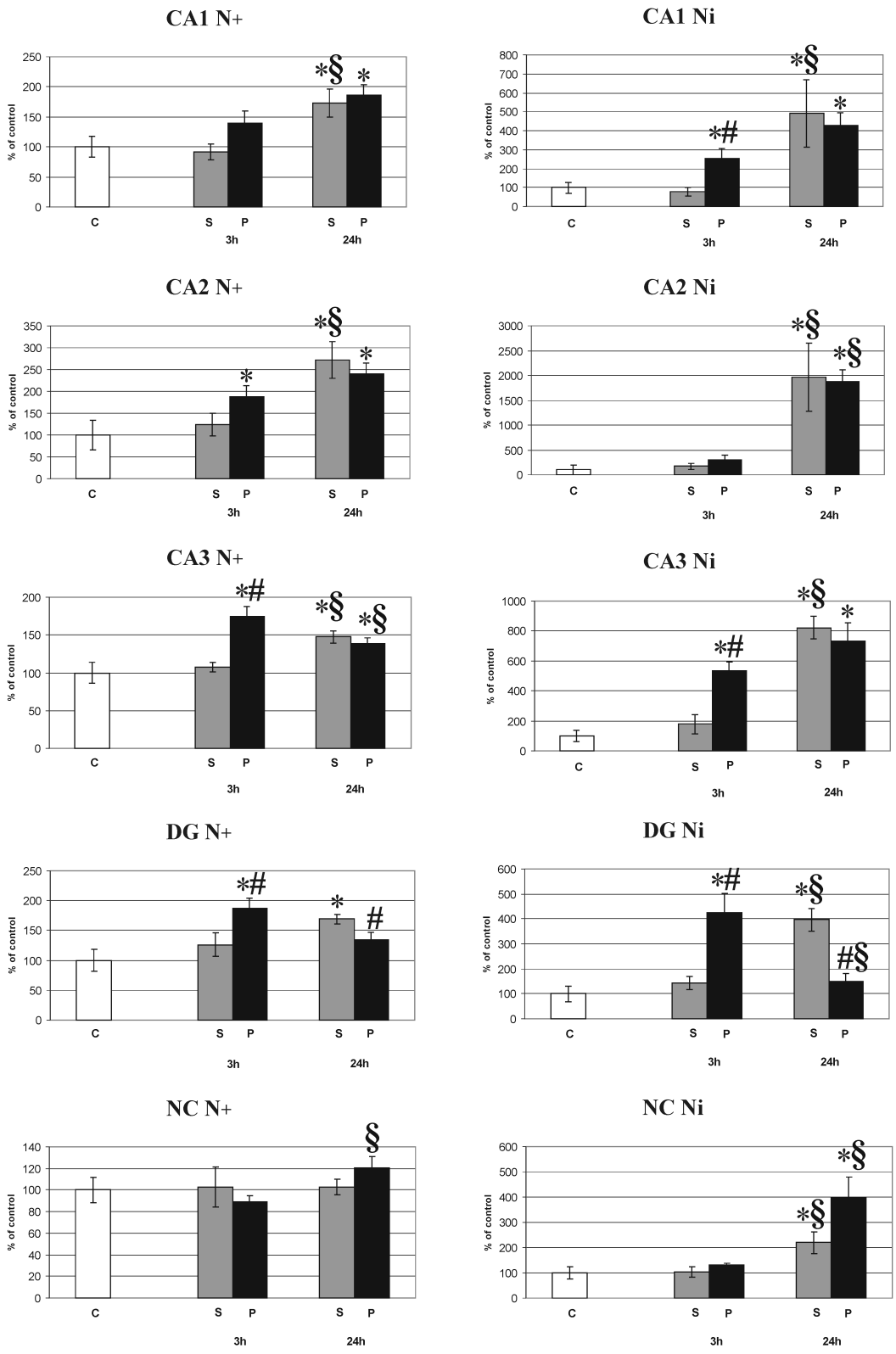



Fig. 8. Changes in the total number of Cu, Zn-SOD-immunoreactive cells ($N_+$) and the number of intensely expressing Cu, Zn-SOD cells (Ni) in the various formations of the brain in non- (S) and preconditioned (P) rats at 3 h and 24 h after severe hypoxia as a percentage of the control (C). The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – in preconditioned rats compared with non-preconditioned rats; and § – at the 24 h time-point compared with the 3 h time-point. These data were published in “Neuroscience Research” (III).

## 5.2.1. Effect of non-preconditioned severe hypoxia compared with the control (I-III)

Immunocytochemistry revealed that the expression levels of all of the antioxidants studied in the hippocampus and neocortex were affected by non-preconditioned severe hypoxia (I-III; Stroev *et al.*, 2005).

At 3 h after the severe hypoxia, a notable increase in Trx-1 (I), Trx-2 (II) and Mn-SOD (Stroev *et al.*, 2005) expression compared with the control was detected in all of the hippocampal areas examined. The expression of Trx-2 (but not the other antioxidants) was also increased in the neocortex (Table 8). The changes in Cu, Zn-SOD (III) expression were not statistically significant in the hippocampal areas nor in the neocortex.

Table 8. Effect of non-preconditioned severe hypobaric hypoxia at 3 h after reoxygenation on the expression of antioxidants compared with the control (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | +++ 129±8 | +++ 238 ±24 | +++ 120±6 | +++ 532 ±53 | +++ 157±9 | +++ 197 ±14 | - 92±13 | - 77±24 |
| CA2 | +++ 145±6 | +++ 776 ±145 | +++ 130±8 | +++ 9750 ±991 | +++ 127±4 | +++ 143±9 | + 124 ±26 | = 170 ±59 |
| CA3 | = 101±9 | +++ 469 ±81 | +++ 260 ±23 | +++ 700 ±127 | + 123±8 | +++ 170 ±20 | = 107±7 | + 178 ±68 |
| DG | + 124±8 | +++ 259 ±45 | +++ 150±3 | +++ 5611 ±657 | +++ 151 ±13 | = 110 ±15 | + 126 ±20 | + 143 ±27 |
| Neo-cortex | - 84±5 | = 115 ±28 | + 119±7 | +++ 355 ±45 | = 103±7 | + 116 ±12 | = 103 ±18 | = 104 ±22 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with the control; + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control; - (light blue) – tended to decrease compared with the control but was not statistically significant. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

At 24 h after the severe hypoxia, the expression of all of the antioxidants studied was notably increased compared with the control in all of the hippocampal areas as well as in the neocortex (I-III; Stroev *et al.*, 2005) (Table 9). The only exception was the expression of Trx-2 in the CA1 area where the number of intensely stained cells was increased, but the total number of cells immunoreactive to Trx-2 was significantly decreased compared with the control (II) (Table 9).

Table 9. Effect of non-preconditioned severe hypobaric hypoxia at 24 h after reoxygenation on the expression of antioxidants compared with the control (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | + 125±8 | +++ 421 ±91 | --- 70±6 | +++ 1091 ±142 | = 104±9 | +++ 147 ±14 | +++ 173 ±23 | +++ 493 ±180 |
| CA2 | +++ 159±7 | +++ 1027 ±141 | +++ 117±3 | +++ 9100 ±640 | +++ 177 ±11 | +++ 299 ±32 | +++ 172 ±42 | +++ 1958 ±682 |
| CA3 | +++ 131 ±11 | +++ 1185 ±110 | +++ 195 ±13 | +++ 1633 ±177 | + 117±7 | +++ 163 ±15 | +++ 147±9 | +++ 822 ±78 |
| DG | +++ 129±8 | +++ 223 ±28 | +++ 146±7 | +++ 2852 ±392 | +++ 145±6 | = 102 ±12 | +++ 169±8 | +++ 397 ±47 |
| Neo-cortex | + 114±8 | +++ 337 ±80 | +++ 143±6 | +++ 663 ±81 | + 124±7 | +++ 173 ±24 | = 103±7 | +++ 221 ±43 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with the control; + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control. Green – the opposite direction of the $N_+$ and Ni changes. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

The dynamics of the antioxidant expression between the 3 h and 24 h time-points were variable in different areas for the individual antioxidants (Table 10). The expression of both cytosolic antioxidants was increased at 24 h compared with the 3 h time-point. Cu, Zn-SOD expression was increased significantly in all of the areas studied (III) (Table 10).

Trx-1 expression was increased significantly only in the neocortex and CA3, but in CA1 and CA2, it tended toward an increase (I) (Table 10). The expression of both mitochondrial antioxidants was significantly increased in some areas and significantly decreased or was not changed in other areas (II; Stroev *et al.*, 2005) (Table 10).

Table 10. Effect of non-preconditioned severe hypobaric hypoxia at 24 h after reoxygenation on the expression of antioxidants compared with the 3 h time-point (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | = | + | --- | +++ | --- | --- | +++ | +++ |
| CA2 | = | + | = | = | +++ | +++ | +++ | +++ |
| CA3 | + | +++ | - | +++ | = | = | +++ | +++ |
| DG | = | - | = | --- | = | = | + | +++ |
| Neo-cortex | +++ | +++ | +++ | +++ | + | + | = | +++ |

$N_+$ – the total number of immunoreactive cells expressed as a percentage of the control; Ni – the number of intensely labelled cells expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase at 24 h compared with the 3 h time-point; + (pink) – tended to increase at 24 h compared with the 3 h time-point but was not statistically significant; = (white) – no differences between the 3 h and 24 h time-points; - (light blue) – tended to decrease at 24 h compared with the 3 h time-point but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease at 24 h compared with the 3 h time-point. Green – the opposite direction of the $N_+$ and Ni changes. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

### 5.2.2. Effect of preconditioned severe hypobaric hypoxia compared with the control (I-III)

In the brains of preconditioned rats at 3 h after severe hypoxia, a notable increase in the expression of both Trxs compared with the control was detected in all of the hippocampal areas examined as well as in the neocortex (I-II) (Table 11). Expression of

both SODs was significantly increased compared with the control in all of the hippocampal areas examined but not in the neocortex (III; Stroev *et al.*, 2005) (Table 11).

Table 11. Effect of preconditioned severe hypobaric hypoxia at 3 h after reoxygenation on the expression of antioxidants compared with the control (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | +++ 159 ±10 | +++ 536 ±57 | +++ 193 ±13 | +++ 2435 ±142 | +++ 188 ±12 | +++ 213 ±19 | + 140 ±20 | +++ 151 ±55 |
| CA2 | +++ 207±7 | +++ 1158 ±101 | +++ 163±4 | +++ 17500 ±1204 | +++ 187 ±11 | +++ 210 ±16 | +++ 189 ±26 | + 294 ±110 |
| CA3 | + 118±4 | +++ 898 ±108 | +++ 417 ±21 | +++ 4550 ±188 | +++ 155 ±11 | +++ 237±9 | +++ 175 ±14 | +++ 536 ±58 |
| DG | +++ 154±8 | +++ 296 ±39 | +++ 152±6 | +++ 9238 ±431 | +++ 167±5 | +++ 141±8 | +++ 187 ±18 | +++ 427 ±77 |
| Neo-cortex | + 107±5 | +++ 244 ±29 | +++ 135±4 | +++ 563 ±27 | = 103 ±11 | - 85±4 | - 89±5 | + 131±7 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with the control; + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control; - (light blue) – tended to decrease compared with the control but was not statistically significant. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

At 24 h after preconditioned severe hypoxia, the expression of Trx-1 was significantly increased compared with the control in all of the hippocampal areas and in the neocortex (I). The expression of Trx-2 was significantly increased in the neocortex and in all of the hippocampal areas except CA1 (II). The expression of Mn-SOD was significantly increased only in CA2 and the DG (Stroev *et al.*, 2005). The expression of Cu, Zn-SOD was significantly increased in CA1, CA2, and CA3 and the neocortex; in the DG the tendency to increase was not statistically significant (III) (Table 12).

Table 12. Effect of preconditioned severe hypobaric hypoxia at 24 h after reoxygenation on the expression of antioxidants compared with the control (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | +++ 136±4 | +++ 584 ±90 | = 102±9 | + 314 ±233 | = 94±8 | - 85±14 | +++ 186 ±17 | +++ 426 ±70 |
| CA2 | +++ 157±8 | +++ 1511 ±270 | +++ 200 ±16 | +++ 2500 ±1000 | +++ 154±9 | +++ 222 ±38 | +++ 241 ±26 | +++ 1875 ±250 |
| CA3 | = 103±7 | +++ 1111 ±236 | +++ 246 ±17 | +++ 817 ±316 | = 103±7 | + 135 ±37 | +++ 138±9 | +++ 733 ±122 |
| DG | +++ 132±6 | + 166 ±31 | +++ 154±2 | - 74±37 | +++ 144±7 | - 64±11 | + 134 ±14 | + 148 ±33 |
| Neo-cortex | + 110±6 | +++ 526 ±80 | +++ 164 ±22 | +++ 670 ±138 | - 84±5 | + 127 ±24 | + 121 ±10 | +++ 397 ±81 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant increase compared with the control ($p<0.05$; F-criteria); + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control; - (light blue) – tended to decrease compared with the control but was not statistically significant. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

The dynamics of the Trx-1, Trx-2 and Mn-SOD expression in hippocampal areas from the 3 h to the 24 h time-point in preconditioned rats was mostly statistically significantly decreased (I-II; Stroev *et al.*, 2005). Only in the CA2 area, the expression of Trx-2 showed the opposite trends of $N_+$ and Ni (II); and in the CA3 area, the expression of Trx-1 was changed not significantly. The expression of Cu, Zn-SOD changed differently in individual areas (III) (Table 13). In the neocortex, the expression of both cytosolic antioxidants was increased significantly (I, III), but the expression of both mitochondrial antioxidants was changed not significantly (II; Stroev *et al.*, 2005) (Table 13).

Table 13. Effect of preconditioned (by 3 sessions of mild hypobaric hypoxia) severe hypoxia on the expression of antioxidants at the 24 h time-point compared with the 3 h time-point (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | --- | = | --- | --- | --- | --- | + | + |
| CA2 | --- | = | +++ | --- | --- | = | + | +++ |
| CA3 | - | = | --- | --- | --- | --- | --- | + |
| DG | --- | --- | = | --- | --- | --- | - | --- |
| Neo-cortex | = | +++ | + | + | - | + | +++ | +++ |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase at 24 h compared with 3 h; + (pink) – tended to increase at 24 h compared with 3 h but was not statistically significant; = (white) – no differences between the 24 h and 3 h time-points; - (light blue) – tended to decrease at 24 h compared with 3 h but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease at 24 h compared with 3 h. Green – the opposite direction of the $N_+$ and Ni changes. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

## 5.2.3. Effect of preconditioning with 3 sessions of mild hypobaric hypoxia after subsequent severe hypoxia compared with non-preconditioning (I-III)

Preconditioning with 3 sessions of mild repetitive hypoxia markedly augmented the severe hypoxia-induced expression of antioxidant proteins in the brain at the 3 h time-point compared with non-preconditioning (I-III; Stroev *et al.*, 2005) (Table 14). For both Trxs, this increase was statistically significant in all of the hippocampal areas studied (I-II). For the SODs, this increase was statistically significant in some areas of the hippocampus, but in other areas, there was only a tendency to increase (III; Stroev *et al.*, 2005). In the neocortex, the expression of both Trxs was significantly increased, but the expression of both SODs was not changed compared with non-preconditioning (I-III; Stroev *et al.*, 2005) (Table 14).

Table 14. Effect of preconditioning by 3 sessions of mild hypobaric hypoxia on the expression of antioxidants at 3 h after a subsequent severe hypoxia compared with non-preconditioning (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | +++ | +++ | +++ | +++ | + | + | + | +++ |
| CA2 | +++ | +++ | +++ | +++ | +++ | +++ | + | + |
| CA3 | + | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| DG | +++ | + | = | +++ | + | + | +++ | +++ |
| Neo-cortex | +++ | +++ | + | +++ | = | - | - | + |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with non-preconditioning; + (pink) – tended to increase compared with non-preconditioning but was not statistically significant; = (white) – no differences between preconditioning and non-preconditioning; - (light blue) – tended to decrease compared with non-preconditioning but was not statistically significant. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

At 24 h after severe hypoxia, the effect of preconditioning was not so clear. In most cases, there was a down-regulation of antioxidant expression or no significant changes compared with non-preconditioning (I-III; Stroev *et al.*, 2005) (Table 15). The significant increase in expression as a result of preconditioning in this period was shown only in Trx-2 in CA3 (II), but significant decrease in Trx-1 in CA3 (I), Trx-2 in the DG (II), Mn-SOD in CA1 and the neocortex (Stroev *et al.*, 2005) and Cu, Zn-SOD in the DG (III) was observed. In some cases (the expression of Trx-2 in CA1 and CA2), the up-regulation in the total number of immunoreactive cells was accompanied by a down-regulation in the number of intensely labelled cells (II). In other cases, the changes only occurred at the level of statistically non-significant trends (I-III; Stroev *et al.*, 2005). In the hippocampus, both SODs showed a downward general trend (III; Stroev *et al.*, 2005), but the Trxs in different areas showed different trends in dynamics (I, II). In the neocortex, the expression of all of the antioxidants studied except Mn-SOD tended to increase in

preconditioned rats compared with non-preconditioned ones (I-III; Stroev *et al.*, 2005) (Table 15).

Table 15. Effect of preconditioning by 3 sessions of mild hypobaric hypoxia on the expression of antioxidants at 24 h after a subsequent severe hypoxia compared with non-preconditioning (I-III; Stroev *et al.*, 2005).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | + | + | +++ | --- | = | --- | + | - |
| CA2 | = | + | +++ | --- | - | - | - | = |
| CA3 | --- | = | +++ | - | - | - | - | - |
| DG | = | - | = | --- | = | - | --- | --- |
| Neo-cortex | = | + | + | = | --- | - | + | + |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with non-preconditioning; + (pink) – tended to increase compared with non-preconditioning but was not statistically significant; = (white) – no differences between preconditioning and non-preconditioning; - (light blue) – tended to decrease compared with non-preconditioning but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease compared with non-preconditioning. Green – the opposite direction of the $N_+$ and Ni changes. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

## 5.3. Effect of 3 sessions and 1 session of mild hypobaric hypoxia (preconditioning itself without severe hypoxia) on the expression of antioxidants (IV-X)

The results of studying of the effects of 1 session and 3 sessions of mild hypobaric hypoxia on the expression of endogenous antioxidant proteins in the CA1, CA2, and CA3 hippocampal fields and in the DG 3 h and 24 h after the end of hypoxic exposure are summarised in Figures 9-12: Trx-1 (V, X) (Fig. 9), Trx-2 (VII) (Fig. 10), Mn-SOD (IV, VIII) (Fig. 11) and Cu, Zn-SOD (VI, IX) (Fig. 12). These results are described in more detail below.

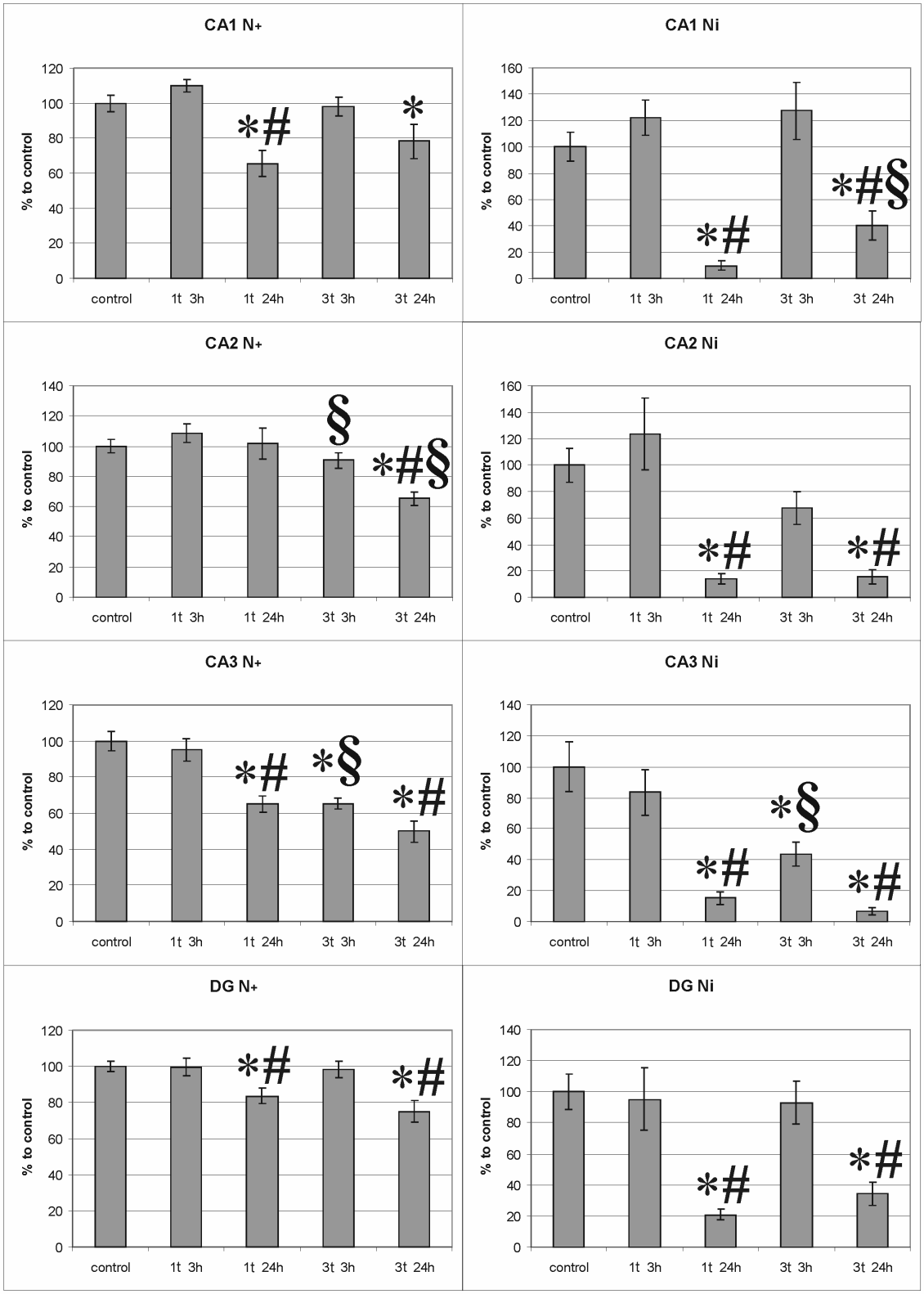



Fig. 9. Changes in the total number of Trx-1-immunoreactive cells ($N_+$) and the number of intensely expressing Trx-1 cells (Ni) in the various areas of the hippocampus at 3 h and 24 h after 1 session (1 t) and at 3 h and 24 h after 3 sessions (3 t) of mild hypoxia as a percentage of the control. The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – at the 24 h time-point compared with the 3 h time-point; and § – 3 sessions of hypoxia compared with 1 session. These data were published in “Neuroscience and Behavioral Physiology” (X).

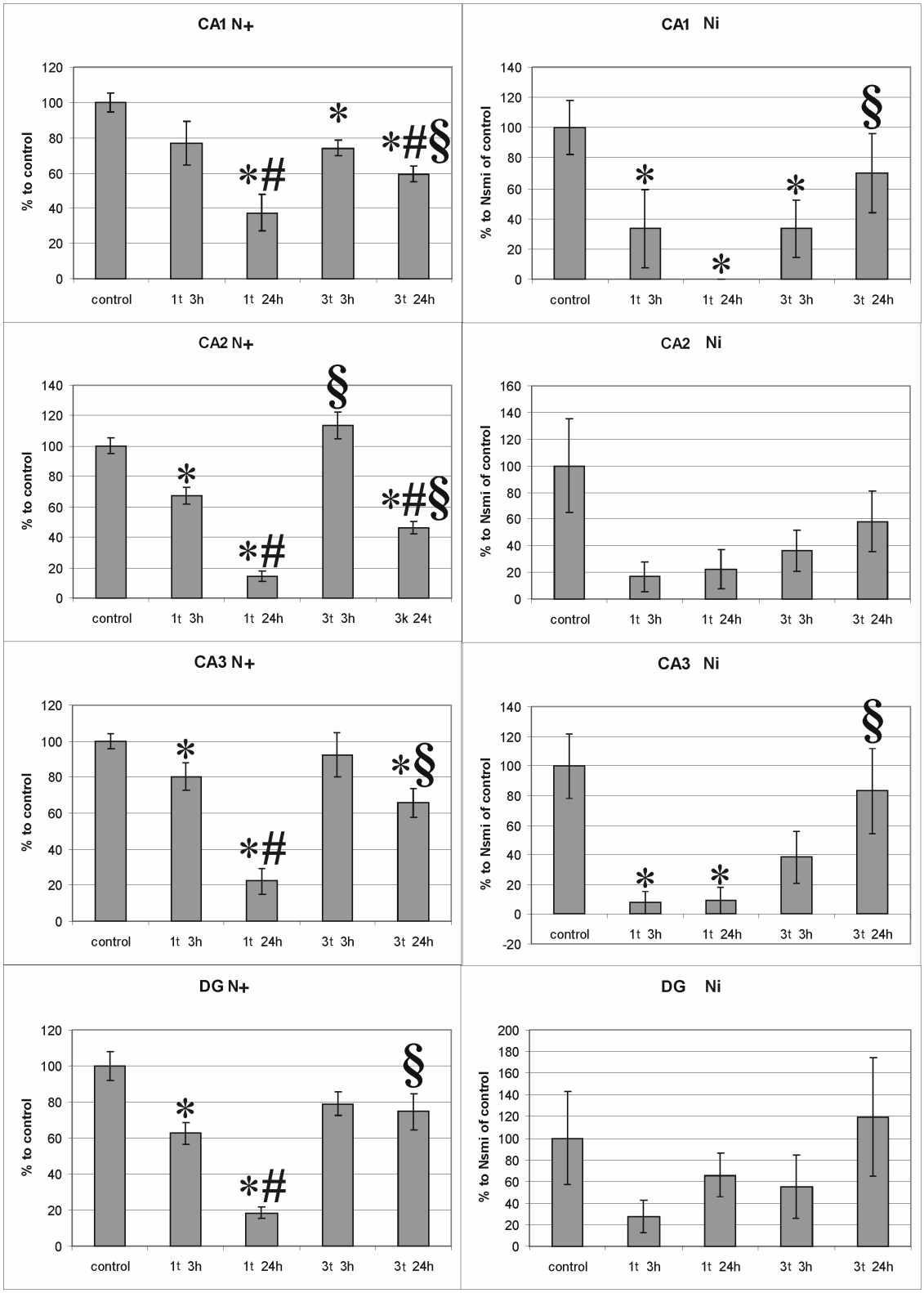



Fig. 10. Changes in the total number of Trx-2-immunoreactive cells ($N_+$) and the number of intensely expressing Trx-2 cells (Ni) in the various areas of the hippocampus at 3 h and 24 h after 1 session (1 t) and at 3 h and 24 h after 3 sessions (3 t) of mild hypoxia as a percentage of the control. The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – at the 24 h time-point compared with the 3 h time-point; and § – 3 sessions of hypoxia compared with 1 session. These data were published in “Acta Neurobiologiae Experimentalis” (VII).

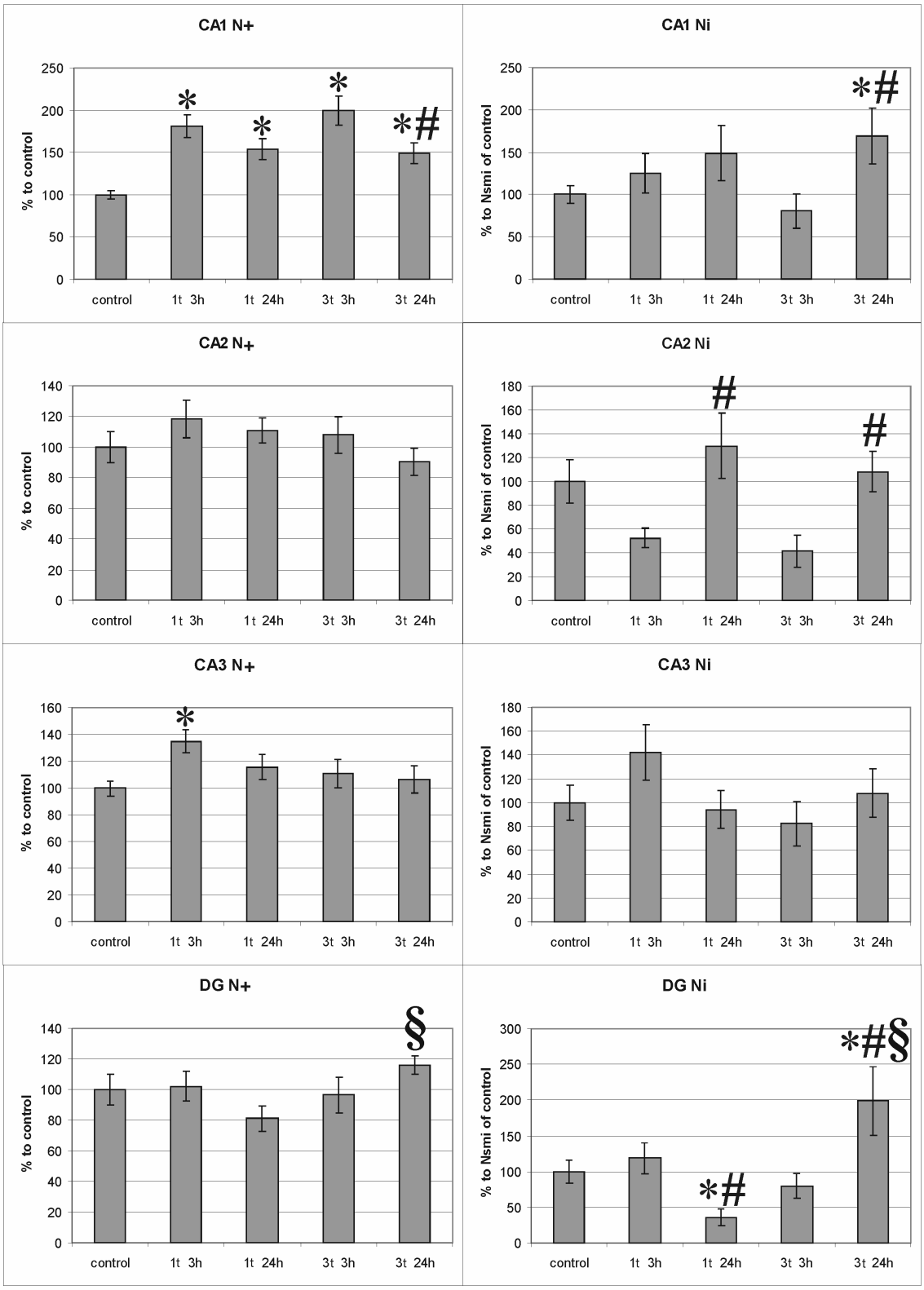



Fig. 11. Changes in the total number of Mn-SOD-immunoreactive cells ($N_+$) and the number of intensely expressing Mn-SOD cells (Ni) in the various areas of the hippocampus at 3 h and 24 h after 1 session (1 t) and at 3 h and 24 h after 3 sessions (3 t) of mild hypoxia as a percentage of the control. The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – at the 24 h time-point compared with the 3 h time-point; and § – 3 sessions of hypoxia compared with 1 session. These data were published in “Neuroscience and Behavioral Physiology” (VIII).

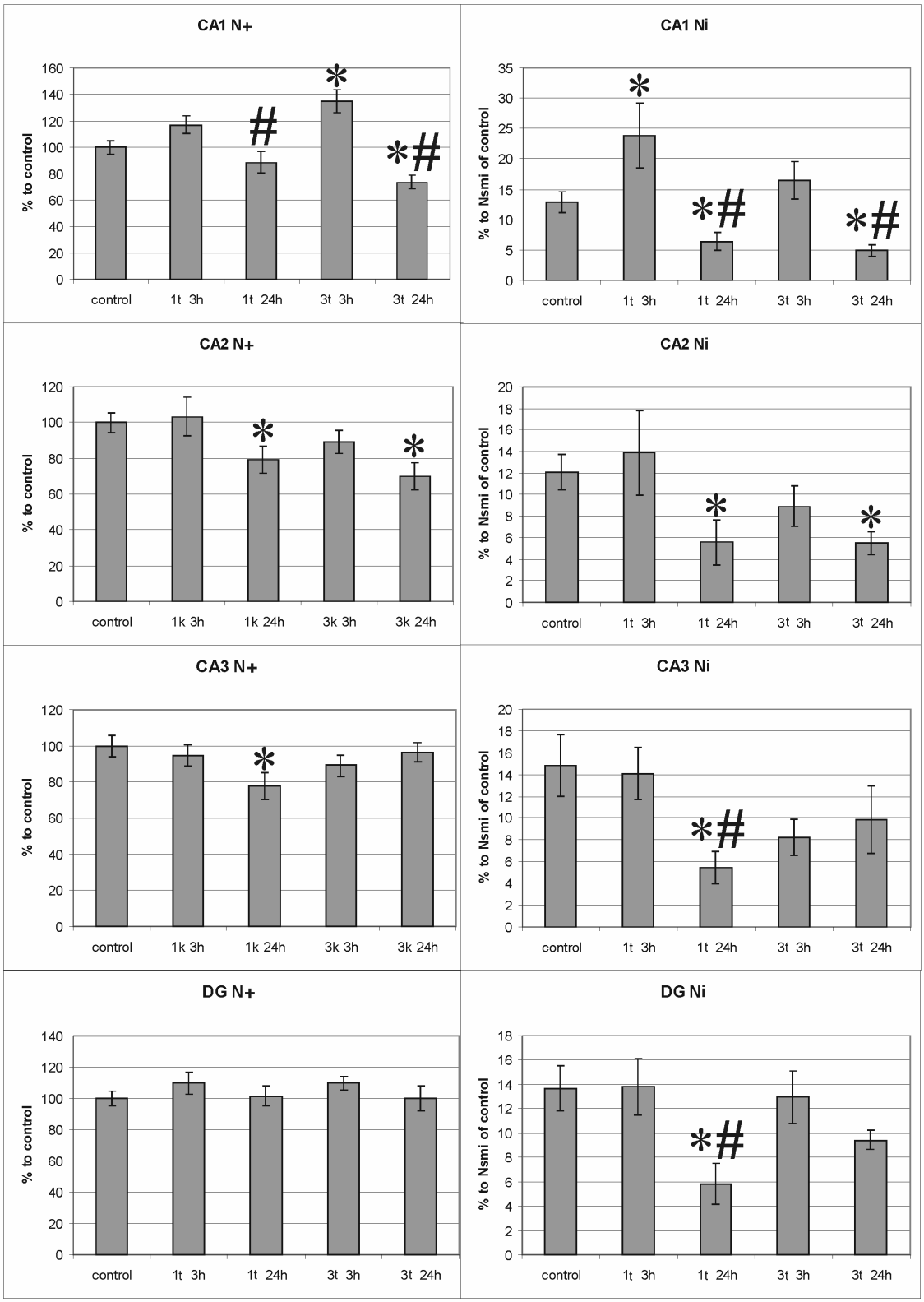



Fig. 12. Changes in the total number of Cu, Zn-SOD-immunoreactive cells ($N_+$) and the number of intensely expressing Cu, Zn-SOD cells (Ni) in the various areas of the hippocampus at 3 h and 24 h after 1 session (1 t) and at 3 h and 24 h after 3 sessions (3 t) of mild hypoxia as a percentage of the control. The statistical significance is as follows ($p<0.05$, F-test): * – compared with the control; # – at the 24 h time-point compared with the 3 h time-point; and § – 3 sessions of hypoxia compared with 1 session. These data were published in “Neurochemical Journal” (IX).

## 5.3.1. Effect of 3 sessions of mild hypoxia (IV-VII)

At 3 h after the last of the 3 sessions of mild hypobaric hypoxia (preconditioning), in most cases, there were no statistically significant differences in the expression of the antioxidants compared with the control (IV-VII). Only the expression of both SODs was significantly increased in CA1 (IV, VI); the expression of Trx-1 was significantly decreased in CA3 (V), and the expression of Trx-2 was significantly decreased in CA1 (VII) (Table 16).

Table 16. Effect of 3 sessions of mild hypobaric hypoxia itself at the 3 h time-point after the end of the last session on the expression of antioxidants compared with the control (IV-VII).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | = 98±5 | + 127 ±22 | --- 74±5 | --- 33±19 | +++ 200 ±17 | - 80±21 | +++ 135±9 | + 129 ±24 |
| CA2 | - 91±5 | - 68±12 | + 113±9 | - 36±15 | = 108 ±12 | - 41±14 | - 89±6 | - 73±16 |
| CA3 | --- 65±3 | --- 44±8 | = 93±12 | - 38±18 | + 111 ±11 | - 82±18 | - 89±6 | - 56±12 |
| DG | = 98±5 | = 93±14 | - 79±7 | - 55±29 | = 96±12 | - 80±17 | + 110±4 | = 95±16 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with the control; + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control; - (light blue) – tended to decrease compared with the control but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease compared with the control. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

At 24 h after the last of the 3 sessions of mild hypoxia, the expression of Trx-1 (both $N_+$ and Ni) was significantly decreased in all of the hippocampal areas studied (V). The expression of Trx-2 was significantly decreased in CA1, CA2 and CA3, but only in terms

of $N_+$; the changes in Ni in comparison with the control were not significant in any of the fields studied (VII). The expression of Cu, Zn-SOD (both $N_+$ and Ni) was significantly decreased in CA1 and CA2 (in CA3 and the DG, the downward trend for Ni was not statistically significant, and for $N_+$ there was virtually no difference compared with control) (VI) (Table 17). Only the expression of Mn-SOD was significantly increased in CA1 and the DG (IV) (Table 17).

Table 17. Effect of 3 sessions of mild hypobaric hypoxia itself at the 24 h time-point after the end of the last session on the expression of antioxidants compared with the control (IV-VII).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | --- 78±10 | --- 40±11 | --- 59±5 | - 70±26 | +++ 149 ±13 | +++ 169 ±33 | --- 74±5 | --- 39±7 |
| CA2 | --- 65±5 | --- 16±5 | --- 46±4 | - 58±23 | - 90±9 | = 108 ±17 | --- 70±8 | --- 45±9 |
| CA3 | --- 50±6 | --- 7±3 | --- 66±8 | - 83±29 | = 106 ±11 | = 108 ±20 | = 96±5 | - 67±21 |
| DG | --- 75±6 | --- 34±8 | - 75±10 | + 119 ±55 | + 116±6 | +++ 199 ±49 | = 100±8 | - 69±6 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with the control; + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control; - (light blue) – tended to decrease compared with the control but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease compared with the control. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

Compared with the 3 h time-point, at 24 h after the last of the 3 sessions of mild hypoxia, the expression of Trx-1 was significantly decreased in all of the hippocampal areas studied (V). The expression of Trx-2 was significantly decreased compared with the 3 h time-point in CA1 and CA2 (VII). The expression of Cu, Zn-SOD was significantly

decreased compared with the 3 h time-point in CA1 (VI) (Table 18). The number of intensely labelled cells immunoreactive to Mn-SOD was significantly increased in CA1, CA2 and the DG and tended to increase in CA3 compared with the 3 h time-point (IV). However, the total number of cells immunoreactive to Mn-SOD was significantly decreased in CA1 and tended to increase in CA2 (IV) (Table 18).

Table 18. Effect of 3 sessions of mild hypobaric hypoxia itself at the 24 h time-point after the end of the last session on the expression of antioxidants compared with the 3 h time-point (IV-VII).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | - | --- | --- | + | --- | +++ | --- | --- |
| CA2 | --- | --- | --- | = | - | +++ | - | - |
| CA3 | --- | --- | - | + | = | + | = | = |
| DG | --- | --- | = | + | + | +++ | = | - |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase at 24 h compared with 3 h; + (pink) – tended to increase at 24 h compared with 3 h but was not statistically significant; = (white) – no differences between the 24 h and 3 h time-points; - (light blue) – tended to decrease at 24 h compared with 3 h but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease at 24 h compared with 3 h. Green – the opposite direction of the $N_+$ and Ni changes. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

## 5.3.2. Effect of 1 session of mild hypoxia (VII-X)

At 3 h after 1 session of mild hypobaric hypoxia, the Ni to Trx-2 in CA1 and CA3 and the $N_+$ to Trx-2 in CA2, CA3 and the DG were significantly decreased compared with the control (VII). Thus, the expression of Trx-2 was significantly reduced in all of the hippocampal areas studied (VII). The Ni and $N_+$ to Trx-1 expression did not have significant differences compared with the control (X). At the same time, the Ni to Cu, Zn-SOD in CA1 (IX) and $N_+$ to Mn-SOD in CA1 and CA3 (VIII) were significantly

increased compared with the control. In other cases (VII-X), the level of expression of the antioxidants was not significantly different compared with the control (Table 19).

Table 19. Effect of 1 session of mild hypobaric hypoxia itself at the 3 h time-point after reoxygenation on the expression of antioxidants compared with the control (VII-X).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | + 110±4 | + 122 ±14 | - 77±12 | --- 33±26 | +++ 181 ±13 | + 125 ±23 | + 117±7 | +++ 187 ±41 |
| CA2 | + 109±6 | + 124 ±27 | --- 67±5 | - 17 ±11 | + 118 ±12 | - 53±8 | = 103 ±11 | = 114 ±32 |
| CA3 | = 95±6 | - 84±15 | --- 80±8 | --- 8±8 | +++ 135±9 | + 142 ±23 | = 95±6 | = 96±17 |
| DG | = 100±5 | = 95±20 | --- 63±6 | - 27±15 | = 102±9 | = 119 ±21 | + 110±7 | = 101 ±17 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with the control; + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control; - (light blue) – tended to decrease compared with the control but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease compared with the control. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

At 24 h after 1 session of mild hypobaric hypoxia, the expression of Trx-1 (Ni in CA1, CA2, CA3 and the DG; $N_+$ in CA1, CA3 and the DG) (X), Trx-2 (Ni in CA1 and CA3; $N_+$ in CA1, CA2, CA3 and the DG) (VII) and Cu, Zn-SOD (Ni in CA1, CA2, CA3 and the DG; $N_+$ in CA2 and CA3) (IX) was significantly decreased compared with the control. Thus, the expression of both Trxs (VII, X) and Cu, Zn-SOD (IX) was significantly decreased in all of the hippocampal areas studied. The $N_+$ to Mn-SOD was significantly increased in CA1 and tended to increase (without statistical significance) in CA2 and CA3; the Ni to Mn-SOD also had a tendency, although not statistically

significant, to increase in CA1 and CA2 (VIII). At the same time, in the DG, the Ni to Mn-SOD was significantly decreased, and the $N_+$ had a non-significant tendency to decrease (VIII) (Table 20).

Table 20. Effect of 1 session of mild hypobaric hypoxia itself at the 24 h time-point after reoxygenation on the expression of antioxidants compared with the control (VII-X).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | --- 66±8 | --- 10±4 | --- 38±10 | --- 0±0 | +++ 154 ±12 | + 149 ±33 | - 89±8 | --- 50±11 |
| CA2 | = 102 ±10 | --- 14±4 | --- 14±3 | - 22±15 | + 111±8 | + 130 ±28 | --- 79±7 | --- 46±17 |
| CA3 | --- 65±5 | --- 15±4 | --- 22±7 | --- 9±9 | + 116 ±10 | = 94±16 | --- 78±7 | --- 37±10 |
| DG | --- 84±4 | --- 21±4 | --- 18±3 | - 66±20 | - 81±8 | --- 36±12 | = 102±6 | --- 43±13 |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase compared with the control; + (pink) – tended to increase compared with the control but was not statistically significant; = (white) – no differences compared with the control; - (light blue) – tended to decrease compared with the control but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease compared with the control. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

Compared with the 3 h time-point, at 24 h after 1 session of mild hypoxia, the expression of Trx-1 (X) and Trx-2 (VII) was significantly decreased in all of the hippocampal areas studied. The expression of Cu, Zn-SOD (IX) was significantly decreased compared with the 3 h time-point in all of the hippocampal areas except CA2. The expression of Mn-SOD was significantly increased in CA2 and significantly decreased in the DG compared with the 3 h time-point (VIII) (Table 21).

Table 21. Effect of 1 session of mild hypobaric hypoxia itself at the 24 h time-point after reoxygenation on the expression of antioxidants compared with the 3 h time-point (VII-X).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | --- | --- | --- | - | - | = | --- | --- |
| CA2 | = | --- | --- | = | = | +++ | - | - |
| CA3 | --- | --- | --- | = | - | - | - | --- |
| DG | --- | --- | --- | + | - | --- | - | --- |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase at 24 h compared with 3 h; + (pink) – tended to increase at 24 h compared with 3 h but was not statistically significant; = (white) – no differences between the 24 h and 3 h time-points; - (light blue) – tended to decrease at 24 h compared with 3 h but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease at 24 h compared with 3 h. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

## 5.3.3. Effect of 3 sessions of mild hypoxia compared with the effect of 1 session of mild hypoxia (VII-X)

There were some statistically significant differences between the effects of 1 session and 3 sessions of mild hypoxia at the 3 h time-point. The expression of Trx-1 in the CA2 and CA3 areas after 3 sessions of hypoxia was significantly lower than after 1 session of hypoxia (X). In contrast, the expression of Trx-2 in CA2 after 3 sessions of hypoxia was significantly higher (VII) (Table 22). At 3 h after the last of the 3 sessions of mild hypobaric hypoxia, there were no statistically significant differences in the expression of both SODs compared with 1 session of mild hypoxia (VIII, IX) (Table 22). However, the $N_+$ to Mn-SOD in CA3 was increased significantly compared with the control at 3 h after a single session (Table 19) but non-significantly after 3 sessions (Table 16) (VIII). In the CA1 area, the Ni to Cu, Zn-SOD but not the $N_+$ was significantly increased at 3 h after 1 session of hypoxia compared with the control; in contrast, the $N_+$ to Cu, Zn-SOD, but

not the Ni, was significantly increased at 3 h after 3 sessions of hypoxia compared with the control (VIII).

Table 22. Effect of 3 sessions of mild hypobaric hypoxia itself at the 3 h time-point after the end of the last session on the expression of antioxidants compared with effect of 1 session of mild hypobaric hypoxia itself at the 3 h time-point after reoxygenation (VII-X).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | - | = | = | = | + | - | + | - |
| CA2 | --- | - | +++ | + | = | = | - | - |
| CA3 | --- | --- | = | + | - | - | = | - |
| DG | = | = | + | + | = | - | = | = |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase at 3 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia; + (pink) – tended to increase at 3 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia but was not statistically significant; = (white) – no differences in expression after 3 sessions and after 1 session of mild hypoxia; - (light blue) – tended to decrease at 3 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease at 3 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

At 24 h after the last of the 3 sessions of mild hypobaric hypoxia, compared with 1 session of mild hypoxia, the Ni to Trx-1 was significantly increased in CA1, but the $N_+$ to Trx-1 was significantly decreased in CA2 (X) (Table 23). The $N_+$ to Trx-2 was significantly increased in all of the hippocampal areas studied; the Ni to Trx-2 was significantly increased in CA1 and CA3 and tended to increase in CA2 and the DG (VII) (Table 23). The expression of Mn-SOD in the DG (both the $N_+$ and Ni) after 3 sessions of mild hypobaric hypoxia was significantly higher than after 1 session of mild hypoxia (VIII) (Table 23). Moreover, the Ni to Mn-SOD in the DG at 24 h after 3 sessions of mild hypoxia was significantly increased compared with the control (Table 17), but after 1 session of mild hypoxia, it was significantly decreased compared with the control (Table

20). The expression of Cu, Zn-SOD (both the $N_+$ and Ni) was not significantly different at 24 h after the last of the 3 sessions of mild hypobaric hypoxia compared with 1 session of mild hypoxia (IX) (Table 23).

Table 23. Effect of 3 sessions of mild hypobaric hypoxia itself at the 24 h time-point after the end of the last session on the expression of antioxidants compared with effect of 1 session of mild hypobaric hypoxia itself at the 24 h time-point after reoxygenation (VII-X).

| | Trx-1 | | Trx-2 | | Mn-SOD | | Cu, Zn-SOD | |
|---|---|---|---|---|---|---|---|---|
| | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni | $N_+$ | Ni |
| CA1 | + | +++ | +++ | +++ | = | = | - | - |
| CA2 | --- | = | +++ | + | - | - | - | = |
| CA3 | - | - | +++ | +++ | = | = | + | + |
| DG | - | + | +++ | + | +++ | +++ | = | + |

$N_+$ – the total number of immunoreactive cells ±SEM expressed as a percentage of the control; Ni – the number of intensely labelled cells ±SEM expressed as a percentage of the control. +++ (red) – statistically significant ($p<0.05$; F-criteria) increase at 24 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia; + (pink) – tended to increase at 24 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia but was not statistically significant; = (white) – no differences in expression after 3 sessions and after 1 session of mild hypoxia; - (light blue) – tended to decrease at 24 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia but was not statistically significant; --- (dark blue) – statistically significant ($p<0.05$; F-criteria) decrease at 24 h after 3 sessions of mild hypoxia compared with 1 session of mild hypoxia. CA1-CA3 – areas of the *cornu Ammonis*; DG – *dentate gyrus*.

# 6. Discussion

Summarising the obtained results, it is possible to note the most important trends. The non-preconditioned severe hypobaric hypoxia increased the expression of the antioxidant proteins studied (I-III; Stroev *et al.*, 2005). However, this increase was not sufficient to prevent mass delayed neuronal death (Rybnikova *et al.*, 2005a, b, 2006b). The preconditioning markedly augmented the increase of the antioxidant expression during the early period (3 h) after a subsequent severe hypoxia compared with non-preconditioning in most cases (I-III; Stroev *et al.*, 2005). This enhancement of the antioxidant expression induction correlated with the reduction of neuronal death and amelioration of functional disorders (Rybnikova *et al.*, 2005a, b, 2006b). However, the preconditioning by 3 sessions of mild hypoxia itself did not increase the expression of antioxidants in most cases at the starting point of the severe hypoxia. Moreover, in many cases, the preconditioning itself significantly reduced the expression of antioxidants (IV-VII). It was also interesting that 1 session of mild hypoxia itself (without severe hypoxia) modified the expression of antioxidants similarly to the 3 sessions of mild hypoxia (VII-X). However, the 3 sessions of preconditioning had neuroprotective effects in cases of subsequent severe hypoxia, but 1 session of preconditioning had no such effect (Churilova *et al.*, 2012).

## 6.1. The effect of non-preconditioned severe hypoxia on the expression of antioxidant proteins

According to our data, severe hypobaric hypoxia increased the protein expression of Trx-1 and Trx-2, Cu, Zn-SOD and Mn-SOD in the brain structures studied. Although the timing and intensity of this induction for the different antioxidants and various brain structures differed, the overall directionality of the responses to severe hypobaric hypoxia was the same: in all of the cases, there were more or less significant increases in expression, and in none of the cases was suppression of antioxidant protein expression observed (I-III; Stroev *et al.*, 2005).

Oxidative stress caused by the increase in reactive oxygen species (ROS) and other free radical production is one of the major mechanisms of cell damage during hypoxia and subsequent reoxygenation (Choi, 1995; Chan, 1996; Mohri *et al.*, 2001). As noted above, endogenous antioxidants play an important role in neuronal cell protection against oxidative stress induced by hypoxia (Kinoshita *et al.*, 1991; Morita-Fujimura *et al.*, 2001; Sugawara *et al.*, 2002). Whether there will be an adaptive or pathological response to hypoxia is largely determined by the ratio of pro- and antioxidant system activities.

The effect of hypoxia on the expression of antioxidant proteins depends on the experimental model, intensity and duration of the hypoxic/ischaemic episode as well as on the time after reoxygenation when this effect was measured. An increase in the expression of antioxidants as a response to hypoxia is often observed. However, particularly severe form of hypoxia/ischaemia on the contrary can cause the suppression of antioxidant expression. In particular, the suppression of Cu, Zn-SOD and Mn-SOD expression has been observed 4 h after 10 minutes of focal cerebral ischaemia (Liu *et al.*, 1993a) and after 3 minutes of global ischaemia (Kato *et al.*, 1995). The induction of Mn-SOD expression has been observed in various tissues under hypobaric hypoxia (Nakanishi *et al.*, 1995) and in hippocampal neurons as a result of hypoxia caused by reduced oxygen concentration (Pohle, Rauca, 1997). In experiments on gerbils, it has been shown that the enzymatic activity of Mn-SOD increases significantly at 24 h and remains elevated until at least 72 h after 5 minutes of temporary global ischaemia in the hippocampus. However, the activity of Mn-SOD remains unchanged in the cortex and striatum, while the activity of Cu, Zn -SOD in the striatum markedly decreases at 12 h but does not change substantially in the hippocampus and cortex (Candelario-Jalil *et al.*, 2001). In a similar model (a temporary global ischaemia in gerbils), the induction of Trx-1 (adult T cell leukaemia-derived factor) appears in the hippocampus up to 24 h after reperfusion and remains increased up to 72 h (Tomimoto *et al.*, 1993). Recently, it has also been shown that the Trx-2 immunoreactivity in pyramidal neurons in gerbils is increased at 30 minutes and 1 day after 5 minutes of transient cerebral ischaemia and reperfusion but is decreased at 6 h after such ischaemia; in this model, the Trx-2 immunoreactivity is expressed in astrocytes at 3 days after ischaemia (Hwang *et al.*, 2010).

Representative results have been obtained by several investigators in experiments with focal ischaemia. After focal ischaemia of the cortex, the neurons degenerate in the

ischaemic core. Both SODs (Liu *et al.*, 1994a) and Trx (Takagi *et al.*, 1998a, b; Hattori *et al.*, 2002) suppress this degeneration. At the same time, at the border between the ischaemic core and intact tissue, where only a few cells are dying although the majority of cells survive, the expression of Trx is increased during the early stages (Takagi *et al.*, 1998a, b; Hattori *et al.*, 2002). The Cu, Zn-SOD expression is decreased during the early stages, but then expression of both SODs is increased (Liu *et al.*, 1994a). Increased expression of Mn-SOD in the border area has also been shown in a similar model of focal cerebral thrombosis (Bidmon *et al.*, 1998). The enzymatic activity of SOD is not noticeably changed in the ischaemic core, but it is increased in the border region (Toyoda, Lee, 1997). A significant increase in Trx has been shown in the mouse brain at 24-72 h after ischaemia/reperfusion, herewith the increased Trx expression level in the peri-infarct region is significantly higher than in the region destined to infarct (Tanaka *et al.*, 2011).

The same principle has been demonstrated in completely different experimental models. Melatonin treatment attenuates cerebral ischaemic injury; this attenuation correlates with the significant increase in Trx expression in a melatonin-treated group compared with a vehicle-treated group (Sung *et al.*, 2009). Similarly, tetramethyl pyrazine treatment significantly decreases the cerebral infarction volume and neurological deficit scores; in this model, the expression of Trx-1/Trx-2 is significantly decreased in rats with ischaemia/reperfusion injury, while it is increased by tetramethyl pyrazine administration (Jia *et al.*, 2009; Zhu *et al.*, 2009). Pre- and post-hypoxia/ischemia treatment by the N-acetylcysteine reduces lipopolysaccharide-sensitised hypoxic/ischaemic brain injury; this protection is associated with increased levels of antioxidants, including Trx-2 (Wang *et al.*, 2007). The overexpression of X chromosome-linked inhibitor of apoptosis protein (XIAP) induces an up-regulation of certain mitochondrial antioxidants, including Mn-SOD and Trx-2; this correlates with the enhanced tissue protection and reduction of hypoxia/ischaemia-induced oxidative stress (Zhu *et al.*, 2007).

Interesting results have been obtained using mutant stroke-prone spontaneously hypertensive rats that are especially non-resistant to hypoxia/ischaemia. It has been shown that the neuronal expression of Trx genes is significantly decreased in these rats compared with normotensive rats (Yamagata *et al.*, 2010).

All of these facts suggest that the activation of antioxidant systems is generally correlated with an adaptive response to damaging effects, and their suppression is generally correlated with non-adaptive pathological processes that lead to the massive death of neurons.

In our study, the expression of both Trxs and Mn-SOD (I-II, Stroev *et al.*, 2005) was already significantly increased in all of the hippocampal areas studied as early as 3 h after end of non-preconditioned severe hypoxia (Table 8). It should be noted that both Trxs (I-II) and Mn-SOD (Stroev *et al.*, 2005) exhibited a more rapid response to hypobaric hypoxia than Cu, Zn-SOD (III) because Cu, Zn-SOD expression was increased only at 24 h. Thus, our experiments with hypobaric hypoxia confirmed the idea, established on the basis of experiments with different models of hypoxia/ischaemia, that mitochondrial Mn-SOD is more inducible, while cytosolic Cu, Zn-SOD is the more constitutive form of SOD (Kato *et al.*, 1995; Takeuchi *et al.*, 2000). Presumably, this difference is because mitochondria are the major source of ROS (Ueda *et al.*, 2002) and are a key organelle in the induction of cell death (Mishra, Kumar, 2005; Tait, Green, 2010; Dodson *et al.*, 2013), so mitochondrial antioxidants represent the forefront of the defence against oxidative stress.

In addition, we showed that up-regulation in the number (Ni) of cells intensely expressing mitochondrial Trx-2 (II) was more pronounced than for Mn-SOD (Stroev *et al.*, 2005). This fact was consistent with previously obtained data on the expression of genes of these proteins (Samoilov *et al.*, 2002). It is known that the content and activity of most antioxidant enzymes is much lower in the brain compared with tissues such as the liver, kidney or heart (Hayes *et al.*, 1989; Miroshnichenko, 1992; L'vova, Abaeva, 1996; Levadnaya *et al.*, 1998). For example, the brain content of SOD is 3-7 times lower on average than the liver content. Similarly, the catalase activity is 50-70 times lower. Consequently, in brain tissues, the thiol antioxidants are probably more important in the implementation of protective mechanisms compared with SODs, which was confirmed by our data.

In our experiments, the induction of antioxidants in the neocortex was observed later compared with the hippocampus (I-III; Stroev *et al.*, 2005). In the neocortex, only Trx-2 expression was increased at the 3 h time-point, whereas the induction of the other

antioxidants developed at the 24 h time-point. This finding is also in agreement with data on the expression of the mRNAs for Trx-2 and Mn-SOD (Samoilov *et al.*, 2002).

The role of different antioxidants likely varied in neurons of distinct brain areas. For example, the total number of cells immunoreactive to Trx-2 (II) was most noticeably increased in CA3 (1.5-2 times higher than in the other studied areas of the brain), while an increase in the number of cells immunoreactive to Cu, Zn-SOD (III) was most significant in CA2.

It should be noted that both the total number of immunopositive cells ($N_+$) and the number of intensely stained cells (Ni) was expressed as a percentage of the control. The number of cells intensely expressing Cu, Zn-SOD (III) and Trx-2 (II) was very small in the controls, but after the hypoxic exposure, the number of intensely stained cells reached a significant portion of the total number of immunopositive cells. Accordingly, the Ni to these antioxidants after hypoxia increased and reached values of hundreds or thousands of percent compared with the control.

## 6.2. The effect of preconditioning on the expression of antioxidant proteins after severe hypoxia

According to our data, preconditioning significantly increased the expression of Trx-1, Trx-2, Mn-SOD and Cu, Zn-SOD during the early period (3 h) after subsequent severe hypoxia in the hippocampus compared with non-preconditioning (I-III; Stroev *et al.*, 2005) (Table 14).

As already noted, the sharp increase in free radical production induced by hypoxia and subsequent reoxygenation causes severe functional and structural damages in cells and can lead to cell death by apoptosis or necrosis. In both cases (Lewen *et al.*, 2001; Sims, Anderson, 2002; Sugawara *et al.*, 2002), the key step in cell death is the release of cytochrome *c* from mitochondria. In the case of apoptosis, the released cytochrome *c* forms a complex with Apaf-1 and procaspase-9, which leads to the transformation of the procaspase to active caspase-9 (Bratton *et al.*, 2010; Reubold, Eschenburg, 2012; Yuan, Akey, 2013). Apaf-1 and caspase-9 play an important role in the realisation of the

cytochrome *c*-dependent apoptotic programme (Zou *et al.*, 1997, 1999; Hakem *et al.*, 1998; Kuida *et al.*, 1998; Würstle *et al.*, 2012; Marek, 2013). Initiator caspase-9 activates then effector (executioner) caspase-3 (Li *et al.*, 1997; Yuan, Yankner, 2000; Chan, 2001; Riedl *et al.*, 2007; Ola *et al.*, 2011) and effector caspases-6 and -7 (Slee *et al.*, 1999), which leads to cell death by apoptosis. Initiator caspase-9 via activation of effector caspase-3 can also activate initiator caspases-2, -8, and -10 that probably represents the additional feedback pathway for amplification of apoptotic signal (Slee *et al.*, 1999). It is known that antioxidant systems, including the proteins of the Trx family (Sachi *et al.*, 1995; Andoh *et al.*, 2002b; Ueda *et al.*, 2002), Mn-SOD (Fujimura *et al.*, 1999a; Noshita *et al.*, 2001) and Cu, Zn-SOD (Fujimura *et al.*, 2000; Sugawara *et al.*, 2002) prevent the release of cytochrome *c* from mitochondria into the cytosol.

The critical period for cytochrome *c* release and consequently for the start of the apoptotic programme is the earliest period after reoxygenation/reperfusion – the first 2-4 h after the end of hypoxic exposure (Fujimura *et al.*, 2000). Nevertheless, the expression of antioxidants in these early periods that determine the death or survival of cells still remains poorly studied. Expression of Cu, Zn-SOD and Mn-SOD in the first hours after hypoxia/ischaemia has been studied only in a few works in ischaemic models (Ohtsuki *et al.*, 1993; Liu *et al.*, 1993a, 1994a; Kato *et al.*, 1995). The expression of Trx-1 has been studied in a model of focal ischaemia. A decrease in Trx-1 expression in the ischaemic core and an increase in the border region were found (Takagi *et al.*, 1998a, b; Hattori *et al.*, 2002). Any data on the effect of hypoxia or ischaemia on the expression of Trx-2, which was cloned in 1997 (Spyrou *et al.*, 1997), including during the first hours after exposure, were absent from the literature up to our study (II). The early expression of Trx-2 in model of transient cerebral ischaemia and reperfusion has been studied only in 2010 (Hwang *et al.*, 2010).

We showed an increase in the expression of the antioxidants studied (except Cu, Zn-SOD) after non-preconditioned severe hypobaric hypoxia/reoxygenation during the early period (I-III; Stroev *et al.*, 2005), which is critical for apoptosis induction (Fujimura *et al.*, 2000). It is important to note that preconditioning significantly enhanced this increase (or in the case of Cu, Zn-SOD, significantly induced the expression) precisely at this critical period (I-III; Stroev *et al.*, 2005). This enhancement of antioxidant expression induction

by preconditioning in the early hours after reoxygenation was probably one of the essential mechanisms of hypoxic tolerance induced by the preconditioning of neurons.

Reduction in the expression of the antioxidants at the 24 h time-point compared with the 3 h time-point in preconditioned animals (Table 13) could be explained by the fact that this period, apparently, is not critical for the initiation of the apoptotic programme, and the adaptive stress response induced by preconditioned severe hypoxia had begun to fade. At the same time, in non-preconditioned animals for which hypoxia was apparently much more severe stressor, the expression of the antioxidants lasted longer, and the disappearance of the induced effect was slower. Perhaps, this may reflect less adaptive nature of the response.

In connection with this observation, it was interesting to pay particular attention to the changes in the expression of the antioxidants in the cells of the *dentate gyrus*, which is the most resistant area of the hippocampus to hypoxia/ischaemia (Abe *et al.*, 2004). In the DG, the differences in the number of immunoreactive cells between non- and preconditioned rats were smaller than in more sensitive areas of the hippocampus (I-III; Stroev *et al.*, 2005). It appears that this distinction between the DG and the more sensitive hippocampal areas indicated that the stress response to non-preconditioned severe hypoxic exposure in the DG (compared with other hippocampal regions) was more similar to the adaptive response observed in preconditioned rats. This finding correlated well with the fact of the relatively high resistance of this area to hypoxia. At the 24 h time-point in the DG, a substantial (more noticeable than in the other areas) decrease in the Ni to Trx-2 and Cu, Zn-SOD occurred in preconditioned rats compared with non-preconditioned animals (Fig. 6, 8; Table 15). Thus, the reduction in the Ni at 24 h on the background of a relatively stable $N_+$ was observed in this most resistant area of the hippocampus. Accordingly, we concluded that a substantial decrease in the Ni at 24 h correlated with the relatively adaptive character of the response to hypoxia. This observation was interesting to compare with the following specific feature of the dynamics of Trx-2 expression.

A characteristic feature of the expression of Trx-2 was the fact that the trend in the changes in the total number of immunoreactive cells did not always coincide with the trend in the changes in the intensely stained cells. In some areas of the hippocampus, these two parameters of the Trx-2 expression could change in different directions (II).

Such opposite directions for changes in the $N_+$ and Ni after non- and preconditioned severe hypoxia were not observed in the 3 other investigated antioxidants. The possibility of such a mixed character of expression determined the need for quantitative methods of analysis because simple qualitative assessments of the overall protein expression would not detect such details. At 24 h after non-preconditioned severe hypoxia in CA1, the total number of cells immunoreactive to Trx-2 ($N_+$) was decreased, but the number of cells actively expressing Trx-2 (Ni) was increased compared with the control (Table 9). In contrast, preconditioning at the same time-point significantly increased the $N_+$ but significantly reduced the Ni in the CA1 and CA2 areas compared with non-preconditioned animals (Table 15).

It can be assumed that the overall increase in the number of cells moderately expressing antioxidants is an adaptive response, whereas the long-term and high levels of expression in individual cells, especially against the background of reduction in the total number of expressing cells, was perhaps an indication of damage. This assumption was in accordance with the above-noted pattern: a significant reduction in the Ni in preconditioned rats at 24 h (not only for Trx-2, but also for other antioxidants) in the DG as the most resistant area of the hippocampus compared with areas that were more sensitive to hypoxia.

The characteristic and exclusive feature of the expression of Cu, Zn-SOD (III) was the fact that it was not induced at the 3 h time-point after reoxygenation in non-preconditioned rats unlike the other investigated antioxidants. Preconditioning in this case not only enhanced, but also caused the induction of antioxidant expression as a response to severe hypoxia at the early period after reoxygenation. In certain brain structures of preconditioned rats, the expression of Cu, Zn-SOD continued to increase at 24 h (compared with the 3 h time-point), whereas the expression of other antioxidants at this time in most cases was reduced. Thus, the lag in the reaction of Cu, Zn-SOD expression compared with other antioxidants that was noted in the analysis of non-preconditioned hypoxia was also manifested in the analysis of the preconditioning effect.

## 6.3. The effect of 3 sessions of mild hypoxia on the expression of antioxidant proteins (IV-VII)

Thus, as was shown above, the neuroprotective effect of preconditioning in cases of subsequent severe hypoxia was associated with a significant increase in the expression of the antioxidants at the very early (3 h) period after the end of severe hypoxia (I-III; Stroev *et al.*, 2005), which is critical for the initiation of the delayed death of neurons (Fujimura *et al.*, 2000).

However, the mechanism of this effect was not quite clear. The increased content of endogenous antioxidants at 3 h after severe hypoxia in preconditioned animals compared with non-preconditioned ones could be the result of either their accumulation during preconditioning before the severe impact or modification of the reaction to severe hypoxia itself. To check these two alternative hypotheses, we studied the expression of antioxidants at 3 h and 24 h after a 3 sessions of mild hypoxia itself, i.e., in the interval between the end of preconditioning and the beginning of a severe impact (IV-VII).

Based on both data in the literature and our own results on the effects of non- and preconditioned severe hypoxia, it can be assumed that all hypoxic exposures (adaptive as well as moderately damaging), excepting only the extremely severe variants, cause the defensive reactions associated with increased expression of the antioxidants.

We assumed that 3 sessions of mild hypoxia, i.e., preconditioning itself without a subsequent severe hypoxia, would also increase the expression of endogenous antioxidant proteins. We assumed also that the significant increase in antioxidant expression 3 h after a preconditioned severe hypoxia was a result of a summation of the up-regulation effects of both severe hypoxia and preconditioning individually. However, the results presented in this work refuted these assumptions. It was found that 3 sessions of mild hypoxia did not increase but, in many cases, instead reduced the expression of Trx-1 (V), Trx-2 (VII) and Cu, Zn-SOD (VI) in hippocampal neurons of rats at the starting time-point of the severe hypoxia.

At 3 h after the last (third) session of mild hypoxia, the expression of the antioxidant proteins in most cases had no statistically significant differences compared with the control. Only in a few cases there was a statistically significant increase (both SODs in

CA1) in the total number of immunoreactive cells ($N_+$) or a significant decrease (Trx-1 in CA3 and Trx-2 in CA1) both in the total number of immunoreactive cells ($N_+$) and the number of intensely labelled cells (Ni). In the other cases studied, the differences compared with the control were not statistically significant (IV - VII).

However, at the 24 h time-point, which was the starting point of the severe hypoxia in the previous series of experiments, the expression of the antioxidants was significantly decreased in a number of hippocampal areas (V-VII). Thus, the preconditioning itself in many cases did not cause an increase but on the contrary caused a significant reduction both in the total number of cells expressing the antioxidants studied ($N_+$) and in the number of cells with a high level of expression (Ni). These results were quite unexpected, both because of the assumption made based on our previous data on the effect of preconditioning on the expression of antioxidants after subsequent severe hypoxia and because of the paradigm established in the literature. How can these "paradoxical" results on the possibility of reducing of the expression of the antioxidants after mild adaptive hypoxia be logically interpreted?

It is known that ROS are not only damaging agents but are also mediators of many normal cellular processes (Dröge, 2002; Cross, Templeton, 2006; Valko *et al.*, 2007; Covarrubias *et al.*, 2008; Patten *et al.*, 2010; Brieger *et al.*, 2012; Maron, Michel, 2012; Miki, Funato, 2012; Ray *et al.*, 2012). In particular, ROS mediate the preconditioning-induced rescue pathways (Marini *et al.*, 1996; Rauca *et al.*, 2000; Ravati *et al.*, 2000, 2001; Rudiger *et al.*, 2003), and exposure to exogenous antioxidants such as N-acetyl cysteine, 2-mercaptopropionyl glycine, dimethyl thiourea, N-t-butyl-alpha-phenylnitrone, 2-hydroxyoestradiol and vitamin E before or during preconditioning at least partially abolished its beneficial effect (Baines *et al.*, 1997; Kaeffer *et al.*, 1997; Vanden Hoek *et al.*, 1998; Das *et al.*, 1999; Rauca *et al.*, 2000; Ravati *et al.*, 2000, 2001; Leak *et al.*, 2006). Thus, the increase in the ROS content during preconditioning, i.e., a moderate oxidative stress, is the necessary signal for the development of the protective effect. The suppression of ROS by exogenous antioxidants blocks the effect of preconditioning including, probably, the induction of the expression of endogenous antioxidants.

The explanation for our findings was probably that a similar feedback system could also operated in the mutual regulation of antioxidant genes and their protein products. Perhaps a change in functional state, which determines the ability of preconditioned cells

to more rapidly and intensely respond to severe hypoxia, was mediated by ROS. In our experiments, the low background proteins expression levels of both Trxs and Cu, Zn-SOD at 24 h after the first and third sessions of mild preconditioning hypoxia could have resulted in increased ROS levels at these time-points and most likely provided favourable conditions for ROS signal implementation. One could assume that the increased level of ROS during preconditioning by feedback mechanism induced changes in the expression of regulators of antioxidants because it is known that ROS can at least induce Trx mRNA transcription (Taniguchi *et al.*, 1996; Moon *et al.*, 2005). Obviously, this modification of the expression of antioxidant regulators or/and up-regulation of antioxidant mRNA transcription could later cause augmentation in the antioxidant protein expression levels after a subsequent severe hypoxia.

The character of the response of Mn-SOD expression (IV) was different in several ways. At 24 h after the last of the 3 sessions of mild hypoxia, the $N_+$ and Ni to Mn-SOD were significantly increased compared with the control in CA1, and the Ni was also significantly increased in the DG. At the same time, in CA2 and CA3, the $N_+$ and Ni were similar to control (IV). However, as was noted above, the effect of preconditioning after a subsequent severe hypoxia was manifested, on the contrary, in CA2 and CA3 but did not appear in CA1 or the DG (Stroev *et al.*, 2005). From a formal point of view, the response of Mn-SOD expression was different from the other antioxidants studied: the Mn-SOD expression after 3 sessions of mild hypoxia was increased or not changed (IV) but was never reduced as was the case for Cu, Zn-SOD and both Trxs (V-VII) in some areas.

However, despite this difference, the result confirmed the same fundamental conclusion that the neuroprotective effect of preconditioning during the early stages (in those areas where it is found, i.e., in CA2 and CA3) after severe hypoxia was caused not by the accumulation of antioxidant proteins during preconditioning, but rather it was due to modification of the reaction to severe hypoxia. In contrast, in areas where preconditioning by moderate hypoxia increased the expression of Mn-SOD at the starting time-point of the severe hypoxia (i.e., in CA1 and the DG), the effect of preconditioning on its expression after severe hypoxia was absent.

# 6.4. The effect of 1 session of mild hypoxia on the expression of antioxidant proteins (VII-X)

In our experimental model, it has been shown that resistance to a subsequent severe hypoxia is induced by 3 sessions but not by 1 session of preconditioning (Churilova *et al.*, 2012). It also has been found that the 3 sessions of preconditioning by mild hypobaric hypoxia *in vivo* inhibits the development of the abnormal "calcium overload" in brain slices in response to a subsequent 10 min of anoxia *in vitro*, while 1 session of preconditioning does not provide the same neuroprotective effect (Semenov et al, 2004). This finding was in agreement with the literature (Kitagawa *et al.*, 1991). In this regard, it was interesting to analyse the effect of 1 session of mild hypoxia on the expression of endogenous protein antioxidants.

At the 3 h and at the 24 h time-points, there were a number of statistically significant differences between the effects of 1 session and 3 sessions of mild hypoxia on the expression of both Trxs and both SODs (VII-X). Nevertheless, we observed a general trend toward a decrease in the expression of both Trxs and Cu, Zn-SOD in hippocampal neurons at 24 h after both 1 session and 3 sessions of mild hypoxia (VII, IX, X). Thus, although 3 sessions of preconditioning yielded a neuroprotective effect and 1 session of preconditioning was insufficient, their effects on the levels of antioxidant protein expression in most cases were more or less similar and had the same direction. This fact confirmed the earlier conclusion that the neuroprotective effect of preconditioning was not caused by achievement of the specific "baseline" level of antioxidant protein expression at the starting time-point of a subsequent severe hypoxia but by a transition of the neuronal cells to a particular state of readiness for rapid and intense expression of antioxidants in response to a heavy stress load.

1 session of hypoxia is the first stage of treatment with 3 sessions of hypoxia. It is likely that there are no significant differences in antioxidant expression at the time-point 24 h after the beginning of the first session of mild hypoxia (that is the starting point of second session in case of preconditioning by 3 sessions of mild hypoxia) and at the time-point 24 h after the end of the 1 session of mild hypoxia (which was studied). Given this assumed lack of difference, we could consider the changes in the expression of the

antioxidants at the 4 time-points studied (3 h after 1 session, 24 h after 1 session, 3 h after 3 sessions and 24 h after 3 sessions) as a continuous dynamic process. These changes in the expression of the antioxidants were repeated more or less similarly after the first and third sessions of hypoxia (VII-X). It is possible that this periodic recurrence was the factor that converted the cell to the functional state of preparedness for a rapid response of the antioxidant systems to a severe hypoxia. The molecular mechanism of this modification of the neuronal cell functional state could be associated with regulation at the level of expression of the relevant genes or with additional factors that specifically regulate the translation of antioxidant proteins from already-existing mRNA.

It was interesting to compare this observation (Stroev *et al.*, 2008, 2009) with data that were obtained later in a model of transient cerebral ischaemia in gerbils (Hwang *et al.*, 2010) in which alternating increases and decreases in Trx-2 expression over time had also been shown. In our experiments, repetition of the periods of increased and decreased antioxidant expression was achieved by repetition of the moderate hypoxic exposures. In the research of Hwang and co-authors, the alternating pattern of increased Trx-2 expression at 30 min after reperfusion, decreased expression at 6 h after reperfusion and a renewed increase at 1 day after reperfusion occurred without the recurrence of the ischaemia. That is, the fluctuations in Trx-2 expression occurred as a spontaneous process caused by a single session of ischaemia. This difference and the differences in the experimental models that were used are certainly significant. However, the data of Hwang and co-authors (Hwang *et al.*, 2010) could be considered an indirect confirmation of our hypothesis (Stroev *et al.*, 2008, 2009) concerning the role of the alternating phases of increased and decreased antioxidant expression in the adaptive response of neurons to hypoxia and in the induction of a hypoxic-tolerant state in the brain.

## 6.5. The effect of non- and preconditioned severe hypoxia on the enzymatic activity of Cu, Zn-SOD (III)

In this study, it was shown that the enzymatic activity of Cu, Zn-SOD in the hippocampus, striatum and piriform cortex of rats was significantly reduced at 3 h after

non-preconditioned severe hypoxia (III). At the 24 h time-point, the activity approached the control values. In the neocortex, a noticeable reaction to hypoxia was not observed (III). The reduction in antioxidant enzyme activity during the early stages after reoxygenation, obviously, was a maladaptive and pathological reaction. As already mentioned, Cu, Zn-SOD prevents the release of cytochrome *c* and the secondary activator of caspase (Smac) from mitochondria into the cytosol (Fujimura *et al.*, 2000; Sugawara *et al.*, 2002) and blocks the cytochrome *c*-dependent apoptosis pathway. Thus, the decline in its activity against the background of the post-hypoxic overproduction of free radicals during the early stages after reoxygenation could contribute to the translocation of cytochrome *c* into the cytosol and lead to the activation of caspases. This hypothesis seems probable because a sharp decrease in the enzymatic activity of Cu, Zn-SOD was observed in our experiments at time-points that coincided with the induction of cytochrome *c* release (Fujimura et al., 2000).

The increase in the Cu, Zn-SOD protein expression compared with the control at 24 h after severe hypoxia (III) could most likely be considered a defensive reaction aimed at compensating for the reduced enzymatic activity. This protective reaction, however, was clearly insufficient and delayed in time because by the 24 h time-point, the basic mechanisms of cellular damage were already running. In this case, during the early stages after reoxygenation when the cell death programme was induced, the hyperproduction of superoxide anions was uncompensated.

On the contrary, in preconditioned rats at 3 h after hypoxia, the enzymatic activity in all of the studied structures (except neocortex) was markedly and significantly increased compared with not only the non-preconditioned animals but also with the controls (III). At the 24 h time-point, the activity was decreased and approached the control values (III). Thus, at the level of enzymatic activity, the protective mechanism of preconditioning appeared more evident than at the level of the protein expression of Cu, Zn-SOD. In this case, the role of preconditioning was, obviously, not only to enhance the neuroprotective reaction but also to switch the response from a significant suppression of antioxidant activity to a significant activation of this activity during the earliest period after reoxygenation, which is the critical time for apoptosis induction.

It seems that the regulation of Cu, Zn-SOD enzyme activity was related to its posttranslational modifications, which can occur significantly faster than the induction of

protein expression. At the level of enzymatic activity, the reaction developed within the first few hours after the exposure, but by the 24 h time-point, it had almost completely faded.

## 6.6. Protein and mRNA expression of antioxidants after non- and preconditioned severe hypoxia

It seems interesting to compare the data on the expression of antioxidant proteins after non- and preconditioned severe hypoxia presented in this paper (I-III; Stroev *et al.*, 2005) with the published data on the expression of their mRNAs obtained using the same hypoxic model. The mRNA expression levels in this model have only been investigated for the mitochondrial isoforms Mn-SOD and Trx-2 (Samoilov *et al.*, 2002).

The expression of Mn-SOD mRNA 3 h after non-preconditioned severe hypoxia did not change either in the neocortex, *dentate gyrus*, or the field of CA1. Preconditioning resulted in a marked increase in the expression of Mn-SOD mRNA during this period in CA1 and the neocortex, but not in the DG. At 24 h after non-preconditioned hypoxia, a marked increase in Mn-SOD mRNA expression was observed in CA1, but preconditioning inhibited this increase. In contrast, the expression was slightly reduced in the neocortex, but preconditioning increased it (Samoilov *et al.*, 2002).

Comparing these data (Samoilov *et al.*, 2002) with the results of our work (Stroev *et al.*, 2005), we noted that the induction of Mn-SOD protein expression occurred markedly earlier than the induction of the expression of its mRNA because at 3 h after non-preconditioned severe hypoxia, the protein expression in the hippocampus was significantly increased: the total number of immunopositive to Mn-SOD cells in CA1, CA2 and the DG was 1.2-1.6 times higher than in the controls and the number of only the intensely labelled cells in CA1, CA2 and CA3 was 1.4-2 times higher than in the controls (Fig. 7, Table 8). Thus, the temporal expression patterns of Mn-SOD immunoreactivity and the expression of its mRNA in various brain structures did not coincide. This lack of correlation between the expression of the gene and its product, which appeared at first sight to be paradoxical, was not actually unique. In particular, a

study on the changes in the levels of expression of the Mn-SOD gene and its protein product during ontogenesis showed that their expression patterns varied in different directions: the level of mRNA expression increased with age, but the level of protein expression fell (Sugaya *et al.*, 1996).

A similar scenario can be observed when comparing the expression of Trx-2 (II) and its gene (Samoilov *et al.*, 2002). The induction of Trx-2 mRNA in CA1 was noticeable only at 24 h after non-preconditioned hypoxia (Samoilov *et al.*, 2002), but the protein expression (both $N_+$ and Ni) in all of the hippocampal areas studied including CA1 was significantly increased compared with the control by as early as 3 h (II) (Fig. 6, Table 8). In the DG, the gene expression was increased at 24 h after non-preconditioned hypoxia compared with the 3 h time-point (Samoilov *et al.*, 2002). However, the expression of the protein product (Ni in the DG) was on the contrary markedly reduced at 24 h compared with the 3 h time-point (II) (Fig. 6, Table 10). Based on our own results and earlier publication by other authors (Samoilov *et al.*, 2002), it seems that the regulation of protein expression was significantly faster than of gene expression.

In this case the regulation probably occurred at the posttranscriptional level, most likely at the level of translation. However, the molecular mechanism of this regulation, particularly in the case of the early induction of protein expression without changes in its gene expression, remains unclear and represents an important and interesting research problem that requires further investigation. It is possible that this phenomenon may be related to the changes in mRNA localisation and the transfer of translation processing from the rough endoplasmic reticulum to polysomes. It may also be related to the effect of specific translation factors. For example, the heterogeneous ribonucleoprotein A18 (hnRNP A18) can play the role of such a posttranscriptional regulator in thioredoxin expression (Yang *et al.*, 2006). In contrast to most members of the hnRNP family, which are expressed constitutively, hnRNP A18 is induced by stress factors including hypoxia (Wellmann *et al.*, 2004). During stress, hnRNP A18 translocates from the nucleus to the cytosol. There, it binds with specific mRNA transcripts, including the mRNA of Trx. As a result, hnRNP A18 specifically enhances the activity of Trx translation (Yang, Carrier, 2001). It is possible that the translation speed of other antioxidant proteins is regulated in a similar way. As a result, the induction of protein synthesis may occur earlier than the increase in the expression of the corresponding mRNA.

## 6.7. Methodological limitations of the research and future directions

This work had several limitations. In particular, the mRNA expression levels of the antioxidant proteins were not examined. Therefore, the data on the mRNA expression levels were taken from the literature (Samoilov *et al.*, 2002) and were only available for the mitochondrial antioxidants Trx-2 and Mn-SOD. The specific regulators of the expression of the antioxidants such as hnRNP A18 were also not studied. It would be interesting and useful to conduct such a study for our understanding of the molecular mechanisms of hypoxic tolerance. However, such a study would be laborious. It would also be interesting to determine the expression of antioxidants at more than two time-points to more clearly understand the dynamics of the changes in expression. The immunocytochemical study of the expression of the antioxidant proteins could be supplemented by electrophoresis and Western blot analysis. However, it should be noted that the immunocytochemical method is significantly more informative. Thus, the addition of the Western blot method was not considered necessary for the purposes of this study. The enzymatic activity was only determined for Cu, Zn-SOD. It would also be interesting to study the enzymatic activity of Mn-SOD and the Trxs in the same experimental model.

In some cases, there is a discussion on the "background" against which the immunoreactivity of the cells was determined using immunocytochemistry. The level of staining of a slice outside the visible cell bodies was taken as the background (zero value) in this method. The subject of criticism is that such "backgrounds" may have contained not only the extracellular space but also axons, dendrites and sometimes glial cells or their fragments. Accordingly, these "backgrounds" were different in the control and experimental samples. However, it would be incorrect to compare the staining of neurons in the experimental samples with the “background” of the control samples because the immune reactions in each sample occur with different intensities. It is a real problem, but it is an inevitable limitation of the conventional method of determining of a specific protein’s expression level. Fortunately, it can be reasonably assumed that this limitation in the method could lead to the concealment of differences between the experimental and

the control samples but not to the appearance of artefactual differences that do not exist in reality.

## 6.8. Summary

In summary, we can conclude the following main results. Severe hypobaric hypoxia and subsequent reoxygenation caused both adaptive (induction of the expression of antioxidants) and maladaptive (inhibition of Cu, Zn-SOD enzymatic activity) reactions in vulnerable structures in the rat brain. The adaptive compensatory mechanisms induced by non-preconditioned severe hypoxia, however, appeared in this case to be insufficient to effectively protect neurons against oxidative stress. Preconditioning significantly enhanced the adaptive response to subsequent severe hypoxia and eliminated the maladaptive: it amplified the increase of protein antioxidant expression and switched changes in the enzymatic activity of Cu, Zn-SOD from decrease to increase. The effect of preconditioning was essential during the early period of reoxygenation after a subsequent severe hypoxia (3 h time-point), wich is critical for the initiation of an apoptotic programme (Fujimura *et al.*, 2000). At 24 h after severe hypoxia, the differences in the expression of the antioxidants between the non- and preconditioned animals were reduced in many cases, and in some cases, in preconditioned rats at this time-point, the expression of the antioxidants began to decline.

There were specific features of the expression dynamics of the various antioxidants in different areas of the hippocampus and neocortex in the non- and preconditioned animals. In particular, up-regulation of Trx-2 expression induced by non- and preconditioned severe hypoxia was the most intense (maximum values of Ni). It appears that this mitochondrial thiol antioxidant plays a particularly important role at protecting neurons against oxidative stress.

The increase in the expression of the antioxidants after severe hypoxia in preconditioned rats compared with non-preconditioned was caused by modification in the response to severe hypoxia; it was not caused by the accumulation of antioxidants during preconditioning. The expression of 3 of the 4 antioxidant proteins studied (both Trxs and Cu, Zn-SOD) was decreased in all or, at least, in some of the studied

hippocampal areas compared with the control at 24 h after 3 sessions of preconditioning that is the start time-point of severe hypoxia. The expression of the fourth antioxidant (Mn-SOD) was increased at 24 h after preconditioning, but only in those hippocampal areas (CA1 and the DG) where the effect of preconditioning was absent at 3 h after subsequent severe hypoxia. In contrast, the enhancement of Mn-SOD expression by preconditioning at 3 h after a subsequent severe hypoxia was manifested in those hippocampal areas (CA2 and CA3) where preconditioning itself (before the severe impact) did not increase the Mn-SOD level. This result was one of the unexpected and totally new findings of the present research. To interpret this phenomenon, one can assume that the decrease in the antioxidant level during preconditioning is important for an optimal implementation of the ROS-mediated signal transduction that then modifies the response of the antioxidant systems to the subsequent severe hypoxia.

Another important and new result of this study was that 1 session of mild hypoxia modified the levels of the 4 antioxidants at 24 h comparably with 3 sessions of mild hypoxia. However, as has been shown in the same model (Churilova *et al.*, 2012), 3 sessions of mild hypoxia had a significant neuroprotective effect in case of a subsequent severe hypoxia, but 1 session of mild hypoxia had no such ameliorative effect. It could be speculated that the hypoxic tolerance depended not on the static levels of the antioxidants at the fixed time-point at the beginning of the severe hypoxia but on a dynamic process of changes in their expression during preconditioning. In the present work, we showed that the changes in antioxidant expression were repeated more or less similarly after the first and the third sessions of mild hypoxia. It is possible that namely dynamics of these repetitive changes were important for the mechanism of hypoxic tolerance induced by 3 sessions of preconditioning.

The results of this work could be of importance for finding and developing new ways to increase the adaptive capacity of brain neurons and to the prevention and treatment of neurodegenerative brain diseases induced by severe hypoxia/ischaemia.

# 7. Conclusions

The main aim of present work was to study the effects of non- and preconditioned severe hypobaric hypoxia and preconditioning itself on the expression of 4 protein antioxidants (Trx-1, Trx-2, Mn-SOD and Cu, Zn-SOD) in neurons of the rat brain. It was found that the expression of the antioxidants was increased following non-preconditioned severe hypoxia, but this increase was not sufficient or too late to prevent massive cell death. Preconditioning significantly enhanced the increase in antioxidant expression after subsequent severe hypoxia, but this effect was not caused by the accumulation of antioxidant proteins during or after the preconditioning itself.

The main findings of this study were as follows:

1. Non-preconditioned severe hypobaric hypoxia and subsequent reoxygenation induced the expression of Trx-1, Trx-2, Mn-SOD and Cu, Zn-SOD in neurons of the hippocampus and neocortex. Increased expression of Mn-SOD and both Trxs in the hippocampal neurons was observed during the early phase (3 h) after reoxygenation, while increased expression of Cu, Zn-SOD in the hippocampus was observed only at 24 h after reoxygenation. In the neocortex, a significant increase in the expression of both Trxs and both SODs was observed at 24 h, but only the expression of Trx-2 was increased at 3 h.

2. The preconditioning markedly enhanced the induction of the expression of Trx-1, Trx-2 and Mn-SOD and induced the expression of Cu, Zn-SOD in all or at least in some of the hippocampal areas at 3 h (but not at 24 h) after a subsequent severe hypoxia compared with non-preconditioned rats.

3. The enzymatic activity of Cu, Zn-SOD was markedly reduced in the hippocampus, striatum and piriform cortex of the rats at 3 h after non-preconditioned severe hypoxia. In preconditioned rats, in contrast, the enzymatic activity was significantly increased in these brain structures at 3 h after severe hypoxia compared with the control. At 24 h after both non- and preconditioned severe hypoxia, the activity more or less approached the control value.

4. 3 sessions of mild hypoxia itself did not increase the expression of Trx-1, Trx-2 or Cu, Zn-SOD in any area of the hippocampus at 24 h after the last session (i.e., at the starting point of the severe hypoxia). Moreover, in many cases, 3 sessions of mild hypoxia significantly reduced the expression of these antioxidants. At the same time, 3 sessions of mild hypoxia increased the expression of Mn-SOD, but only in the hippocampal areas in which there was no effect of preconditioning on Mn-SOD expression after a subsequent severe hypoxia. Thus, the neuroprotective effect of preconditioning was not caused by the accumulation of antioxidants to the starting point of the severe hypoxia but rather by modification of the neuronal response during or immediately after severe hypoxia.

5. 1 session of mild hypoxia itself also, in many cases, significantly reduced the expression of Trx-1, Trx-2 and Cu, Zn-SOD at the 24 h time-point. Thus, the effect of 1 session of mild hypoxia on the expression of the antioxidants was similar to the effect of 3 sessions of mild hypoxia. However, 1 session of mild hypoxia did not have a neuroprotective preconditioning effect in cases of a subsequent severe hypoxia. Thus, it appears that neuroprotective effect of preconditioning was not determined by achievement of any specific static increased or decreased "baseline" level of antioxidant protein expression at the starting time-point of a subsequent severe hypoxia but was caused by dynamic changes of the expression, which switched the neuronal cells into a functional state of readiness for rapid and intense expression of antioxidants in response to severe hypoxia.

6. In some cases, the changes in the expression of the antioxidants at the 3 h and 24 h time-points were repeated in a similar way after 1 session and 3 sessions of mild hypoxia. It is possible that namely these more or less regular repetitions in the changes in expression of the antioxidants modified the reaction to severe hypoxia and induced hypoxic tolerance.

Taken together, the changes in the expression and activity of the endogenous protein antioxidants are an important molecular mechanism of neuronal adaptation to acute hypobaric hypoxia. However, the patterns and dynamics of these changes are complex and non-linear. The process of neuronal adaption to hypoxia does not always correlate with increased levels of the antioxidants.

# Acknowledgements

The present study was carried out during the years 2001-2013 in the Department of Developmental Biology and the Department of Anatomy of the Medical School (School of Medicine) of the University of Tampere (Finland) and in the Laboratory of the Regulation of the Functions of Brain Neurons of the Federal State Budgetary Institution of Science "I.P. Pavlov Institute of Physiology" of the Russian Academy of Sciences (Koltushi – St. Petersburg, Russia). This work has been financially supported by the Medical Research Fund of Tampere University Hospital (PSHP-EVO grants 9A044, 9A181, 9B064, 9C056, 9C183, 9D053, 9E051, 9E174, 9F056, 9F219, 9G056, 9H059, 9J088, 9K090, 9L072 and 9M076), grants from Tampere University (Jan-Dec 2010; Jan-Feb 2011), the Finnish Centre for International Mobility (grant HA1 072 / L8261 48000 mk 01 T2V110 TO4707 OS5707), the Russian Science Support Foundation – Regional Public Fund for Assistance to Native Science (The programme "Outstanding scientists. Candidates and doctors of sciences, RAS", 2008-2009), the Russian Fund for Basic Research (projects 01-04-49575, 04-04-48242, 06-04-49650, 07-04-00664-a, 08-04-00363, 10-04-00371, 11-04-00677-a), Innovation grants INTAS (2001-0245, 05-1000007-422) and the St. Petersburg Competitive Centre of Basic Natural Sciences (grants M03-2.6K-297 and M04-2.6K-303). I thank also Kela and "Työ- ja Elinkeinotoimisto" for financial support during the period when I did not have any grant.

Finishing this work, I would like to express my deepest gratitude to Lord God, Who created me and gave me everything I needed for my life and work, and to the people who helped me during the present study or on my way preceding it.

First of all, I express my deepest gratitude to my supervisor, Professor Markku Tapio Miettinen (Pelto-Huikko) MD, PhD, for his patient guidance, participation in the organisation of my work, and his invaluable support of my study. In 2001, Professor Markku Miettinen gave me a happy opportunity to work in the Department of Developmental Biology that he headed. Since my the first day in Finland and through the final years of my work when he saved my ability to stay in Finland and to finish this thesis, he exercised an unfailing kindness and willingness to help in a difficult situation.

I express my deepest appreciation to my co-supervisor, Professor Mikhail Olegovich Samoilov MD, PhD, the head of the Laboratory of the Regulation of the Functions of Brain

Neurons in the I.P. Pavlov Institute of Physiology of RAS. He made a great conceptual and organisational contribution to the present study. All of these years, I have always felt his constant benevolent support, constructive interest and participation in my work.

I express my deep gratitude to the scientists on my follow-up committee, Professor Seppo Parkkila MD, PhD and Dr Heimo Syvälä PhD, for their support during the preparation of this thesis and to Mrs Sari Maarit Orhanen for organisational help and advice. I also thank Professor Seppo Parkkila for his kind permission to complete the present work in the laboratory that he heads and for his kind consent to be a Chairman (custos) at the defence of my thesis. I would like to express my deep appreciation to my reviewers, Professor Irma Elina Holopainen MD, PhD and Docent Jouni Saulo Ilmari Sirviö PhD. Their great work, interest, benevolent exactingness and constructive criticism, their valuable questions, comments, observations and suggestions gave me the opportunity to significantly improve the text of this thesis and the presentation of my data. I am grateful to Professor Jari Koistinaho MD, PhD for his kind consent to be my opponent during the defence of this thesis. I am grateful to the high-level professionals from the "American Journal Experts" who greatly improved the overall readability of this thesis.

I am very grateful to my colleagues and co-authors of the articles that formed my thesis: Dr Ekaterina Iosifovna Tyulkova, Dr Tatiana Sergeevna Gluschenko and Dr Elena Alexandrovna Rybnikova. Each of these high-level scientists has made an important and valuable contribution to this study. I also thank them for their goodwill, unselfishness and willingness to share knowledge and practical research experience.

I am deeply grateful to Dr Ling Yi L. Chang, Dr Yumiko Nishinaka, Professor Junji Yodoi and Professor Giannis Spyrou for kindly providing me with antibodies for this research and to Mr Ivan Anatolievich Tugoy for the wonderful computer programme "Morphix", which was created by him especially for my work.

I want to thank and acknowledge Mrs Ulla-Margit Jukarainen, Mrs Elena Viktorovna Aksenova and Mrs Tatiana Evgenievna Omelyanenko for excellent technical assistance and for their permanent goodwill and willingness to help.

I thank Mrs Arja Ahola, Mrs Hilkka Mäkinen, Professor Timo Ylikomi MD, PhD, Dr Nadežhda Yurievna Nazarova PhD, Mr Reza Zolfaghari Emameh MSc and all my the other colleagues in the Anatomy Department for creating a friendly working atmosphere. I thank my colleagues in the Laboratory of the Regulation of the Functions of Brain Neurons, Dr Dmitriy Germanovich Semenov, Dr Larisa Nikolaevna Grinkevich, Dr Ksenia

Aleksandrovna Baranova, Dr Dmitriy Yurievich Romanovskiy, Dr Larisa Ivanovna Pavlinova, Dr Irina Alexandrovna Gerasimova, and all of the others, for the friendly work environment and productive cooperation. I thank my colleagues from other laboratories of the I.P. Pavlov Institute of Physiology of RAS with whom I had the pleasure to carry out collaborative investigations in previous years: Dr Ludmila Anatolievna Vataeva, Dr Alexander Ivanovich Vaido, Dr Natalia Viktorovna Shiryaeva, Professor Nina Georgievna Lopatina and Professor Vladimir Alexandrovich Otellin.

Taking this opportunity, I would like to thank all of the scientists, high-level specialists and brilliant experts who participated in the preliminary discussion of my work and who have made valuable suggestions and comments: Dr Faina Evgeniena Putilina, Professor Alexander Vartanovich Arutyunyan, Professor Mikhail Alexandrovich Flerov, Dr Igor Alexandrovich Žhuravin, Dr Tatiana Gennadievna Sazontova, Professor Yuriy Vladimirovich Arkhipenko, Professor Natalia Dmitrievna Eschenko, Dr Olga Georgievna Chivileva, Dr Natalia Borisovna Saulskaya, Professor Oleg Semenovich Sotnikov, Dr Kirill Nikolaevich Dudkin, Dr Andrey Andreevich Gruzdkov, Dr Vyacheslav Timofeevich Shuvaev, Professor Ludmila Dmitrievna Lukyanova, Dr Ideya Nikolaevna Yanvaryova, Dr Rimma Ivanovna Kovalenko, Dr Leonid Georgievich Khaspekov, Dr Vladimir Pavlovich Nikitin, Professor Vladimir Adamovich Kulchitskiy, Dr Natalia Fedorovna Avrova, Dr Natalia Nikolaevna Nalivaeva, Dr Svetlana Alexandrovna Plesneva, Professor Yuriy Petrovich Pushkarev, Dr Nikolay Grigorievich Kamyshev, Professor Ilya Vasilievich Viktorov, Dr Alexander Evgenievich Chuykin, Dr Irina Pavlovna Butkevich, Dr Yuriy Nikolavich Andrianov, Dr Ruben Sergeevich Arutyunyan, Dr Vera Vasilievna Egorova, Professor Gennadiy Arkadievich Kulikov, Professor Valeriy Pavlovich Lebedev, Dr Evgeniy L'vovich Patkin, Dr Sergei Georgievich Tsikunov, Professor Mikhail Trofimovich Gengin, Professor Vladimir Vyacheslavovich Sherstnev, Professor Natalia Valerievna Gulyaeva, Professor Anthony J. Turner, Professor Jerzy W. Łazarewicz and all of the others.

I am grateful to the editors and referees of all of the journals where my articles have been accepted and published, especially to the editors and referees of "Acta Neurobiologiae Experimentalis (Warsaw)", "Neurochemistry (Moscow)" and "Morphology (St. Petersburg)" who published my results even though, at that time, they seemed too disputable and paradoxical and were rejected on this basis by several editorial boards.

I thank Dr Irina Mikhailovna Podkletnova (Djatchkova) and Dr Andrey Grigoryevich Taranukhin for their help in adapting to life in Finland.

My sincere thanks to Professor Gustavo Zubieta-Castillo (Sr.), Professor Gustavo R. Zubieta-Calleja and to the entire Zubieta family for organising the wonderful Chronic Hypoxia Symposium in August 2008 in La Paz and for the warm, hearty welcome that they have gave us. It was one of the most memorable and successful scientific conferences of my life, and it provided me with rich opportunities for fruitful discussions and to establish useful contacts with colleagues from all around the world in an unforgettable and friendly atmosphere.

My special thanks and deep appreciation to the people who were my research supervisors during my school and student years and who were my teachers during my first steps in science: Valentina Alekseevna Kolosova, Alexandra Valentinovna Sulyalina, Dr Alexey Valerievich Smirnov, Dr Natalia Valerievna Lentsman, and Dr Tamara Efimovna Raize who was my supervisor for my Bachelor's and Master's theses.

I want to warmly acknowledge the excellent lecturers, the teachers of workshops and the benevolent administrators of St. Petersburg State University who were my teachers and to whom I am greatly indebted: Dr Andrey Alexandrovich Dobrovolskiy, Dr Andrey Ekskustadianovich Vishnyakov, Dr Natalia Borisovna Balashova, Professor Elena Evgenievna Kovalenko, Professor Boris Vasilievich Gromov, Professor Alexandra (Alla) Davydovna Kharazova, Professor Olga Igorevna Podgornaya, Dr Alexander Vitalievich Polevschikov, Dr Irina Sergeevna Antonova, Professor Zoya Irinarkhovna Krutetskaya, Dr Nadežhda Gennadievna Andreeva, Professor Marina Pavlovna Chernysheva, Dr Alexey Vladimirovich Grishankov, Professor Dmitriy Konstantinovich Obuhov, Dr Mikhail Nikolaevich Molitvin, Dr Oleg Yurievich Chaga, Professor Archil Karpezovich Dondua, Dr Maxim Pavlovich Baranov, Professor Valentin Germanovich Borkhvardt, Professor Alexander Sergeevich Batuev, Dr Yuriy Alexandrovich Yunatov, Professor Evgeniy Georgievich Skvortsevich, Dr Susanna Yuvenalievna Tumanova, Dr Galina Pavlovna Sokolova, Dr Georgiy Georgievich Volskiy, Dr Ludmila Mikhailovna Osadchaya, Dr Valentina Pavlovna Goncharova, Dr Alexandr Alfredovich Myul'berg, Dr Andrey Yurievich Lyanguzov, Professor Nadežhda Vladimirovna Kuleva, Dr Natalia Evgenievna Voinova, Dr Florentina Alexeevna Belinskaya, Dr Olga Vyacheslavovna Galkina, Dr Alexey Alexandrovich Sazanov, Dr Elena Yurievna Dmitrieva, Dr Svetlana Gennadievna Averina, Dr Valeriya Frantsevna Sinitsina, Dr Tatiana Vasilievna Sergovskaya, Dr Andrey Borisovich Savchenko, Dr Sergei Gennadievich Kuznetsov, Dr Ivan Dmitrievich Kvasov, Dr Ivan Alexeevich Tikhomirov, Dr Pavel Mikhailovich Nikolaev, Dr Alexander Valentinovich Žhuk,

Dr Alexey Alexeevich Zavarzin, Dr Vladimir Igorevich Gusarov, Dr Alexey Valerievich Kupriyanov, Dr Georgiy Sergeevich Slyusarev, Professor Andrey Igorevich Granovich, Dr Alexander Vasilievich Bardin, Dr Alexey Valerievich Poloskin, Dr Vadim Mikhailovich Khaitov, Dr Alexander Mikhailovich Gorbushin, Dr Larisa Vladimirovna Barabanova, Dr Tatiana Germanovna Aksenova, Professor Natalia Vladimirovna Chežhina, Dr Ludmila Mikhailovna Kuznetsova, Dr Yuriy Dmitrievich Mikhailov, Dr Dina Nikolaevna Nikolaeva, Professor Vladimir Anatolievich Prozorovskiy, Dr Andrey Viktorovich Shvidkiy, Dr Vladimir Evgenievich Kholmogorov, Dr Natalia Mikhailovna Saltykova, Dr Sergei Alexeevich Kovalev, Dr Valeriy Vladimirovich Bykov, Professor Fedor Fedorovich Vyakkerev, Dr Alexander Grigorievich Konfisakhor, Dr Vsevolod Vladimirovich Zelchenko, Professor Nelli Vladimirovna Kozyreva, Dr Tatiana Vadimovna Burkova, Dr Sergei Mikhailovich Žhestokanov, Dr Maxim Vladimirovich Belkin, Professor Andrey Yurievich Prokopiev, Professor Sergei Egorovich Fedorov, Professor Dmitriy Glebovich Savinov, Professor Abram Davidovich Stolyar, Dr Alexander Mikhailovich Zakharov, Dr Ariadna Alexandrovna Petrova, Dr Svetlana Vladimirovna Shershneva, Dr Alexander Nikolaevich Dmitriev, Professor Yuriy Germanovich Akimov, Dr Sergei Nikolaevich Korotkov, Professor Ruslan Vasilievich Kostyuk, Professor Vladimir Germanovich Burkov and many others. Unfortunately, it is not possible to mention everyone who certainly deserves it, including all of the people who generously shared with me by their knowledge and provided me organisational support in my student years.

I am deeply grateful to my teachers from the Anichkov Lyceum (St. Petersburg): Nina Valerievna Guschina, Ilya Alexandrovich Chistyakov, Dr Evgeniy Georgievich Krasnodembskiy, Vladimir Borisovich Volovik, Alexandra Nikolaevna Simankova, Sergei L'vovich Ilyin, Dr Andrey Valentinovich Petrov, Dr Vladimir Romanovich Arseniev, Dr Ludmila Petrovna Sergeeva, Dr Dmitriy Nikolaevich Kopelev, Dr Olga Alexeevna Scheglova, Valentina Nikolaevna Mazurina, Alexander Mikhailovich Bianki, Galina Viktorovna Yakovleva, Ludmila Viktorovna Makhova, and Nikolay Fedorovich Trubitsyn, the director of the Lyceum. My two years at the Anichkov Lyceum were instrumental in all of my subsequent studies and scientific work, and the teachers of the Lyceum were very influential in my development. I would also like to thank my school teachers Valeriy Genrikhovich Suhanovskiy, Vera Dmitrievna Stepanovskaya and Ksenia Petrovna Dementyeva, the teachers of the St. Petersburg City Palace of Youth Creativity – Andrey Alexeevich Burov, Nina Yakovlevna Masharskaya and Dr Marina Alexandrovna Volkova, and my aunt Valentina

Borisovna Sirotina, who first showed me a work in a cytological laboratory during my school years.

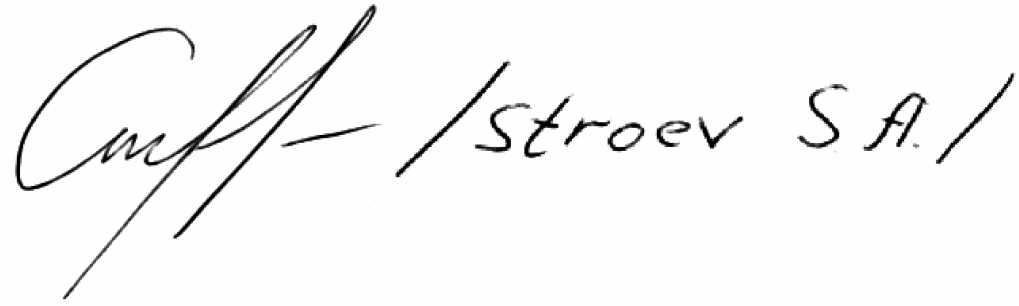
I take this opportunity to thank the musicians of the folk-rock group "Rada & Ternovnik" ("Rada and the Blackthorn") and specifically Radislava Alexandrovna Anchevskaya whose wonderful songs saved me from depression and gave me back the ability to continue my work.

I thank all of the people whose company lifted my morale and spirits, who helped me or provided me moral support during different periods of my life, or with whom I have had fruitful cooperation: my school friends Sergei Borisovich Nazartsev and Pavel Evgenievich Rezvov; my student friends and comrades from the University Dr Alexander Sergeevich Kukalev, Dr Ksenia Vladimirovna Karpisheva, Konstantin Eduardovich Martens, Nikita Nikolaevich Shumov, Alexey Yurievich Ibragimov, Dr Tatiana Pavlovna Petrova, Nadežhda Vladimirovna Nikolaeva, Petr Nikolaevich Gamanilov, Vladimir Alexandrovich Nosov, Alexander Yurievich Karyakin, Mikhail Avenirovich Savinov, Dr Nikolay Viktorovich Fedorov and many others; my colleague in interdisciplinary studies Dr Ilya Borisovich Gubanov; Ekaterina Konstantinovna Mashkova for her interesting lectures and hospitality; my good comrades in travels and tourism Petr Mikhailovich Knopf, Natalia Konstantinovna Zaychikova, Denis Valerievich Gorenoy, Dr Alexey Sergeevich Andreev, Alexey Vladimirovich Sigachev, Vladimir Alexandrovich Gerasimov, Sergei Viktorovich Nazarkin, Roman Sergeevich Osykin, Lev Mikhailovich Selivanov, Mikhail Anatolievich Evstratov, Galina Vikentievna Minina, Dr Vladimir Stanislavovich Sibirtsev, Irina Alexandrovna Andrianova, Petr Alexandrovich Baranov and many others; the friends and colleagues on my political activity Mikhail Mikhailovich Lalochkin, Anna Nikolaevna Vasiltsova (Klyueva), Alexey Mikhailovich Bogachev, Alexey Vladimirovich Makukhin, Kirill Dmitrievich Popov; the people who have provided me valuable aid in the publication of my social and political works and speeches Alexander Romanovich Kontarev, Yuriy Vasilievich Erdjakov, Andrey Yurievich Popov, Vyacheslav Prokofievich Suvorov, Dr Anatoliy Nikolaevich Samarin, Dr Sergei Viktorovich Gorin, Dr Igor Pavlovich Smirnov, Valeriy Vasilievich Khatyushin, Konstantin Yurievich Gordeev, Professor Elena Georgievna Borisova, Dionys Leonidovich Kaptar, Olga Vladimirovna Kozyreva, Evgeniy Nikolaevich Karamyshev, Sergei Valerievich Aristov, Dr Viktor Borisovich Shvedov, Dr Tatiana Nikolaevna Fedorova, Dr Valentin Evgenievich Semenov, Dr Anatoliy Dmitrievich Stepanov, Dr Andrey Leonidovich Vassoevich, Dr Vladimir Vladimirovich Vasilik, Alexander Evgenievich Molotkov, Dr

Alexander Leonidovich Kazin, Professor Elena (Helen) Vladimirovna Shelestiuk, Dr Alexey Vladislavovich Shustov; and my elder party and political comrades Vladimir Panasovich Brodovskiy, Anatoliy Egorovich Sterlikov, Nikolay Sergeevich Markov, Nikolay Evgenievich Popov, Vladimir Vasilievich Mamushkin, Professor Alexey Vasilievich Vorontsov, Dr Sergei Pavlovich Obukhov and Dr Sergei Ivanovich Vasiltsov. I also take this opportunity to thank Ekaterina Yurievna Bobrovskaya and Vladimir Nikolaevich Mertsalov for their help in a difficult situation. I thank the physicians who took care of my health, the drivers who helped me to regularly travel by hitchhiking between my Finnish and Russian places of work, and all the good people who helped me in any way.

In conclusion, I express my special and deepest gratitude to my mother Galina Afanasievna Stroeva (Sotnikova) who formed me as a person, taught me to see the beauty of the world, and who instilled in me in my childhood an interest in knowledge in general and in medicine, biology and chemistry in particular. She has supported me throughout my entire life, and each of my results includes her participation and her merit. I also express my gratitude to my grandmother Vera Alexandrovna Sotnikova (Poyarkova), to my grandfather Afanasiy Mikhailovich Sotnikov, and to all my ancestors who I know and who I do not know. Without them, I would not be here, and therefore, neither would this work.

Tampere, 12 December 2013




Sergei Alexandrovich Stroev (born 25.06.1977, Leningrad, USSR)
[Сергей Александрович Строев]

# References


1. Abe T., Takagi N., Nakano M., Furuya M., Takeo S. Altered Bad localization and interaction between Bad and Bcl-xL in the hippocampus after transient global ischemia. Brain Res. 2004. 1009(1-2): 159-168.

2. Abunasra H.J., Smolenski R.T., Morrison K., Yap J., Sheppard M.N., O'Brien T., Suzuki K., Jayakumar J., Yacoub M.H. Efficacy of adenoviral gene transfer with manganese superoxide dismutase and endothelial nitric oxide synthase in reducing ischemia and reperfusion injury. Eur J Cardiothorac Surg. 2001. 20(1): 153-158.

3. Adam-Vizi V. Production of reactive oxygen species in brain mitochondria: contribution by electron transport chain and non-electron transport chain sources. Antioxid Redox Signal. 2005. 7(9-10): 1140-1149.

4. Adam-Vizi V., Tretter L. The role of mitochondrial dehydrogenases in the generation of oxidative stress. Neurochem Int. 2013. 62(5): 757-763.

5. Agarkov F.T. Materials to explore of possible ways to improve the heat resistance of the organism. Materials of the conference on preblem of adaptation, training and other ways to increase of resistance of organism. Donetsk. 1960. 7-9. (In Russian)

6. Akamatsu Y., Ohno T., Hirota K., Kagoshima H., Yodoi J., Shigesada K. Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues. J Biol Chem. 1997. 272(23): 14497-14500.

7. Aketa S., Nakase H., Kamada Y., Hiramatsu K., Sakaki T. Chemical preconditioning with 3-nitropropionic acid in gerbil hippocampal slices: therapeutic window and the participation of adenosine receptor. Exp Neurol. 2000. 166(2): 385-391.

8. Akhavan M.M., Foroutan T., Safari M., Sadighi-Moghaddam B., Emami-Abarghoie M., Rashidy-Pour A. Prenatal exposure to maternal voluntary exercise during pregnancy provides protection against mild chronic postnatal hypoxia in rat offspring. Pak J Pharm Sci. 2012. 25(1): 233-238.

9. Alanen H.I., Williamson R.A., Howard M.J., Lappi A.K., Jantti H.P., Rautio S.M., Kellokumpu S., Ruddock L.W. Functional characterisation of ERp18, a new endoplasmic reticulum located thioredoxin superfamily member. J Biol Chem. 2003. 278(31): 28912-28920.

10. Altschmied J., Haendeler J. Thioredoxin-1 and endothelial cell aging: role in cardiovascular diseases. Antioxid Redox Signal. 2009. 11(7): 1733-1740.

11. Amundson S.A., Myers T.G., Fornace A.J. Jr. Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene. 1998. 17(25): 3287-3299.

12. Andoh T., Chiueh C.C., Chock P.B. Cyclic GMP-dependent protein kinase regulates the expression of thioredoxin and thioredoxin peroxidase-1 during hormesis in response to oxidative stress-induced apoptosis. J Biol Chem. 2003. 278(2): 885-890.

13. Andoh T., Chock P.B., Chiueh C.C. Preconditioning-mediated neuroprotection: role of nitric oxide, cGMP, and new protein expression. Ann N Y Acad Sci. 2002 a. 962: 1-7.

14. Andoh T., Chock P.B., Chiueh C.C. The roles of thioredoxin in protection against oxidative stress-induced apoptosis in SH-SY5Y cells. J Biol Chem. 2002 b. 277(12): 9655-9660.

15. Andoh T., Chock P.B., Murphy D.L., Chiueh C.C. Role of the redox protein thioredoxin in cytoprotective mechanism evoked by (-)-deprenyl. Mol Pharmacol. 2005. 68(5): 1408-1414.

16. Antico Arciuch V.G., Elguero M.E., Poderoso J.J., Carreras M.C. Mitochondrial regulation of cell cycle and proliferation. Antioxid Redox Signal. 2012. 16(10): 1150-1180.

17. Aon-Bertolino M.L., Romero J.I., Galeano P., Holubiec M., Badorrey M.S., Saraceno G.E., Hanschmann E.M., Lillig C.H., Capani F. Thioredoxin and glutaredoxin system proteins-immunolocalization in the rat central nervous system. Biochim Biophys Acta. 2011. 1810(1): 93-110.

18. Arnér E.S. Focus on mammalian thioredoxin reductases--important selenoproteins with versatile functions. Biochim Biophys Acta. 2009. 1790(6): 495-526.

19. Arnér E.S.J., Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem. 2000. 267(20): 6102-6109.

20. Arnér E.S., Holmgren A. The thioredoxin system in cancer. Semin Cancer Biol. 2006. 16(6): 420-426.

21. Arnoult D., Gaume B., Karbowski M., Sharpe J.C., Cecconi F., Youle R.J. Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization. EMBO J. 2003. 22(17): 4385-4399.

22. Aruoma O. Free radicals, oxidative stress, and antioxidants in human heals and disease. J Amer Oil Chem Soc. 1998. 75(2): 199-212.

23. Bai J., Nakamura H., Kwon Y.W., Hattori I., Yamaguchi Y., Kim Y.C., Kondo N., Oka S., Ueda S., Masutani H., Yodoi J. Critical roles of thioredoxin in nerve growth factor-mediated signal transduction and neurite outgrowth in PC12 cells. J Neurosci. 2003. 23(2): 503-509.

24. Baines C.P., Goto M., Downey J.M. Oxygen radicals released during ischemic preconditioning contribute to cardioprotection in the rabbit myocardium. J Mol Cell Cardiol. 1997. 29(1): 207-216.

25. Banasiak K.J., Haddad G.G. Hypoxia-induced apoptosis: effect of hypoxic severity and role of p53 in neuronal cell death. Brain Res. 1998. 797(2): 295-304.

26. Banerjee R. Redox outside the box: linking extracellular redox remodeling with intracellular redox metabolism. J Biol Chem. 2012. 287(7): 4397-4402.

27. Bannister J.V., Bannister W.H., Rotilio G. Aspects of the structure, function, and applications of superoxide dismutase. CRC Crit Rev Biochem. 1987. 22(2): 111-180.

28. Bao Q., Shi Y. Apoptosome: a platform for the activation of initiator caspases. Cell Death Differ. 2007. 14(1): 56-65.

29. Baranova K.A., Rybnikova E.A., Mironova V.I., Samoĭlov M.O. The hypoxic preconditioning effect upon expression of the NGFI-A transcription factor in the rat brain following an unavoidable stess in the "learned helpnessness" model. Ross Fiziol Zh Im I M Sechenova. 2009. 95(4): 405-416 (In Russian, summary in English).

30. Baranova K.A., Rybnikova E.A., Mironova V.I., Samoilov M.O. Effects of hypoxic preconditioning on expression of transcription factor NGFI-A in the rat brain after unavoidable stress in the "learned helplessness" model. Neurosci Behav Physiol. 2010. 40(6): 693-700.

31. Baranova K.A., Rybnikova E.A., Samoilov M.O. Antidepressant effect of hypoxic preconditioning is associated with modification of expression of transcription factor c-Fos in rat brain in response to unavoidable stress. Bull Exp Biol Med. 2012. 152(5): 564-567.

32. Barbashova Z.I. Tissue processes during the acclimatization to oxygen deficiency. In: Materials on the evolutionary physiology. Edited by L.A. Orbeli. Moscow, Leningrad. 1956. Volume 1: 12-35.

33. Barbashova Z.I. Acclimatization to hypoxia and its physiological mechanisms. Moscow, Leningrad. Publishing house of the Academy of Science of the USSR. 1960. 216 p.

34. Barbashova Z.I., Ginetsinskiy A.G. The effect of acclimatization on the intravital colourability of tissues. In: Materials on the evolutionary physiology. Edited by L.A. Orbeli. Moscow, Leningrad. 1956. Volume 1: 36-40.

35. Barhwal K., Hota S.K., Jain V., Prasad D., Singh S.B., Ilavazhagan G. Acetyl-l-carnitine (ALCAR) prevents hypobaric hypoxia-induced spatial memory impairment through extracellular related kinase-mediated nuclear factor erythroid 2-related factor 2 phosphorylation. Neuroscience. 2009. 161(2): 501-514.

36. Belizário J.E., Alves J., Occhiucci J.M., Garay-Malpartida M., Sesso A. A mechanistic view of mitochondrial death decision pores. Braz J Med Biol Res. 2007. 40(8): 1011-1024.

37. Berggren M., Gallegos A., Gasdaska J.R., Gasdaska P.Y., Warneke J., Powis G. Thioredoxin and thioredoxin reductase gene expression in human tumors and cell lines, and the effects of serum stimulation and hypoxia. Anticancer Res. 1996. 16(6B): 3459-3466.

38. Biaglow J.E., Miller R.A. The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy. Cancer Biol Ther. 2005. 4(1): 6-13.

39. Bianciardi P., Fantacci M., Caretti A., Ronchi R., Milano G., Morel S., von Segesser L., Corno A., Samaja M. Chronic in vivo hypoxia in various organs: hypoxia-inducible factor-1alpha and apoptosis. Biochem Biophys Res Commun. 2006. 342(3): 875-880.

40. Bidmon H.-J., Kato K., Schleicher A., Witte O. W., Zilles K. Transient increase of manganese-superoxide dismutase in remote brain areas after focal photothrombotic cortical lesion. Stroke. 1998. 29(1): 203-211.

41. Björnstedt M., Kumar S., Björkhem L., Spyrou G., Holmgren A. Selenium and the thioredoxin and glutaredoxin systems. Biomed Environ Sci. 1997. 10(2-3): 271-279.

42. Bolaños J.P., Moro M.A., Lizasoain I., Almeida A. Mitochondria and reactive oxygen and nitrogen species in neurological disorders and stroke: Therapeutic implications. Adv Drug Deliv Rev. 2009. 61(14): 1299-1315.

43. Boldyrev A.A. Carnosine. The biological significance and possible use in medicine. Moscow: Moscow State University Press, 1998. 320 pp. (In Russian)

44. Bordet R., Deplanque D., Maboudou P., Puisieux F., Pu Q., Robin E., Martin A., Bastide M., Leys D., Lhermitte M., Dupuis B. Increase in endogenous brain superoxide dismutase as a potential mechanism of lipopolysaccharide-induced brain ischemic tolerance. J. Cereb. Blood. Flow. Metab. 2000. 20(8): 1190-1196.

45. Bratton S.B., Salvesen G.S. Regulation of the Apaf-1-caspase-9 apoptosome. J Cell Sci. 2010. 123(Pt 19): 3209-3214.

46. Breckenridge D.G., Xue D. Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases. Curr Opin Cell Biol. 2004. 16(6): 647-652.

47. Brieger K., Schiavone S., Miller F.J. Jr., Krause K.H. Reactive oxygen species: from health to disease. Swiss Med Wkly. 2012. 142: w13659.

48. Brown D.M., Donaldson K., Borm P.J., Schins R.P., Dehnhardt M., Gilmour P., Jimenez L.A., Stone V. Calcium and ROS-mediated activation of transcription factors and TNF-alpha

cytokine gene expression in macrophages exposed to ultrafine particles. Am J Physiol Lung Cell Mol Physiol. 2004. 286(2): L344- L353.

49. Budanov A.V. Stress-responsive sestrins link p53 with redox regulation and mammalian target of rapamycin signaling. Antioxid Redox Signal. 2011. 15(6): 1679-1690.

50. Bulkley G.B. Free radical-mediated reperfusion injury: a selective review. Br J Cancer Suppl. 1987. 8: 66-73.

51. Caballero B., Tomás-Zapico C., Vega-Naredo I., Sierra V., Tolivia D., Hardeland R., Rodríguez-Colunga M.J., Joel A., Nevo E., Avivi A., Coto-Montes A. Antioxidant activity in Spalax ehrenbergi: a possible adaptation to underground stress. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2006. 192(7): 753-759.

52. Cadet J.L., Krasnova I.N. Cellular and molecular neurobiology of brain preconditioning. Mol Neurobiol. 2009. 39(1): 50-61.

53. Cain K. Chemical-induced apoptosis: formation of the Apaf-1 apoptosome. Drug Metab Rev. 2003. 35(4): 337-363.

54. Cain K., Bratton S.B., Cohen G.M. The Apaf-1 apoptosome: a large caspase-activating complex. Biochimie. 2002. 84(2-3): 203-214.

55. Calabrese V., Cornelius C., Dinkova-Kostova A.T., Calabrese E.J. Vitagenes, cellular stress response, and acetylcarnitine: relevance to hormesis. Biofactors. 2009. 35(2): 146-160.

56. Calabrese V., Cornelius C., Dinkova-Kostova A.T., Calabrese E.J., Mattson M.P. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. Antioxid Redox Signal. 2010. 13(11): 1763-1811.

57. Candelario-Jalil E., Mhadu N.H., Al-Dalain S.M., Martinez G., Leon O.S. Time course of oxidative damage in different brain regions following transient cerebral ischemia in gerbils. Neurosci Res. 2001. 41(3): 233-241.

58. Cao Z., Lindsay J.G., Isaacs N.W. Mitochondrial peroxiredoxins. Subcell Biochem. 2007. 44: 295-315.

59. Carmody R.J., Cotter T.G. Signalling apoptosis: a radical approach. Redox Rep. 2001. 6(2): 77-90.

60. Chae H.Z., Robison K., Poole L.B., Church G., Storz G., Rhee S.G. Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. Proc Natl Acad Sci U S A. 1994. 91(15): 7017-7021.

61. Chan P.H. Role of oxidants in ischemic brain damage. Stroke. 1996. 27(6): 1124-1129.

62. Chan P.H. Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab. 2001. 21(1): 2-14.

63. Chan P.H., Epstein C.J., Kinouchi H., Kamii H., Chen S.F., Carlson E., Gafni J., Yang G., Reola L. Neuroprotective role of Cu Zn-superoxide dismutase in ischemic brain damage. Adv Neurol. 1996. 71: 271-280.

64. Chan P.H., Kamii H., Yang G., Gafni J., Epstein C.J., Carlson E., Reola L. Brain infarction is not reduced in SOD-1 transgenic mice after a permanent focal cerebral ischemia. Neuroreport. 1993. 5(3): 293-296.

65. Chan P.H., Kawase M., Murakami K., Chen S.F., Li Y., Calagui B., Reola L., Carlson E., Epstein C.J. Overexpression of SOD1 in transgenic rats protects vulnerable neurons against

ischemic damage after global cerebral ischemia and reperfusion. J Neurosci. 1998. 18(20): 8292-8299.

66. Chen J., Gao Y., Liao W., Huang J., Gao W. Hypoxia affects mitochondrial protein expression in rat skeletal muscle. OMICS. 2012. 16(3): 98-104.

67. Chen J., Graham S.H., Zhu R.L., Simon R.P. Stress proteins and tolerance to focal cerebral ischemia. J Cereb Blood Flow Metab. 1996. 16(4): 566-577.

68. Chen Y., Cai J., Murphy T.J., Jones D.P. Overexpressed human mitochondrial thioredoxin confers resistance to oxidant-induced apoptosis in human osteosarcoma cells. J Biol Chem. 2002. 277(36): 33242-33248.

69. Chen Z., Siu B., Ho Y.-S., Vincent R., Chua C.C., Hamdy R.C., Chua B.H.L. Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. J Mol Cell Cardiol. 1998. 30(11): 2281-2289.

70. Chevari S., Chaba I., Sekeĭ I. Role of superoxide dismutase in cellular oxidative processes and method of its determination in biological materials. Lab. Delo. 1985. 11: 678-681 (In Russian).

71. Chiueh C., Lee S., Andoh T., Murphy D. Induction of antioxidative and antiapoptotic thioredoxin supports neuroprotective hypothesis of estrogen. Endocrine. 2003. 21(1): 27-31.

72. Choi D.W. Calcium: still center-stage in hypoxic-ischemic cell death. Trends Neurosci. 1995. 18(2): 58-60.

73. Chung J.Y., Yi J.W., Kim S.M., Lim Y.J., Chung J.H., Jo D.J. Changes in gene expression in the rat hippocampus after focal cerebral ischemia. J Korean Neurosurg Soc. 2011. 50(3): 173-178.

74. Churilova A.V., Glushchenko T.S., Samoĭlov M.O. Changes in hippocampal and neocortical rat neurons induced by different regimes of hypobaric hypoxia. Morfologiia. 2012. 141(1): 7-11 (In Russian, summary in English).

75. Churilova A.V., Rybnikova E.A., Glushchenko T.S., Tiul'kova E.I., Samoĭlov M.O. Effect of mild hypobaric hypoxia in the preconditioning regime on expression of pCREB and NF-kappaB transcription factors in the rat hippocampus before and after severe hypoxia. Morfologiia. 2009. 136(6): 38-42 (In Russian, summary in English).

76. Churilova A.V., Rybnikova E.A., Glushchenko T.S., Tyulkova E.I., Samoilov M.O. Effects of moderate hypobaric hypoxic preconditioning on the expression of the transcription factors pCREB and NF-kappaB in the rat hippocampus before and after severe hypoxia. Neurosci Behav Physiol. 2010. 40(8): 852-857.

77. Clarke P.G., Clarke S. Nineteenth century research on naturally occurring cell death and related phenomena. Anat Embryol (Berl). 1996. 193(2): 81-99.

78. Clarke P.G., Clarke S. Nineteenth century research on cell death. Exp Oncol. 2012. 34(3): 139-145.

79. Clemens J.A. Cerebral ischemia: gene activation, neuronal injury, and the protective role of antioxidants. Free Radic Biol Med. 2000. 28(10): 1526-1531.

80. Conrad M., Schick J., Angeli J.P. Glutathione and thioredoxin dependent systems in neurodegenerative disease: What can be learned from reverse genetics in mice. Neurochem Int. 2013. 62(5): 738-749.

81. Cooke M.S., Evans M.D., Dizdaroglu M., Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J. 2003. 17 (10): 1195-214.

82. Copin J.-C., Gasche Y., Chan P.H. Overexpression of copper/zinc superoxide dismutase does not prevent neonatal lethality in mutant mice that lack manganese superoxide dismutase. Free Radic Biol Med. 2000. 28(10): 1571-1576.

83. Corbett D., Crooks P. Ischemic preconditioning: a long term survival study using behavioural and histological endpoints. Brain Res. 1997. 760(1-2): 129-136.

84. Cory S., Adams J.M. The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer. 2002. 2(9): 647-656.

85. Costa D.C., Alva N., Trigueros L., Gamez A., Carbonell T., Rama R. Intermittent hypobaric hypoxia induces neuroprotection in kainate-induced oxidative stress in rats. J Mol Neurosci. 2013. 50(3): 402-410.

86. Covarrubias L., Hernández-García D., Schnabel D., Salas-Vidal E., Castro-Obregón S. Function of reactive oxygen species during animal development: passive or active? Dev Biol. 2008. 320(1): 1-11.

87. Coyle D.W., Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science. 1993. 262(5134): 689-695.

88. Crack P.J., Taylor J.M., de Haan J.B., Kola I., Hertzog P., Iannello R.C. Glutathione peroxidase-1 contributes to the neuroprotection seen in the superoxide dismutase-1 transgenic mouse in response to ischemia/reperfusion injury. J Cereb Blood Flow Metab. 2003. 23(1): 19-22.

89. Crapo J.D., Oury T., Rabouille C., Slot J.W., Chang L.Y. Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci USA. 1992. 89(21): 10405-10409.

90. Cregan S.P., Dawson V.L., Slack R.S. Role of AIF in caspase-dependent and caspase-independent cell death. Oncogene. 2004. 23(16): 2785-2796.

91. Cross J.V., Templeton D.J. Regulation of signal transduction through protein cysteine oxidation. Antioxid Redox Signal. 2006. 8(9-10): 1819-1827.

92. Cunnea P.M., Miranda-Vizuete A., Bertoli G., Simmen T., Damdimopoulos A.E., Hermann S., Leinonen S., Huikko M.P., Gustafsson J.A., Sitia R., Spyrou G. ERdj5, an endoplasmic reticulum (ER)-resident protein containing DnaJ and thioredoxin domains, is expressed in secretory cells or following ER stress. J Biol Chem. 2003. 278(2): 1059-1066.

93. Dai Y., Li W., Zhong M., Chen J., Liu Y., Cheng Q., Li T. Preconditioning and post-treatment with cobalt chloride in rat model of perinatal hypoxic-ischemic encephalopathy. Brain Dev. 2013. pii: S0387-7604(13)00156-3. doi: 10.1016/j.braindev.2013.04.007 [Epub ahead of print].

94. Damdimopoulos A.E., Miranda-Vizuete A., Pelto-Huikko M., Gustafsson J.-Å., Spyrou G. Human mitochondrial thioredoxin. Involvement in mitochondrial membrane potential and cell death. J Biol Chem. 2002. 277(36): 33249-33257.

95. Dana A., Jonassen A.K., Yamashita N., Yellon D.M. Adenosine A(1) receptor activation induces delayed preconditioning in rats mediated by manganese superoxide dismutase. Circulation. 2000. 101(24): 2841-2848.

96. Das D.K., Maulik N., Sato M., Ray P.S. Reactive oxygen species function as second messenger during ischemic preconditioning of heart. Mol Cell Biochem. 1999. 196(1-2): 59-67.

97. Das K.C., Das C.K. Thioredoxin, a singlet oxygen quencher and hydroxyl radical scavenger: redox independent functions. Biochem Biophys Res Commun. 2000. 277(2): 443-447.

98. Das K.C., Guo X., White C.W. Protein kinase Cδ-dependent induction of manganese superoxide dismutase gene expression by microtubule-active anticancer drugs. J Biol Chem. 1998. 273(51): 34639-34645.

99. Das K.C., Lewis-Molock Y., White C.W. Elevation of manganese superoxide dismutase gene expression by thioredoxin. Am J Respir Cell Mol Biol. 1997. 17(6): 713-726.

100. Das S., Khan N., Mukherjee S., Bagchi D., Gurusamy N., Swartz H., Das DK. Redox regulation of resveratrol-mediated switching of death signal into survival signal. Free Radic Biol Med. 2008. 44(1): 82-90.

101. Dash P.K., Blum S., Moore A.N. Caspase activity plays an essential role in long-term memory. Neuroreport. 2000. 11(12): 2811-2816.

102. Dawson V.L., Dawson T.M. Free radicals and neuronal cell death. Cell Death Differentiation. 1996. 3: 71–78.

103. Dayer R., Fischer B.B., Eggen R.I., Lemaire S.D. The peroxiredoxin and glutathione peroxidase families in Chlamydomonas reinhardtii. Genetics. 2008. 179(1): 41-57.

104. de Grey A.D. Reactive oxygen species production in the mitochondrial matrix: implications for the mechanism of mitochondrial mutation accumulation. Rejuvenation Res. 2005. 8(1): 13-17.

105. Dodson M., Darley-Usmar V., Zhang J. Cellular metabolic and autophagic pathways: traffic control by redox signaling. Free Radic Biol Med. 2013. 63: 207-221.

106. Donovan M., Cotter T.G. Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. Biochim Biophys Acta. 2004. 1644(2-3): 133-147.

107. Douen A.G., Akiyama K., Hogan M.J., Wang F., Dong L., Chow A.K., Hakim A. Preconditioning with cortical spreading depression decreases intraischemic cerebral glutamate levels and down-regulates excitatory amino acid transporters EAAT1 and EAAT2 from rat cerebal cortex plasma membranes. J Neurochem. 2000. 75(2): 812–818.

108. Drago F., Grassi M., Valerio C., Spadaro F., D'Agata V., Lauria N. Effects of vinburnine on experimental models of learning and memory impairments. Pharmacol Biochem Behav. 1990. 37(1): 53-57.

109. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002. 82(1): 47-95.

110. Duan C., Yan F., Song X., Lu G.W. Changes of superoxide dismutase, glutathione perioxidase and lipid peroxides in the brain of mice preconditioned by hypoxia. Biol Signals Recept. 1999. 8(4-5): 256-260.

111. Dubrovskaya N.M., Zhuravin I.A. Ontogenetic characteristics of behavior in rats subjected to hypoxia on day 14 or day 18 of embryogenesis. Neurosci Behav Physiol. 2010. 40(2): 231-238.

112. Dunn L.L., Buckle A.M., Cooke J.P., Ng M.K. The emerging role of the thioredoxin system in angiogenesis. Arterioscler Thromb Vasc Biol. 2010. 30(11): 2089-2098.

113. Ebrahimian T., Touyz R.M. Thioredoxin in vascular biology: role in hypertension. Antioxid Redox Signal. 2008. 10(6): 1127-1136.

114. Emerson M.R., Nelson S.R., Samson F.E., Pazdernik T.L. A global hypoxia preconditioning model: neuroprotection against seizure-induced specific gravity changes (edema) and brain damage in rats. Brain Res Prot. 1999. 4(3): 360-366.

115. Encinas J.M., Fernandez A.P., Salas E., Castro-Blanco S., Munoz P., Rodrigo J., Serrano J. Nitric oxide synthase and NADPH-diaphorase after acute hypobaric hypoxia in the rat caudate putamen. Exp Neurol. 2004. 186(1): 33-45.

116. Encinas J.M., Serrano J., Bentura M.L., Castro-Blanco S., Fernandez A.P., Rodrigo J. Nitric oxide system and protein nitration are modified by an acute hypobaric hypoxia in the adult rat hippocampus. J Neuropathol Exp Neurol. 2003. 62(8): 863-877.

117. Endo H., Kamada H., Nito C., Nishi T., Chan P.H. Mitochondrial translocation of p53 mediates release of cytochrome c and hippocampal CA1 neuronal death after transient global cerebral ischemia in rats. J Neurosci. 2006 a. 26(30): 7974-7983.

118. Endo H., Nito C., Kamada H., Nishi T., Chan P.H. Activation of the Akt/GSK3beta signaling pathway mediates survival of vulnerable hippocampal neurons after transient global cerebral ischemia in rats. J Cereb Blood Flow Metab. 2006 b. 26(12): 1479-1489.

119. Endo H., Saito A., Chan P.H. Mitochondrial translocation of p53 underlies the selective death of hippocampal CA1 neurons after global cerebral ischaemia. Biochem Soc Trans. 2006 c. 34(Pt 6): 1283-1286.

120. Eshchenko N.D. Energy metabolism in the brain. In: Biochemistry of brain. Ed. I.P. Ashmarin etc. St. Petersburg: St. Petersburg State University Publishing, 1999. p. 124-169. (In Russian).

121. Estaquier J., Vallette F., Vayssiere J.L., Mignotte B. The mitochondrial pathways of apoptosis. Adv Exp Med Biol. 2012. 942: 157-183.

122. Evans M.D., Dizdaroglu M., Cooke M.S. Oxidative DNA damage and disease: induction, repair and significance. Mutat Res. 2004. 567(1): 1-61.

123. Fattman C.L., Schaefer L.M., Oury T.D. Extracellular superoxide dismutase in biology and medicine. Free Radic Biol Med. 2003. 35(3): 236-256.

124. Festjens N., Cornelis S., Lamkanfi M., Vandenabeele P. Caspase-containing complexes in the regulation of cell death and inflammation. Biol Chem. 2006. 387(8): 1005-1016.

125. Fisher A.B. Peroxiredoxin 6: a bifunctional enzyme with glutathione peroxidase and phospholipase $A_2$ activities. Antioxid Redox Signal. 2011. 15(3): 831-844.

126. Flanagan S.W., Anderson R.D., Ross M.A., Oberley L.W. Overexpression of manganese superoxide dismutase attenuates neuronal death in human cells expressing mutant (G37R) Cu/Zn-superoxide dismutase. J Neurochem. 2002. 81(1): 170-177.

127. Fujimura M., Morita-Fujimura Y., Copin J., Yoshimoto T., Chan P.H. Reduction of copper, zinc-superoxide dismutase in knockout mice does not affect edema or infarction volumes and the early release of mitochondrial cytochrome c after permanent focal cerebral ischemia. Brain Res. 2001. 889(1-2): 208-213.

128. Fujimura M., Morita-Fujimura Y., Kawase M., Copin J.C., Calagui B., Epstein C.J., Chan P.H. Manganese superoxide dismutase mediates the early release of mitochondrial cytochrome C and subsequent DNA fragmentation after permanent focal cerebral ischemia in mice. J Neurosci. 1999 a. 19(9): 3414-3422.

129. Fujimura M., Morita-Fujimura Y., Narasimhan P., Copin J.C., Kawase M., Chan P.H. Copper-zinc superoxide dismutase prevents the early decrease of apurinic/apyrimidinic endonuclease and subsequent DNA fragmentation after transient focal cerebral ischemia in mice. Stroke. 1999 b. 30(11): 2408-2415.

130. Fujimura M., Morita-Fujimura Y., Noshita N., Sugawara T., Kawase M., Chan P.H. The cytosolic antioxidant copper/zinc-superoxide dismutase prevents the early release of

mitochondrial cytochrome c in ischemic brain after transient focal cerebral ischemia in mice. J of Neurosci. 2000. 20(8): 2817-2824.

131. Fujino G., Noguchi T., Takeda K., Ichijo H. Thioredoxin and protein kinases in redox signaling. Semin Cancer Biol. 2006. 16(6): 427-435.

132. Galkina O.V. The role of thyroxine and hydrocortisone in the regulation of lipid peroxidation in rat brain. Summary of thesis for the degree of candidate of biological sciences. St. Petersburg, 2000 (In Russian).

133. Galluzzi L., Maiuri M.C., Vitale I., Zischka H., Castedo M., Zitvogel L., Kroemer G. Cell death modalities: classification and pathophysiological implications. Cell Death Differ. 2007. 14(7): 1237-1243.

134. Galluzzi L., Vitale I., Abrams J.M., Alnemri E.S., Baehrecke E.H., Blagosklonny M.V., Dawson T.M., Dawson V.L., El-Deiry W.S., Fulda S., Gottlieb E., Green D.R., Hengartner M.O., Kepp O., Knight R.A., Kumar S., Lipton S.A., Lu X., Madeo F., Malorni W., Mehlen P., Nuñez G., Peter M.E., Piacentini M., Rubinsztein D.C., Shi Y., Simon H.U., Vandenabeele P., White E., Yuan J., Zhivotovsky B., Melino G., Kroemer G. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ. 2012. 19(1): 107-120.

135. Gan Y., Ji X., Hu X., Luo Y., Zhang L., Li P., Liu X., Yan F., Vosler P., Gao Y., Stetler R.A., Chen J. Transgenic overexpression of peroxiredoxin-2 attenuates ischemic neuronal injury via suppression of a redox-sensitive pro-death signaling pathway. Antioxid Redox Signal. 2012. 17(5): 719-732.

136. Ganther H.E. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis. 1999. 20(9): 1657-1666.

137. Garnier P., Demougeot C., Bertrand N., Prigent-Tessier A., Marie C., Beley A. Stress response to hypoxia in gerbil brain: HO-1 and Mn SOD expression and glial activation. Brain Res. 2001. 893(1-2): 301-309.

138. Garrido C., Galluzzi L., Brunet M., Puig P.E., Didelot C., Kroemer G. Mechanisms of cytochrome c release from mitochondria. Cell Death Differ. 2006. 13(9): 1423-1433.

139. Geleziunas R., Xu W., Takeda K., Ichijo H., Greene W.C. HIV-1 Nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. Nature. 2001. 410(6830): 834-838.

140. Gemma C., Fister M., Hudson C., Bickford P.C. Improvement of memory for context by inhibition of caspase-1 in aged rats. Eur J Neurosci. 2005. 22(7): 1751-1756.

141. Ghosh I., Eisinger S.W., Raghavan N., Scott A.L. Thioredoxin peroxidases from Brugia malayi. Mol Biochem Parasitol. 1998. 91(2): 207-220.

142. Girouard H., Iadecola C. Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. J Appl Physiol. 2006. 100(1): 328-335.

143. Glazier S.S., O'Rourke D.M., Graham D.I., Welsh F.A. Induction of ischemic tolerance following brief focal ischemia in rat brain. J Cereb Blood Flow Metab. 1994. 14(4): 545-553.

144. Gleason F.K., Holmgren A. Thioredoxin and related proteins in procaryotes. FEMS Microbiol Rev. 1988. 4(4): 271-297.

145. Go Y.M., Ziegler T.R., Johnson J.M., Gu L., Hansen J.M., Jones D.P. Selective protection of nuclear thioredoxin-1 and glutathione redox systems against oxidation during glucose and glutamine deficiency in human colonic epithelial cells. Free Radic Biol Med. 2007. 42(3): 363-370.

146. Gogvadze V., Orrenius S., Zhivotovsky B. Multiple pathways of cytochrome c release from mitochondria in apoptosis. Biochim Biophys Acta. 2006. 1757(5-6): 639-647.

147. Golubeva L.Yu., Dvoryantsev S.N., Khatkevich A.N., Malyshev I.Yu., Meerson F.Z. Adaptation to hypoxia in contrast to the adaptation to stress does not protect the isolated heart from reperfusion after total ischemia (the study by nuclear magnetic resonance). Bull Exp Biol Med. 1995. 120(11): 481-484 (In Russian, summary in English).

148. Gong S.J., Chen L.Y., Zhang M., Gong J.X., Ma Y.X., Zhang J.M., Wang Y.J., Hu Y.Y., Sun X.C., Li W.B., Zhang Y. Intermittent hypobaric hypoxia preconditioning induced brain ischemic tolerance by up-regulating glial glutamate transporter-1 in rats. Neurochem Res. 2012. 37(3): 527-537.

149. Green D.R., Ferguson T., Zitvogel L., Kroemer G. Immunogenic and tolerogenic cell death. Nat Rev Immunol. 2009. 9(5): 353-363.

150. Greenlund L.J., Deckwerth T.L., Johnson E.M. Jr. Superoxide dismutase delays neuronal apoptosis: a role for reactive oxygen species in programmed neuronal death. Neuron. 1995. 14(2): 303-315.

151. Grimm C., Hermann D.M., Bogdanova A., Hotop S., Kilic U., Wenzel A., Kilic E., Gassmann M. Neuroprotection by hypoxic preconditioning: HIF-1 and erythropoietin protect from retinal degeneration. Semin Cell Dev Biol. 2005. 16(4-5): 531-538.

152. Gulyaeva N.V. Non-apoptotic functions of caspase-3 in nervous tissue. Biochemistry (Mosc). 2003. 68(11): 1171-1180.

153. Gulyaeva N.V., Kudryashov I.E., Kudryashova I.V. Caspase activity is essential for long-term potentiation. J Neurosci Res. 2003. 73(6): 853-864.

154. Hakem R., Hakem A., Duncan G.S., Henderson J.T., Woo M., Soengas M.S., Elia A., de la Pompa J.L., Kagi D., Khoo W., Potter J., Yoshida R., Kaufman S.A., Lowe S.W., Penninger J.M., Mak T.W. Differential requirement for caspase-9 in apoptotic pathways in vivo. Cell. 1998. 94(3): 339-352.

155. Halliwell B., Gutteridge J.M.C. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J. 1984. 219(1): 1-14.

156. Hamakawa M., Ishida A., Tamakoshi K., Shimada H., Nakashima H., Noguchi T., Toyokuni S., Ishida K. Repeated short-term daily exercise ameliorates oxidative cerebral damage and the resultant motor dysfunction after transient ischemia in rats. J Clin Biochem Nutr. 2013. 53(1): 8-14.

157. Hanschmann E.M., Godoy J.R., Berndt C., Hudemann C., Lillig C.H. Thioredoxins, glutaredoxins, and peroxiredoxins-molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. Antioxid Redox Signal. 2013. 19(13): 1539-1605.

158. Hansen J.M., Zhang H., Jones D.P. Mitochondrial thioredoxin-2 has a key role in determining tumor necrosis factor-alpha-induced reactive oxygen species generation, NF-kappaB activation, and apoptosis. Toxicol Sci. 2006 a. 91(2): 643-650.

159. Hansen J.M., Zhang H., Jones D.P. Differential oxidation of thioredoxin-1, thioredoxin-2, and glutathione by metal ions. Free Radic Biol Med. 2006 b. 40(1): 138-145.

160. Harper J.W., Adami G.R., Wei N., Keyomarsi K., Elledge S.J. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell. 1993. 75(4): 805-816.

161. Harris M.H., Thompson C.B. The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability. Cell Death Differ. 2000. 7(12): 1182-1191.

162. Hartwell L.H., Kastan M.B. Cell cycle control and cancer. Science. 1994. 266(5192): 1821-1828.

163. Hashimoto S., Matsumoto K., Gon Y., Furuichi S., Maruoka S., Takeshita I., Hirota K., Yodoi J., Horie T. Thioredoxin negatively regulates p38 MAP kinase activation and IL-6 production by tumor necrosis factor-alpha. Biochem Biophys Res Commun. 1999. 258(2): 443-447.

164. Hatai T., Matsuzawa A., Inoshita S., Mochida Y., Kuroda T., Sakamaki K., Kuida K., Yonehara S., Ichijo H., Takeda K. Execution of apoptosis signal-regulating kinase 1 (ASK1)-induced apoptosis by the mitochondria-dependent caspase activation. J Biol Chem. 2000. 275(34): 26576-26581.

165. Hattori F., Oikawa S. Peroxiredoxins in the central nervous system. Subcell Biochem. 2007. 44: 357-374.

166. Hattori I., Takagi Y., Nakamura H., Nozaki K., Bai J., Kondo N., Sugino T., Nishimura M., Hashimoto N., Yodoi J. Intravenous administration of thioredoxin decreases brain damage following transient focal cerebral ischemia in mice. Antioxid Redox Signal. 2004. 6(1): 81-87.

167. Hattori I., Takagi Y., Nozaki K., Kondo N., Bai J., Nakamura H., Hashimoto N., Yodoi J. Hypoxia-ischemia induces thioredoxin expression and nitrotyrosine formation in new-born rat brain. Redox Rep. 2002. 7(5): 256-259.

168. Hayashi S., Hajiro-Nakanishi K., Makino Y., Eguchi H., Yodoi J., Tanaka H. Functional modulation of estrogen receptor by redox state with reference to thioredoxin as a mediator. Nucleic Acids Res. 1997. 25(20): 4035-4040.

169. Hayashi T., Saito A., Okuno S., Ferrand-Drake M., Dodd R.L., Chan P.H. Oxidative injury to the endoplasmic reticulum in mouse brains after transient focal ischemia. Neurobiol Dis. 2004. 15(2): 229-239.

170. Hayashi T., Saito A., Okuno S., Ferrand-Drake M., Dodd R.L., Chan P.H. Damage to the endoplasmic reticulum and activation of apoptotic machinery by oxidative stress in ischemic neurons. J Cereb Blood Flow Metab. 2005. 25(1): 41-53.

171. Hayashi T., Saito A., Okuno S., Ferrand-Drake M., Dodd R.L., Nishi T., Maier C.M., Kinouchi H., Chan P.H. Oxidative damage to the endoplasmic reticulum is implicated in ischemic neuronal cell death. J Cereb Blood Flow Metab. 2003. 23(10): 1117-1128.

172. Hayes J.D., Milner S.W., Walker S.W. Expression of glyoxalase, glutathione peroxidase and glutathione S-transferase isoenzymes in different bovine tissues. Biochim Biophys Acta. 1989. 994(1): 21-29.

173. Higuchi M., Celino F.T., Shimizu-Yamaguchi S., Miura C., Miura T. Taurine plays an important role in the protection of spermatogonia from oxidative stress. Amino Acids. 2012. 43(6): 2359-2369.

174. Hirano N., Shibasaki F., Kato H., Sakai R., Tanaka T., Nishida J., Yazaki Y., Takenawa T., Hirai H. Molecular cloning and characterization of a cDNA for bovine phospholipase C-alpha: proposal of redesignation of phospholipase C-alpha. Biochem Biophys Res Commun. 1994. 204(1): 375-382.

175. Hirota K., Matsui M., Iwata S., Nishiyama A., Mori K., Yodoi J. AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. Proc Natl Acad Sci U S A. 1997. 94(8): 3633-3638.

176. Hirota K., Matsui M., Murata M., Takashima Y., Cheng F.S., Itoh T., Fukuda K., Yodoi J. Nucleoredoxin, glutaredoxin, and thioredoxin differentially regulate NF-kappaB, AP-1, and CREB activation in HEK293 cells. Biochem Biophys Res Commun. 2000. 274(1): 177-182.

177. Hirota K., Murata M., Sachi Y., Nakamura H., Takeuchi J., Mori K., Yodoi J. Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. J Biol Chem. 1999. 274(39): 27891-27897.

178. Hirota K., Nakamura H., Masutani H., Yodoi J. Thioredoxin superfamily and thioredoxin-inducing agents. Ann N Y Acad Sci. 2002. 957: 189-199.

179. Hochachka P.W. Mechanism and evolution of hypoxia-tolerance in humans. J Exp Biol. 1998. 201(Pt 8): 1243-1254.

180. Holmgren A. Thioredoxin. Annu Rev Biochem. 1985. 54: 237-271.

181. Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem. 1989. 264(24): 13963-13966.

182. Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. Antioxid Redox Signal. 2000. 2(4): 811-820.

183. Holmgren A., Lu J. Thioredoxin and thioredoxin reductase: current research with special reference to human disease. Biochem Biophys Res Commun. 2010. 396 (1): 120-124.

184. Holmgren A., Soderberg B.O., Eklund H., Branden C.I. Three-dimensional structure of Escherichia coli thioredoxin-S2 to 2.8 A resolution. Proc Natl Acad Sci U S A. 1975. 72(6): 2305-2309.

185. Holtzclaw W.D., Dinkova-Kostova A.T., Talalay P. Protection against electrophile and oxidative stress by induction of phase 2 genes: the quest for the elusive sensor that responds to inducers. Adv Enzyme Regul. 2004. 44: 335-367.

186. Homi H.M., Freitas J.J., Curi R., Velasco I.T., Junior B.A. Changes in superoxide dismutase and catalase activities of rat brain regions during early global transient ischemia/reperfusion. Neurosci Lett. 2002. 333(1): 37-40.

187. Hori K., Katayama M., Sato N., Ishii K., Waga S., Yodoi J. Neuroprotection by glial cells through adult T cell leukemia-derived factor/human thioredoxin (ADF/TRX). Brain Res. 1994. 652(2): 304-310.

188. Hoshida S., Yamashita N., Otsu K., Hori M. The importance of manganese superoxide dismutase in delayed preconditioning. Cardiovasc Res. 2002. 55(3): 495-505.

189. Huang X.J., Song C.X., Zhong C.Q., Wang F.S. Research progress in the radioprotective effect of superoxide dismutase. Drug Discov Ther. 2012. 6(4): 169-177.

190. Huesmann G.R., Clayton D.F. Dynamic role of postsynaptic caspase-3 and BIRC4 in zebra finch song-response habituation. Neuron. 2006. 52(6): 1061-1072.

191. Hughes G., Murphy M.P., Ledgerwood E.C. Mitochondrial reactive oxygen species regulate the temporal activation of nuclear factor kappaB to modulate tumour necrosis factor-induced apoptosis: evidence from mitochondria-targeted antioxidants. Biochem J. 2005. 389(Pt 1): 83-89.

192. Hwang I.K., Yoo K.Y., Kim D.W., Lee C.H., Choi J.H., Kwon Y.G., Kim Y.M., Choi S.Y., Won M.H. Changes in the expression of mitochondrial peroxiredoxin and thioredoxin in neurons and glia and their protective effects in experimental cerebral ischemic damage. Free Radic Biol Med. 2010. 48(9): 1242-1251.

193. Häcki J., Egger L., Monney L., Conus S., Rosse T., Fellay I., Borner C. Apoptotic crosstalk between the endoplasmic reticulum and mitochondria controlled by Bcl-2. Oncogene. 2000. 19(19): 2286-2295.

194. Ichijo H., Nishida E., Irie K., ten Dijke P., Saitoh M., Moriguchi T., Takagi M., Matsumoto K., Miyazono K., Gotoh Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science. 1997. 275(5296): 90-94.

195. Ichimiya S., Davis J.G., O'Rourke D.M., Katsumata M., Greene M.I. Murine thioredoxin peroxidase delays neuronal apoptosis and is expressed in areas of the brain most susceptible to hypoxic and ischemic injury. DNA Cell Biol. 1997. 16(3): 311-321.

196. Imray C., Booth A., Wright A., Bradwell A. Acute altitude illnesses. BMJ. 2011. 343: d4943.

197. Iñarrea P., Casanova A., Alava M.A., Iturralde M., Cadenas E. Melatonin and steroid hormones activate intermembrane Cu,Zn-superoxide dismutase by means of mitochondrial cytochrome P450. Free Radic Biol Med. 2011. 50(11): 1575-1581.

198. Ishii T., Miyazawa M., Onouchi H., Yasuda K., Hartman P.S., Ishii N. Model animals for the study of oxidative stress from complex II. Biochim Biophys Acta. 2013. 1827(5): 588-597.

199. Işlekel S., Işlekel H., Güner G., Ozdamar N. Alterations in superoxide dismutase, glutathione peroxidase and catalase activities in experimental cerebral ischemia-reperfusion. Res Exp Med (Berl). 1999. 199(3): 167-176.

200. Isowa N., Yoshimura T., Kosaka S., Liu M., Hitomi S., Yodoi J., Wada H. Human thioredoxin attenuates hypoxia-reoxygenation injury of murine endothelial cells in a thiol-free condition. J Cell Physiol. 2000. 182(1): 33-40.

201. Itoh N., Yonehara S., Ishii A., Yonehara M., Mizushima S., Sameshima M., Hase A., Seto Y., Nagata S. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell. 1991. 66(2): 233-243.

202. Jacotot E., Costantini P., Laboureau E., Zamzami N., Susin S.A., Kroemer G. Mitochondrial membrane permeabilization during the apoptotic process. Ann N Y Acad Sci. 1999. 887: 18-30.

203. Jeong J.S., Kwon S.J., Kang S.W., Rhee S.G., Kim K. Purification and characterization of a second type thioredoxin peroxidase (type II TPx) from Saccharomyces cerevisiae. Biochemistry. 1999. 38(2): 776-783.

204. Jeong W., Bae S.H., Toledano M.B., Rhee S.G. Role of sulfiredoxin as a regulator of peroxiredoxin function and regulation of its expression. Free Radic Biol Med. 2012. 53(3): 447-456.

205. Jeong W., Chang T.S., Boja E.S., Fales H.M., Rhee S.G. Roles of TRP14, a thioredoxin-related protein in tumor necrosis factor-alpha signaling pathways. J Biol Chem. 2004 a. 279(5): 3151-3159.

206. Jeong W., Jung Y., Kim H., Park S.J., Rhee S.G. Thioredoxin-related protein 14, a new member of the thioredoxin family with disulfide reductase activity: implication in the redox regulation of TNF-alpha signaling. Free Radic Biol Med. 2009. 47(9): 1294-1303.

207. Jeong W., Park S.J., Chang T.S., Lee D.Y., Rhee S.G. Molecular mechanism of the reduction of cysteine sulfinic acid of peroxiredoxin to cysteine by mammalian sulfiredoxin. J Biol Chem. 2006. 281(20): 14400-14407.

208. Jeong W., Yoon H.W., Lee S.R., Rhee S.G. Identification and characterization of TRP14, a thioredoxin-related protein of 14 kDa. New insights into the specificity of thioredoxin function. J Biol Chem. 2004 b. 279(5): 3142-3150.

209. Jia J., Zhang X., Hu Y.S., Wu Y., Wang Q.Z., Li N.N., Wu C.Q., Yu H.X., Guo Q.C. Protective effect of tetraethyl pyrazine against focal cerebral ischemia/reperfusion injury in rats: therapeutic time window and its mechanism. Thromb Res. 2009. 123(5): 727-730.

210. Jiang X., Wang X. Cytochrome C-mediated apoptosis. Annu Rev Biochem. 2004. 73: 87-106.

211. Jiménez A., Johansson C., Ljung J., Sagemark J., Berndt K.D., Ren B., Tibbelin G., Ladenstein R., Kieselbach T., Holmgren A., Gustafsson J.A., Miranda-Vizuete A. Human spermatid-specific thioredoxin-1 (Sptrx-1) is a two-domain protein with oxidizing activity. FEBS Lett. 2002 a. 530(1-3): 79-84.

212. Jiménez A., Oko R., Gustafsson J.A., Spyrou G., Pelto-Huikko M., Miranda-Vizuete A. Cloning, expression and characterization of mouse spermatid specific thioredoxin-1 gene and protein. Mol Hum Reprod. 2002 b. 8(8): 710-718.

213. Jiménez A., Pelto-Huikko M., Gustafsson J.A., Miranda-Vizuete A. Characterization of human thioredoxin-like-1: potential involvement in the cellular response against glucose deprivation. FEBS Lett. 2006. 580(3): 960-967.

214. Jiménez A., Zu W., Rawe V.Y., Pelto-Huikko M., Flickinger C.J., Sutovsky P., Gustafsson J.A., Oko R., Miranda-Vizuete A. Spermatocyte/spermatid-specific thioredoxin-3, a novel Golgi apparatus-associated thioredoxin, is a specific marker of aberrant spermatogenesis. J Biol Chem. 2004. 279(33): 34971-34982.

215. Jung J.E., Kim G.S., Chen H., Maier C.M., Narasimhan P., Song Y.S., Niizuma K., Katsu M., Okami N., Yoshioka H., Sakata H., Goeders C.E., Chan P.H. Reperfusion and neurovascular dysfunction in stroke: from basic mechanisms to potential strategies for neuroprotection. Mol. Neurobiol. 2010. 41(2-3): 172-179.

216. Junn E., Han S.H., Im J.Y., Yang Y., Cho E.W., Um H.D., Kim D.K., Lee K.W., Han P.L., Rhee S.G., Choi I. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. J Immunol. 2000. 164(12): 6287-6295.

217. Jäättelä M. Programmed cell death: many ways for cells to die decently. Ann Med. 2002. 34(6): 480-488.

218. Kaeffer N., Richard V., Thuillez C. Delayed coronary endothelial protection 24 hours after preconditioning: role of free radicals. Circulation. 1997. 96(7): 2311-2316.

219. Kaimul A.M., Nakamura H., Masutani H., Yodoi J. Thioredoxin and thioredoxin-binding protein-2 in cancer and metabolic syndrome. Free Radic Biol Med. 2007. 43(6): 861-868.

220. Kalinina E.V., Chernov N.N., Saprin A.N. Involvement of thio-, peroxi-, and glutaredoxins in cellular redox-dependent processes. Biochemistry (Mosc). 2008. 73(13): 1493-1510.

221. Kamada H., Yu F., Nito C., Chan P.H. Influence of hyperglycemia on oxidative stress and matrix metalloproteinase-9 activation after focal cerebral ischemia/reperfusion in rats: relation to blood-brain barrier dysfunction. Stroke. 2007. 38(3): 1044-1049.

222. Kang S.W., Baines I.C., Rhee S.G. Characterization of a mammalian peroxiredoxin that contains one conserved cysteine. J Biol Chem. 1998. 273(11): 6303-6311.

223. Katagiri K., Matsuzawa A., Ichijo H. Regulation of apoptosis signal-regulating kinase 1 in redox signaling. Methods Enzymol. 2010. 474: 277-288.

224. Kato H., Kogure K., Araki T., Liu X.H., Kato K., Itoyama Y. Immunohistochemical localization of superoxide dismutase in the hippocampus following ischemia in a gerbil model of ischemic tolerance. J Cereb Blood Flow Metab. 1995. 15(1): 60-70.

225. Kedzierewicz R., Cabane D. Acute mountain sickness and high altitude cerebral and pulmonary edema. Rev Prat. 2013. 63(1): 18-26 (in French).

226. Keller J.N., Kindy M.S., Holtsberg F.W., St Clair D.K., Yen H.C., Germeyer A., Steiner S.M., Bruce-Keller A.J., Hutchins J.B., Mattson M.P. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J Neurosci. 1998. 18(2): 687-697.

227. Kelley M.R., Georgiadis M.M., Fishel M.L. APE1/Ref-1 role in redox signaling: translational applications of targeting the redox function of the DNA repair/redox protein APE1/Ref-1. Curr Mol Pharmacol. 2012. 5(1): 36-53.

228. Kelly P.J., Morrow J.D., Ning M., Koroshetz W., Lo E.H., Terry E., Milne G.L., Hubbard J., Lee H., Stevenson E., Lederer M., Furie K.L. Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the Biomarker Evaluation for Antioxidant Therapies in Stroke (BEAT-Stroke) study. Stroke. 2008. 39(1): 100-104.

229. Kerr J.F. A histochemical study of hypertrophy and ischaemic injury of rat liver with special reference to changes in lysosomes. J Pathol Bacteriol. 1965. 90(2): 419-435.

230. Kerr J.F. Shrinkage necrosis: a distinct mode of cellular death. J Pathol. 1971. 105(1): 13-20.

231. Kerr J.F. History of the events leading to the formulation of the apoptosis concept. Toxicology. 2002. 181-182: 471-474.

232. Kerr J.F., Wyllie A.H., Currie A.R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972. 26(4): 239-257.

233. Khavinson V. Kh., Barinov V.A., Arutyunyan A.V., Malinin V.V. Free radical oxidation and aging. St. Petersburg: Nauka, 2003. 327 p. (In Russian).

234. Kim G.S., Jung J.E., Narasimhan P., Sakata H., Chan P.H. Induction of thioredoxin-interacting protein is mediated by oxidative stress, calcium, and glucose after brain injury in mice. Neurobiol Dis. 2012. 46(2): 440-449.

235. Kim G.S., Jung J.E., Niizuma K., Chan P.H. CK2 is a novel negative regulator of NADPH oxidase and a neuroprotectant in mice after cerebral ischemia. J Neurosci. 2009. 29(47):14779-14789.

236. Kim G.W., Kondo T., Noshita N., Chan P.H. Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. Stroke. 2002. 33(3): 809-815.

237. Kim I.H., Kim K., Rhee S.G. Induction of an antioxidant protein of Saccharomyces cerevisiae by O2, Fe3+, or 2-mercaptoethanol. Proc Natl Acad Sci U S A. 1989. 86(16): 6018-6022.

238. Kimura H., Shibuya N., Kimura Y. Hydrogen sulfide is a signaling molecule and a cytoprotectant. Antioxid Redox Signal. 2012. 17(1): 45-57.

239. Kiningham K.K., St Clair D.K. Overexpression of manganese superoxide dismutase selectively modulates the activity of Jun-associated transcription factors in fibrosarcoma cells. Cancer Res. 1997. 57(23): 5265-5271.

240. Kiningham K.K., Xu Y., Daosukho C., Popova B., St Clair D.K. Nuclear factor kappaB-dependent mechanisms coordinate the synergistic effect of PMA and cytokines on the induction of superoxide dismutase 2. Biochem J. 2001. 353(Pt 1): 147-156.

241. Kinoshita A., Yamada K., Kohmura E., Hayakawa T. Human recombinant superoxide dismutase protects primary cultured neurons against hypoxic injury. Pathobiology. 1991. 59(5): 340-344.

242. Kinouchi H., Kamii H., Mikawa S., Epstein C.J., Yoshimoto T., Chan P.H. Role of superoxide dismutase in ischemic brain injury: a study using SOD-1 transgenic mice. Cell Mol Neurobiol. 1998. 18(6): 609-620.

243. Kipp A.P., Müller M.F., Göken E.M., Deubel S., Brigelius-Flohé R. The selenoproteins GPx2, TrxR2 and TrxR3 are regulated by Wnt signalling in the intestinal epithelium. Biochim Biophys Acta. 2012. 1820(10):1588-1596.

244. Kirino T. Ischemic tolerance. J Cereb Blood Flow Metab. 2002. 22(11): 1283-1296.

245. Kirino T., Tsujita Y., Tamura A. Induced tolerance to ischemia in gerbil hippocampal neurons. J. Cereb. Blood Flow Metab. 1991. 11(2): 299-307.

246. Kirkland R.A., Windelborn J.A., Kasprzak J.M., Franklin J.L. A Bax-induced pro-oxidant state is critical for cytochrome c release during programmed neuronal death. J Neurosci. 2002. 22(15): 6480-6490.

247. Kirkpatrick D.L., Ehrmantraut G., Stettner S., Kunkel M., Powis G. Redox active disulfides: the thioredoxin system as a drug target. Oncol Res. 1997. 9(6-7): 351-356.

248. Kirova Iu.I. Effect of hypoxia on dynamics of HIF-1alpha level in the cerebral cortex and development of adaptation in rats with different resistance to hypoxia. Patol Fiziol Eksp Ter. 2012. 3: 51-55 (In Russian).

249. Kislin M.S., Stroev S.A., Gluschenko T.S., Tyulkova E.I., Pelto-Huikko M., Samoilov M.O. Hypoxic preconditioning modifies activity of pro- and antioxidant systems in the rat hippocampus. Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry. 2012. 6(4): 333–337.

250. Kislin M.S., Tiul'kova E.I., Samoĭlov M.O. Dynamics of lipid peroxidation of membranes in cells and mitochondrial fraction of neocortex in non- and preconditioned rats after severe hypobaric hypoxia. Zh Evol Biokhim Fiziol. 2011. 47(2): 157-164 (In Russian).

251. Kislin M.S., Tyul'kova E.I., Samoilov M.O. Time course of lipid peroxidation in hippocampal membranes of preconditioned and nonpreconditioned rats subjected to severe hypobaric hypoxia. Neurochemical Journal. 2010. 4(2): 122-127.

252. Kitagawa K., Matsumoto M., Kuwabara K., Tagaya M., Ohtsuki T., Hata R., Ueda H., Handa N., Kimura K., Kamada T. `Ischemic tolerance' phenomenon detected in various brain regions. Brain Res. 1991. 561(2): 203-211.

253. Kitagawa K., Matsumoto M., Tagaya M., Hata R., Ueda H., Niinobe M., Handa N., Fukunaga R., Kimura K., Mikoshiba K., Kamada T. `Ischemic tolerance' phenomenon found in the brain. Brain Res. 1990. 528(1): 21-24.

254. Kleikers P.W., Wingler K., Hermans J.J., Diebold I., Altenhöfer S., Radermacher K.A., Janssen B., Görlach A., Schmidt H.H. NADPH oxidases as a source of oxidative stress and molecular target in ischemia/reperfusion injury. J Mol Med (Berl). 2012. 90(12): 1391-1406.

255. Kloner R.A., Przyklenk K., Whittaker P. Deleterious effects of oxygen radicals in ischemia/reperfusion: resolved and unresolved issues. Circulation. 1989. 80(5): 1115–1127.

256. Knoops B., Goemaere J., Van der Eecken V., Declercq J.P. Peroxiredoxin 5: structure, mechanism, and function of the mammalian atypical 2-Cys peroxiredoxin. Antioxid Redox Signal. 2011. 15(3): 817-829.

257. Kobayashi M., Yamamoto M. Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. Adv Enzyme Regul. 2006. 46: 113-140.

258. Koh P.O. Proteomic analysis of focal cerebral ischemic injury in male rats. J Vet Med Sci. 2010. 72(2): 181-185.

259. Kohárýová M., Kollárová M. Oxidative stress and thioredoxin system. Gen Physiol Biophys. 2008. 27(2): 71-84.

260. Koltover V.K. Reliability of mitochondrial electron-transport membranes and the role of superoxide radicals in aging. Khim Fiz. 1996. 15(1): 101-106. (In Russian).

261. Kondo T., Murakami K., Honkaniemi J., Sharp F.R., Epstein C.J., Chan P.H. Expression of hsp70 mRNA is induced in the brain of transgenic mice overexpressing human CuZn-superoxide dismutase following transient global cerebral ischemia. Brain Res. 1996. 737(1-2): 321-326.

262. Kondo N., Nakamura H., Masutani H., Yodoi J. Redox regulation of human thioredoxin network. Antioxid Redox Signal. 2006. 8(9-10): 1881-1890.

263. Kondo T., Reaume A.G., Huang T.T., Carlson E., Murakami K., Chen S.F., Hoffman E.K., Scott R.W., Epstein C.J., Chan P.H. Reduction of CuZn-superoxide dismutase activity exacerbates neuronal cell injury and edema formation after transient focal cerebral ischemia. J Neurosci. 1997 a. 17(11): 4180-4189.

264. Kondo T., Reaume A.G., Huang T.T., Murakami K., Carlson E., Chen S., Scott R.W., Epstein C.J., Chan P.H. Edema formation exacerbates neurological and histological outcomes after focal cerebral ischemia in CuZn-superoxide dismutase gene knockout mutant mice. Acta Neurochir Suppl (Wien). 1997 b. 70: 62-64.

265. Koopman W.J., Nijtmans L.G., Dieteren C.E., Roestenberg P., Valsecchi F., Smeitink J.A., Willems P.H. Mammalian mitochondrial complex I: biogenesis, regulation, and reactive oxygen species generation. Antioxid Redox Signal. 2010. 12(12): 1431-1470.

266. Koppenol W. Chemistry of iron and copper in radical reactions. In: Free radical damage and its control. New comprehensive biochem. 1994. 28: 3-25.

267. Kosugi T., Kawahara K., Yamada T., Nakajima T., Tanaka M. Functional significance of the preconditioning-induced down-regulation of glutamate transporter GLT-1 in neuron/astrocyte co-cultures. Neurochem Res. 2005. 30(9): 1109-1116.

268. Kreps E.M., Verzhbinskaja N.A., Chenykaeva E.Ju., Chirkovskaja E.V., Govurina Ts.K. About adaptation of animals to chronic hypoxia. Physiol. J. USSR. 1956. 42(2): 149–158 (In Russian).

269. Kroemer G., El-Deiry W.S., Golstein P., Peter M.E., Vaux D., Vandenabeele P., Zhivotovsky B., Blagosklonny M.V., Malorni W., Knight R.A., Piacentini M., Nagata S., Melino G.; Nomenclature Committee on Cell Death. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ. 2005. 12 Suppl 2: 1463-1467.

270. Kroemer G., Galluzzi L., Vandenabeele P., Abrams J., Alnemri E.S., Baehrecke E.H., Blagosklonny M.V., El-Deiry W.S., Golstein P., Green D.R., Hengartner M., Knight R.A., Kumar S., Lipton S.A., Malorni W., Nuñez G., Peter M.E., Tschopp J., Yuan J., Piacentini M., Zhivotovsky B., Melino G.; Nomenclature Committee on Cell Death 2009. Classification of cell

death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ. 2009. 16(1): 3-11.

271. Kudryashov I.E., Yakovlev A.A., Kudryashova I.V., Gulyaeva N.V. Inhibition of caspase-3 blocks long-term potentiation in hippocampal slices. Neurosci Behav Physiol. 2004. 34(9): 877-880.

272. Kudryashova I.V., Onufriev M.V., Kudryashov I.E., Gulyaeva N.V. Caspase-3 activity in hippocampal slices reflects changes in synaptic plasticity. Neurosci Behav Physiol. 2009. 39(1): 13-20.

273. Kuida K., Haydar T.F., Kuan C.Y., Gu Y., Taya C., Karasuyama H., Su M.S., Rakic P., Flavell R.A. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase-9. Cell. 1998. 94(3): 325-337.

274. Kuranaga E., Miura M. Nonapoptotic functions of caspases: caspases as regulatory molecules for immunity and cell-fate determination. Trends Cell Biol. 2007. 17(3): 135-144.

275. Lane T., Flam B., Lockey R., Kolliputi N. TXNIP shuttling: missing link between oxidative stress and inflammasome activation. Front Physiol. 2013. 4: 50.

276. Lappalainen Z., Lappalainen J., Oksala N.K., Laaksonen D.E., Khanna S., Sen C.K., Atalay M. Diabetes impairs exercise training-associated thioredoxin response and glutathione status in rat brain. J Appl Physiol (1985). 2009. 106(2): 461-467.

277. Lassus P., Opitz-Araya X., Lazebnik Y. Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization. Science. 2002. 297(5585): 1352-1354.

278. Laurent T.C., Moore E.C., Reichard P. Enzymatic synthesis of deoxyribonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen donor from Escherichia coli b. J Biol Chem. 1964. 239: 3436-3444.

279. Leak R.K., Liou A.K., Zigmond M.J. Effect of sublethal 6-hydroxydopamine on the response to subsequent oxidative stress in dopaminergic cells: evidence for preconditioning. J Neurochem. 2006. 99(4): 1151-1163.

280. Lebovitz R.M., Zhang H., Vogel H., Cartwright J. Jr, Dionne L., Lu N., Huang S., Matzuk M.M. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci U S A. 1996. 93(18): 9782-9787.

281. Lee S.T., Hoeflich K.P., Wasfy G.W., Woodgett J.R., Leber B., Andrews D.W., Hedley D.W., Penn L.Z. Bcl-2 targeted to the endoplasmic reticulum can inhibit apoptosis induced by Myc but not etoposide in Rat-1 fibroblasts. Oncogene. 1999. 18(23): 3520-3528.

282. Lehotský J., Burda J., Danielisová V., Gottlieb M., Kaplán P., Saniová B. Ischemic tolerance: the mechanisms of neuroprotective strategy. Anat Rec (Hoboken). 2009. 292(12): 2002-2012.

283. Lemasters J., Nieminen A. Mitochondrial oxygen radical formation during reductive and oxidative stress to intact hepatocytes. Biosci rep. 1997. 17(3): 281-291.

284. Levadnaya O.V., Donchenko G.V., Valutsina V.M., Korzh E.V., Hil' Yu.N. Relation between the quantities of enzyme activity of antioxidant system in various tissues of intact rats. Ukr. Biochem. Journ. 1998. 70(6): 53-58 (In Russian).

285. Levin S., Bucci T.J., Cohen S.M., Fix A.S., Hardisty J.F., LeGrand E.K., Maronpot R.R., Trump B.F. The nomenclature of cell death: recommendations of an ad hoc Committee of the Society of Toxicologic Pathologists. Toxicol Pathol. 1999. 27(4): 484-490.

286. Lewen A., Fujimura M., Sugawara T., Matz P., Copin J.C., Chan P.H. Oxidative stress-dependent release of mitochondrial cytochrome c after traumatic brain injury. J. Cereb. Blood Flow Metab. 2001. 21(8): 914-920.

287. Li P., Nijhawan D., Budihardjo I., Srinivasula S.M., Ahmad M., Alnemri E.S., Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 1997. 91(4): 479-489.

288. Li P.F., Dietz R., von Harsdorf R. p53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome c-independent apoptosis blocked by Bcl-2. EMBO J. 1999. 18(21): 6027-6036.

289. Li Y., Huang T.-T., Carlson E.J., Melov S., Ursell P.C., Olson J.L., Noble L.J., Yoshimura M.P., Berger C., Chan P.H., Wallace D.C., Epstein C.J. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. Nat Genet. 1995 11(4): 376-381.

290. Lillig C.H., Holmgren A. Thioredoxin and related molecules – from biology to health and disease. Antioxid Redox Signal. 2007. 9(1): 25-47.

291. Lin A.M., Chen C.F., Ho L.T. Neuroprotective effect of intermittent hypoxia on iron-induced oxidative injury in rat brain. Exp Neurol. 2002. 176(2): 328-335.

292. Lindenau J., Noack H., Possel H., Asayama K., Wolf G. Cellular distribution of superoxide dismutases in the rat CNS. Glia. 2000. 29(1): 25-34.

293. Liu W., Guo M., Xu Y.B., Li D., Zhou Z.N., Wu Y.L., Chen Z., Kogan S.C., Chen G.Q. Induction of tumor arrest and differentiation with prolonged survival by intermittent hypoxia in a mouse model of acute myeloid leukemia. Blood. 2006. 107(2): 698-707.

294. Liu X.H., Kato H., Araki T., Itoyama Y., Kato K., Kogure K. An immunohistochemical study of copper/zinc superoxide dismutase and manganese superoxide dismutase following focal cerebral ischemia in the rat. Brain Res. 1994 a. 644(2): 257-266.

295. Liu X.H., Kato H., Araki T., Itoyama Y., Kato K., Kogure K. An immunohistochemical observation of manganese superoxide dismutase in rat substantia nigra after occlusion of middle cerebral artery. Neurosci. Lett. 1994 b. 173(1-2): 103-106.

296. Liu X.H., Kato H., Nakata N., Kogure K., Kato K. An immunohistochemical study of copper/zinc superoxide dismutase and manganese superoxide dismutase in rat hippocampus after transient cerebral ischemia. Brain Res. 1993 a. 625(1): 29-37.

297. Liu X.Q., Sheng R., Qin Z.H. The neuroprotective mechanism of brain ischemic preconditioning. Acta Pharmacol Sin. 2009. 30(8): 1071-1080.

298. Liu Y., Kato H., Nakata N., Kogure K. Temporal profile of heat shock protein 70 synthesis in ischemic tolerance induced by preconditioning ishemia in rat hippocampus. Neuroscience. 1993 b. 56(4): 921-927.

299. Lockshin R.A., Zakeri Z. Programmed cell death and apoptosis: origins of the theory. Nat Rev Mol Cell Biol. 2001. 2(7): 545-550.

300. Loeffler M., Kroemer G. The mitochondrion in cell death control: certainties and incognita. Exp Cell Res. 2000. 256(1): 19-26.

301. López-Hernández B., Posadas I., Podlesniy P., Abad M.A., Trullas R., Ceña V. HIF-1α is neuroprotective during the early phases of mild hypoxia in rat cortical neurons. Exp Neurol. 2012. 233(1): 543-554.

302. Lu G., Ding D., Shi M. Acute adaptation of mice to hypoxic hypoxia. Biol Signals Recept. 1999. 8(4-5): 247-255.

303. Lu J., Holmgren A. Thioredoxin system in cell death progression. Antioxid Redox Signal. 2012. 17(12): 1738-1747.

304. Lu W., Egerton G.L., Bianco A.E., Williams S.A. Thioredoxin peroxidase from Onchocerca volvulus: a major hydrogen peroxide detoxifying enzyme in filarial parasites. Mol Biochem Parasitol. 1998. 91(2): 221-235.

305. Lukyanova L.D., Sukoyan G.V., Kirova Y.I. Role of proinflammatory factors, nitric oxide, and some parameters of lipid metabolism in the development of immediate adaptation to hypoxia and HIF-1α accumulation. Bull Exp Biol Med. 2013. 154(5): 597-601.

306. L'vova S.P., Abaeva E.M. Antioxidative system of tissues in early postnatal development of rats. Ontogenesis. 1996. 27(3): 204-207 (In Russian).

307. Ma Y.H., Su N., Chao X.D., Zhang Y.Q., Zhang L., Han F., Luo P., Fei Z., Qu Y. Thioredoxin-1 attenuates post-ischemic neuronal apoptosis via reducing oxidative/nitrative stress. Neurochem Int. 2012. 60(5): 475-483.

308. Maehara K., Oh-Hashi K., Isobe K.I. Early growth-responsive-1-dependent manganese superoxide dismutase gene transcription mediated by platelet-derived growth factor. FASEB J. 2001. 15(11): 2025-2026.

309. Maier C.M., Hsieh L., Crandall T., Narasimhan P., Chan P.H. Evaluating therapeutic targets for reperfusion-related brain hemorrhage. Ann Neurol. 2006. 59(6): 929-938.

310. Maier C.M., Hsieh L., Yu F., Bracci P., Chan P.H. Matrix metalloproteinase-9 and myeloperoxidase expression: quantitative analysis by antigen immunohistochemistry in a model of transient focal cerebral ischemia. Stroke. 2004. 35(5): 1169-1174.

311. Maiti P., Muthuraju S., Ilavazhagan G., Singh S.B. Hypobaric hypoxia induces dendritic plasticity in cortical and hippocampal pyramidal neurons in rat brain. Behav Brain Res. 2008 a. 189(2): 233-243.

312. Maiti P., Singh S.B., Mallick B., Muthuraju S., Ilavazhagan G. High altitude memory impairment is due to neuronal apoptosis in hippocampus, cortex and striatum. J Chem Neuroanat. 2008 b. 36(3-4): 227-238.

313. Maiti P., Singh S.B., Muthuraju S., Veleri S., Ilavazhagan G. Hypobaric hypoxia damages the hippocampal pyramidal neurons in the rat brain. Brain Res. 2007. 1175: 1-9.

314. Makino Y., Yoshikawa N., Okamoto K., Hirota K., Yodoi J., Makino I., Tanaka H. Direct association with thioredoxin allows redox regulation of glucocorticoid receptor function. J Biol Chem. 1999. 274(5): 3182-3188.

315. Mammucari C., Rizzuto R. Signaling pathways in mitochondrial dysfunction and aging. Mech Ageing Dev. 2010. 131(7-8): 536-543.

316. Marek Ł. The role of the apoptosome in the activation of procaspase-9. Postepy Hig Med Dosw (Online). 2013. 67: 54-64 (in Polish).

317. Marini M., Frabetti F., Musiani D., Franceschi C. Oxygen radicals induce stress proteins and tolerance to oxidative stress in human lymphocytes. Int J Radiat Biol. 1996. 70(3): 337-350.

318. Markina E.V., Storozheva Z.I., Sherstnev V.V. Study of learning and memory processes in adult rats under conditions of intracerebral caspases inhibitors administration. Ross Fiziol Zh Im I M Sechenova. 2008. 94(4): 361-370 (in Russian).

319. Markina E.V., Storozheva Z.I., Sherstnev V.V. Studies of learning and memory processes in adult rats in conditions of intracerebral administration of caspase inhibitors. Neurosci Behav Physiol. 2009. 39(4): 341-346.

320. Markina E.V., Storozheva Z.I., Yurasov V.V., Sherstnev V.V. Central injection of caspase inhibitors facilitates the formation of freezing behavior in rats. Bull Exp Biol Med. 2007. 143(5): 566-568.

321. Marklund S.L. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. Biochem J. 1984. 222(3): 649-655.

322. Marklund S.L., Holme E., Hellner L. Superoxide dismutase in extracellular fluids. Clin. Chim. Acta. 1982. 126(1): 41-51.

323. Maron B.A., Michel T. Subcellular localization of oxidants and redox modulation of endothelial nitric oxide synthase. Circ J. 2012. 76(11): 2497-2512.

324. Maslova M.V., Zemlyanskii K.S., Shkol'nikova M.V., Maklakova A.S., Krushinskaya Y.V., Sokolova N.A., Ashmarin I.P. Peptidergic correction of the effect of acute hypobaric hypoxia in pregnant rats on progeny. Bull Exp Biol Med. 2001. 131(2): 109-112.

325. Masutani H., Hirota K., Sasada T., Ueda-Taniguchi Y., Taniguchi Y., Sono H., Yodoi J. Transactivation of an inducible anti-oxidative stress protein, human thioredoxin by HTLV-I Tax. Immunol Lett. 1996. 54(2-3): 67-71.

326. McCord J.M., Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969. 244(22): 6049-6055.

327. McLaughlin B., Hartnett K.A., Erhardt J.A., Legos J.J., White R.F., Barone F.C., Aizenman E. Caspase 3 activation is essential for neuroprotection in preconditioning. Proc Natl Acad Sci U S A. 2003.100(2): 715-720.

328. Medico M., De Vivo S., Tomasello C., Grech M., Nicosia A., Castorina M., D'Agata M.A., Rampello L., Lempereur L., Drago F. Behavioral and neurochemical effects of dopaminergic drugs in models of brain injury. Eur Neuropsychopharmacol. 2002. 12(3): 187-194.

329. Meerson F., Pozharov V., Minyailenko T. Superresistance against hypoxia after preliminary adaptation to repeated stress. J Appl Physiol. 1994. 76(5): 1856-1861.

330. Meerson F.Z., Pozharov V.P., Minyaylenko T.D., Golubeva L.Yu. Adaptation to stress can increase the resistance of animals to sublethal hypoxia more than the adaptation to hypoxia. Bull Exp Biol Med. 1993. 116(12): 574-577 (In Russian).

331. Mehta S.L., Lin Y., Chen W., Yu F., Cao L., He Q., Chan P.H., Li P.A. Manganese superoxide dismutase deficiency exacerbates ischemic brain damage under hyperglycemic conditions by altering autophagy. Transl Stroke Res. 2011. 2(1): 42-50.

332. Mergenthaler P., Muselmann C., Sünwoldt J., Isaev N.K., Wieloch T., Dirnagl U., Meisel A., Ruscher K. A functional role of the cyclin-dependent kinase inhibitor 1 (p21(WAF1/CIP1)) for neuronal preconditioning. J Cereb Blood Flow Metab. 2013. 33(3): 351-355.

333. Mignotte B., Vayssiere J.L. Mitochondria and apoptosis. Eur J Biochem. 1998. 252(1): 1-15.

334. Mikawa S., Kinouchi H., Kamii H., Gobbel G.T., Chen S.F., Carlson E., Epstein C.J., Chan P.H. Attenuation of acute and chronic damage following traumatic brain injury in copper, zinc-superoxide dismutase transgenic mice. J Neurosurg. 1996. 85(5): 885-891.

335. Miki H., Funato Y. Regulation of intracellular signalling through cysteine oxidation by reactive oxygen species. J Biochem. 2012. 151(3): 255-261.

336. Miller A.A., Dusting G.J., Roulston C.L., Sobey C.G. NADPH-oxidase activity is elevated in penumbral and non-ischemic cerebral arteries following stroke. Brain Res. 2006. 1111(1):111-116.

337. Miranda-Vizuete A., Damdimopoulos A.E., Spyrou G. The mitochondrial thioredoxin system. Antioxid Redox Signal. 2000. 2(4): 801-810.

338. Miranda-Vizuete A., Gustafsson J.A., Spyrou G. Molecular cloning and expression of a cDNA encoding a human thioredoxin-like protein. Biochem Biophys Res Commun. 1998. 243(1): 284-288.

339. Miranda-Vizuete A., Ljung J., Damdimopoulos A.E., Gustafsson J.A., Oko R., Pelto-Huikko M., Spyrou G. Characterization of Sptrx, a novel member of the thioredoxin family specifically expressed in human spermatozoa. J Biol Chem. 2001. 276(34): 31567-31574.

340. Miranda-Vizuete A., Tsang K., Yu Y., Jiménez A., Pelto-Huikko M., Flickinger C.J., Sutovsky P., Oko R. Cloning and developmental analysis of murid spermatid-specific thioredoxin-2 (SPTRX-2), a novel sperm fibrous sheath protein and autoantigen. J Biol Chem. 2003. 278(45): 44874-44885.

341. Miriyala S., Holley A.K., St Clair D.K. Mitochondrial superoxide dismutase--signals of distinction. Anticancer Agents Med Chem. 2011. 11(2): 181-190.

342. Mirkes P.E. 2001 Warkany lecture: to die or not to die, the role of apoptosis in normal and abnormal mammalian development. Teratology. 2002. 65(5):228-239.

343. Miroshnichenko O.S. Biogenesis, the physiological role and properties of catalase. Biopolymers and cell. 1992. 8(6): 3-25 (In Russian).

344. Mishra N.C, Kumar S. Apoptosis: a mitochondrial perspective on cell death. Indian J Exp Biol. 2005. 43(1): 25-34.

345. Miura M. Apoptotic and nonapoptotic caspase functions in animal development. Cold Spring Harb Perspect Biol. 2012. 4(10): a008664

346. Miyashita T., Reed J.C. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell. 1995. 80(2): 293-299.

347. Mohamad N., Gutiérrez A., Núñez M., Cocca C., Martín G., Cricco G., Medina V., Rivera E., Bergoc R. Mitochondrial apoptotic pathways. Biocell. 2005. 29(2): 149-161.

348. Mohri D., Satomi F., Kondo E., Fukuoka T., Sakagami M., Noguchi K. Change in gene expression in facial nerve nuclei and the effect of superoxide dismutase in a rat model of ischemic facial paralysis. Brain Res. 2001. 893(1-2): 227-236.

349. Moon S., Fernando M.R., Lou M.F. Induction of thioltransferase and thioredoxin/thioredoxin reductase systems in cultured porcine lenses under oxidative stress. Invest Ophthalmol Vis Sci. 2005. 46(10): 3783-3789.

350. Morgan M.J., Liu Z.G. Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res. 2011. 21(1): 103-115.

351. Morita-Fujimura Y., Fujimura M., Yoshimoto T., Chan P.H. Superoxide during reperfusion contributes to caspase-8 expression and apoptosis after transient focal stroke. Stroke. 2001. 32(10): 2356-2361.

352. Moro M.A., Almeida A., Bolaños J.P., Lizasoain I. Mitochondrial respiratory chain and free radical generation in stroke. Free Radic Biol Med. 2005. 39(10): 1291-1304.

353. Motohashi H., Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med. 2004. 10(11): 549-557.

354. Nagata S. Apoptosis by death factor. Cell. 1997. 88(3): 355-365.

355. Nakagawa T., Zhu H., Morishima N., Li E., Xu J., Yankner B.A., Yuan J. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature. 2000. 403(6765): 98-103.

356. Nakamura H. Thioredoxin and its related molecules: update 2005. Antioxid Redox Signal. 2005. 7(5-6): 823-828.

357. Nakamura H., Masutani H., Yodoi J. Extracellular thioredoxin and thioredoxin-binding protein 2 in control of cancer. Semin Cancer Biol. 2006. 16(6): 444-451.

358. Nakamura H., Matsuda M., Furuke K., Kitaoka Y., Iwata S., Toda K., Inamoto T., Yamaoka Y., Ozawa K., Yodoi J. Adult T cell leukemia-derived factor/human thioredoxin protects endothelial F-2 cell injury caused by activated neutrophils or hydrogen peroxide. Immunol Lett. 1994. 42(1-2): 75-80.

359. Nakamura H., Mitsui A., Yodoi J. Thioredoxin overexpression in transgenic mice. Methods Enzymol. 2002. 347: 436-440.

360. Nakamura H., Nakamura K., Yodoi J. Redox regulation of cellular activation. Annu Rev Immunol. 1997. 15: 351-369.

361. Nakanishi K., Tajima F., Nakamura A., Yagura S., Ookawara T., Yamashita H., Suzuki K., Taniguchi N., Ohno H. Effects of hypobaric hypoxia on antioxidant enzymes in rats. J Physiol. 1995. 489(Pt 3): 869-876.

362. Narasimhan P., Sugawara T., Liu J., Hayashi T., Noshita N., Chan P.H. Overexpression of human copper/zinc-superoxide dismutase in transgenic animals attenuates the reduction of apurinic/apyrimidinic endonuclease expression in neurons after in vitro ischemia and after transient global cerebral ischemia. J Neurochem. 2005. 93(2):351-358.

363. Niizuma K., Endo H., Nito C., Myer D.J., Chan P.H. Potential role of PUMA in delayed death of hippocampal CA1 neurons after transient global cerebral ischemia. Stroke. 2009. 40(2): 618-625.

364. Niizuma K., Yoshioka H., Chen H., Kim G.S., Jung J.E., Katsu M., Okami N., Chan P.H. Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. Biochim Biophys Acta. 2010. 1802(1): 92-99.

365. Nikoletopoulou V., Markaki M., Palikaras K., Tavernarakis N. Crosstalk between apoptosis, necrosis and autophagy. Biochim Biophys Acta. 2013. 1833(12): 3448-3459.

366. Nishi T., Maier C.M., Hayashi T., Saito A., Chan P.H. Superoxide dismutase 1 overexpression reduces MCP-1 and MIP-1 alpha expression after transient focal cerebral ischemia. J Cereb Blood Flow Metab. 2005. 25(10): 1312-1324.

367. Nishinaka Y., Masutani H., Nakamura H., Yodoi J. Regulatory roles of thioredoxin in oxidative stress-induced cellular responses. Redox Rep. 2001 a. 6(5): 289-295.

368. Nishinaka Y., Nakamura H., Masutani H., Yodoi J. Redox control of cellular function by thioredoxin; a new therapeutic direction in host defence. Arch Immunol Ther Exp (Warsz). 2001 b. 49(4): 285-292.

369. Nishinaka Y., Nishiyama A., Masutani H., Oka S., Ahsan K.M., Nakayama Y., Ishii Y., Nakamura H., Maeda M., Yodoi J. Loss of thioredoxin-binding protein-2/vitamin D3 up-regulated protein 1 in human T-cell leukemia virus type I-dependent T-cell transformation: implications for adult T-cell leukemia leukemogenesis. Cancer Res. 2004. 64(4): 1287-1292.

370. Nishiyama A., Masutani H., Nakamura H., Nishinaka Y., Yodoi J. Redox regulation by thioredoxin and thioredoxin-binding proteins. IUBMB Life. 2001. 52(1-2): 29-33.

371. Nishiyama A., Matsui M., Iwata S., Hirota K., Masutani H., Nakamura H., Takagi Y., Sono H., Gon Y., Yodoi J. Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. J Biol Chem. 1999. 274(31): 21645-21650.

372. Nito C., Kamada H., Endo H., Niizuma K., Myer D.J., Chan P.H. Role of the p38 mitogen-activated protein kinase/cytosolic phospholipase A2 signaling pathway in blood-brain barrier disruption after focal cerebral ischemia and reperfusion. J Cereb Blood Flow Metab. 2008. 28(10): 1686-1696.

373. Nonn L., Williams R.R., Erickson R.P., Powis G. The absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. Mol Cell Biol. 2003. 23(3): 916-922.

374. Nordberg J., Arnér E.S.J. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med. 2001. 31(11): 1287-1312.

375. Noshita N., Sugawara T., Fujimura M., Morita-Fujimura Y., Chan P.H. Manganese superoxide dismutase affects cytochrome c release and caspase-9 activation after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab. 2001. 21(5): 557-567.

376. Noshita N., Sugawara T., Hayashi T., Lewen A., Omar G., Chan P.H. Copper/zinc superoxide dismutase attenuates neuronal cell death by preventing extracellular signal-regulated kinase activation after transient focal cerebral ischemia in mice. J Neurosci. 2002. 22(18): 7923-7930.

377. Noshita N., Sugawara T., Lewen A., Hayashi T., Chan P.H. Copper-zinc superoxide dismutase affects Akt activation after transient focal cerebral ischemia in mice. Stroke. 2003. 34(6): 1513-1518.

378. Oberley L.W., St. Clair D.K., Autor A.P., Oberley T.D. Increase in manganese superoxide dismutase activity in the mouse heart after X-irradiation. Arch Biochem Biophys. 1987. 254(1): 69-80.

379. Obrenovitch T.P. Molecular physiology of preconditioning-induced brain tolerance to ischemia. Physiol Rev. 2008. 88(1): 211-247.

380. Ohtsuki T., Matsumoto M., Suzuki K., Taniguchi N., Kamada T. Effect of transient forebrain ischemia on superoxide dismutases in gerbil hippocampus. Brain Res. 1993. 620(2): 305-309.

381. Okuyama H., Son A., Ahsan M.K., Masutani H., Nakamura H., Yodoi J. Thioredoxin and thioredoxin binding protein 2 in the liver. IUBMB Life. 2008. 60(10): 656-660.

382. Ola M.S., Nawaz M., Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. Mol Cell Biochem. 2011. 351(1-2): 41-58.

383. Ookawara T., Kizaki T., Ohishi S., Yamamoto M., Matsubara O., Ohno H. Purification and subunit structure of extracellular superoxide dismutase from mouse lung tissue. Arch Biochem Biophys. 1997. 340(2): 299-304.

384. Ouyang L., Shi Z., Zhao S., Wang F.T., Zhou T.T., Liu B., Bao J.K. Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. Cell Prolif. 2012. 45(6): 487-498.

385. Pan G., O'Rourke K., Dixit V.M. Caspase-9, Bcl-XL, and Apaf-1 form a ternary complex. J Biol Chem. 1998. 273(10): 5841-5845.

386. Patenaude A., Murthy M.R., Mirault M.E. Emerging roles of thioredoxin cycle enzymes in the central nervous system. Cell Mol Life Sci. 2005. 62(10): 1063-1080.

387. Patenaude A., Ven Murthy M.R., Mirault M.E. Mitochondrial thioredoxin system: effects of TrxR2 overexpression on redox balance, cell growth, and apoptosis. J Biol Chem. 2004. 279(26): 27302-27314.

388. Patten D.A., Germain M., Kelly M.A., Slack R.S. Reactive oxygen species: stuck in the middle of neurodegeneration. J Alzheimers Dis. 2010. 20 Suppl 2: S357- S367.

389. Patwari P., Higgins L.J., Chutkow W.A., Yoshioka J., Lee R.T. The interaction of thioredoxin with Txnip. Evidence for formation of a mixed disulfide by disulfide exchange. J Biol Chem. 2006. 281(31): 21884-21891.

390. Paxinos G., Watson C. The rat brain in stereotaxic coordinates. Second edition. Sydney, Orlando, San Diego, New York, Austin, London, Montreal, Tokyo, Toronto: Academic press. 1986.

391. Pedrajas J.R., Kosmidou E., Miranda-Vizuete A., Gustafsson J.A., Wright A.P., Spyrou G. Identification and functional characterization of a novel mitochondrial thioredoxin system in Saccharomyces cerevisiae. J Biol Chem. 1999. 274(10): 6366-6373.

392. Pinto M.C., Mourão F.A., Binda N.S., Leite H.R., Gomez M.V., Massensini A.R., Gomez R.S. Pharmacological induction of ischemic tolerance in hippocampal slices by sarcosine preconditioning. Neurochem Int. 2012. 61(5): 713-720.

393. Pohle W., Rauca C. Does a moderate hypoxia induce manganese-superoxide dismutase or heat shock proteins to act as possible protective factors? Naunyn Schmiedebergs Arch Pharmacol. 1997. 356(6): 746-749.

394. Portnichenko V.I., Nosar' V.I., Portnichenko A.G., Drevitskaia T.I., Sidorenko A.M., Man'kovskaia I.N. Phase changes in energy metabolism during periodic hypoxia. Fiziol Zh. 2012 a. 58(4): 3-12 (In Russian, summary in English).

395. Portnichenko V.I., Nosar V.I., Sydorenko A.M., Portnichenko A.H., Man'kovs'ka I.M. Continuous adaptation of rats to hypobaric hypoxia prevents stressor hyperglycemia and optimizes mitochondrial respiration under acute hypoxia. Fiziol Zh. 2012 b. 58(5): 56-64 (In Ukrainian, summary in English).

396. Powis G., Gasdaska J.R., Gasdaska P.Y., Berggren M., Kirkpatrick D.L., Engman L., Cotgreave I.A., Angulo M., Baker A. Selenium and the thioredoxin redox system: effects on cell growth and death. Oncol Res. 1997. 9(6-7): 303-312.

397. Powis G., Kirkpatrick D.L., Angulo M., Baker A. Thioredoxin redox control of cell growth and death and the effects of inhibitors. Chem Biol Interact. 1998. 111-112: 23-34.

398. Powis G., Montfort W.R. Properties and biological activities of thioredoxins. Annu Rev Pharmacol Toxicol. 2001 a. 41: 261-295.

399. Powis G., Montfort W.R. Properties and biological activities of thioredoxins. Annu Rev Biophys Biomol Struct. 2001 b. 30: 421-455.

400. Powis G., Mustacich D., Coon A. The role of the redox protein thioredoxin in cell growth and cancer. Free Radic Biol Med. 2000. 29(3-4): 312-322.

401. Qi S., Zhan R.Z., Wu C., Fujihara H., Yamakura T., Baba H., Taga K., Shimoji K. Sublethal cerebral ischemia inhibits caspase-3 activation induced by subsequent prolonged ischemia in the C57Black/Crj6 strain mouse. Neurosci Lett. 2001. 315(3): 133-136.

402. Qu Z.W., Miao W.Y., Hu S.Q., Li C., Zhuo X.L., Zong Y.Y., Wu Y.P., Zhang G.Y. N-methyl-D-aspartate receptor-dependent denitrosylation of neuronal nitric oxide synthase increase the enzyme activity. PLoS One. 2012. 7(12): e52788.

403. Queiroga C.S., Tomasi S., Widerøe M., Alves P.M., Vercelli A., Vieira H.L. Preconditioning triggered by carbon monoxide (CO) provides neuronal protection following perinatal hypoxia-ischemia. PLoS One. 2012. 7(8): e42632.

404. Quinlan C.L., Perevoshchikova I.V., Hey-Mogensen M., Orr A.L., Brand M.D. Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. Redox Biol. 2013. 1(1): 304-312.

405. Radák Z., Kaneko T., Tahara S., Nakamoto H., Pucsok J., Sasvári M., Nyakas C., Goto S. Regular exercise improves cognitive function and decreases oxidative damage in rat brain. Neurochem Int. 2001. 38(1): 17-23.

406. Radak Z., Koltai E., Taylor A.W., Higuchi M., Kumagai S., Ohno H., Goto S., Boldogh I. Redox-regulating sirtuins in aging, caloric restriction, and exercise. Free Radic Biol Med. 2013. 58: 87-97.

407. Radak Z., Kumagai S., Taylor A.W., Naito H., Goto S. Effects of exercise on brain function: role of free radicals. Appl Physiol Nutr Metab. 2007. 32(5): 942-946.

408. Radak Z., Toldy A., Szabo Z., Siamilis S., Nyakas C., Silye G., Jakus J., Goto S. The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochem Int. 2006. 49(4): 387-392.

409. Raffaello A., Rizzuto R. Mitochondrial longevity pathways. Biochim Biophys Acta. 2011. 1813(1): 260-268.

410. Ralph S.J., Moreno-Sánchez R., Neuzil J., Rodríguez-Enríquez S. Inhibitors of succinate: quinone reductase/Complex II regulate production of mitochondrial reactive oxygen species and protect normal cells from ischemic damage but induce specific cancer cell death. Pharm Res. 2011. 28(11): 2695-2730.

411. Rao R.V., Castro-Obregon S., Frankowski H., Schuler M., Stoka V., del Rio G., Bredesen D.E., Ellerby H.M. Coupling endoplasmic reticulum stress to the cell death program. An Apaf-1-independent intrinsic pathway. J Biol Chem. 2002. 277(24): 21836-21842.

412. Rauca C., Zerbe R., Jantze H., Krug M. The importance of free hydroxyl radicals to hypoxia preconditioning. Brain Res. 2000. 868(1): 147-149.

413. Ravati A., Ahlemeyer B., Becker A., Klumpp S., Krieglstein J. Preconditioning-induced neuroprotection is mediated by reactive oxygen species and activation of the transcription factor nuclear factor-κB. J Neurochem. 2001. 78(4): 909-919.

414. Ravati A., Ahlemeyer B., Becker A., Krieglstein J. Preconditioning-induced neuroprotection is mediated by reactive oxygen species. Brain Res. 2000. 866(1-2): 23-32.

415. Ray P.D., Huang B.W., Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal. 2012. 24(5): 981-990.

416. Reed J.C. Cytochrome c: can't live with it--can't live without it. Cell. 1997. 91(5): 559-562.

417. Reubold T.F., Eschenburg S. A molecular view on signal transduction by the apoptosome. Cell Signal. 2012. 24(7): 1420-1425.

418. Rhee S.G., Chae H.Z., Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic Biol Med. 2005. 38(12): 1543-1552.

419. Rhee S.G., Kang S.W., Chang T.S., Jeong W., Kim K. Peroxiredoxin, a novel family of peroxidases. IUBMB Life. 2001. 52(1-2): 35-41.

420. Rhee S.G., Woo H.A. Multiple functions of peroxiredoxins: peroxidases, sensors and regulators of the intracellular messenger $H_2O_2$, and protein chaperones. Antioxid Redox Signal. 2011. 15(3): 781-794.

421. Rhee S.G., Woo H.A., Kil I.S., Bae S.H. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. J Biol Chem. 2012. 287(7): 4403-4410.

422. Riedl S.J., Salvesen G.S. The apoptosome: signalling platform of cell death. Nat Rev Mol Cell Biol. 2007. 8(5): 405-413.

423. Rigobello M.P., Callegaro M.T., Barzon E., Benetti M., Bindoli A. Purification of mitochondrial thioredoxin reductase and its involvement in the redox regulation of membrane permeability. Free Radic Biol Med. 1998. 24(2): 370-376.

424. Romanovskii D.Y., Tyul'kova E.I., Samoilov M.O. Preconditioning hypobaric hypoxia prevents anoxia-induced inhibition of generation of focal potentials in slices of olfactory cortex from rat brain. Bull Exp Biol Med. 2001. 132(6): 1154-1156.

425. Romera C, Hurtado O, Botella SH, Lizasoain I, Cárdenas A, Fernández-Tomé P, Leza JC, Lorenzo P, Moro MA. In vitro ischemic tolerance involves upregulation of glutamate transport partly mediated by the TACE/ADAM17-tumor necrosis factor-alpha pathway. J Neurosci. 2004. 24(6):1350-1357.

426. Rüdiger H.A., Graf R., Clavien P.A. Sub-lethal oxidative stress triggers the protective effects of ischemic preconditioning in the mouse liver. J Hepatol. 2003. 39(6): 972–977.

427. Rudner J., Lepple-Wienhues A., Budach W., Berschauer J., Friedrich B., Wesselborg S., Schulze-Osthoff K., Belka C. Wild-type, mitochondrial and ER-restricted Bcl-2 inhibit DNA damage-induced apoptosis but do not affect death receptor-induced apoptosis. J Cell Sci. 2001. 114 (Pt 23): 4161-4172.

428. Rupp K., Birnbach U., Lundstrom J., Van P.N., Soling H.D. Effects of CaBP2, the rat analog of ERp72, and of CaBP1 on the refolding of denatured reduced proteins. Comparison with protein disulfide isomerase. J Biol Chem. 1994. 269(4): 2501-2507.

429. Rybnikova E., Glushchenko T., Churilova A., Pivina S., Samoilov M. Expression of glucocorticoid and mineralocorticoid receptors in hippocampus of rats exposed to various modes of hypobaric hypoxia: Putative role in hypoxic preconditioning. Brain Res. 2011. 1381: 66-77.

430. Rybnikova E., Gluschenko T., Tulkova E., Churilova A., Jaroshevich O., Baranova K., Samoilov M. Preconditioning induces prolonged expression of transcription factors pCREB and NF-kappa B in the neocortex of rats before and following severe hypobaric hypoxia. J Neurochem. 2008 a. 106(3): 1450-1458.

431. Rybnikova E., Glushchenko T., Tyulkova E., Baranova K., Samoilov M. Mild hypobaric hypoxia preconditioning up-regulates expression of transcription factors c-Fos and NGFI-A in rat neocortex and hippocampus. Neurosci Res. 2009. 65(4): 360-366.

432. Rybnikova E.A., Khozhai L.I., Tyul'kova E.I., Glushchenko T.S., Sitnik N.A., Pelto-Huikko M., Otellin V.A., Samoilov M.O. Expression of early gene proteins, structural changes in brain neurons in hypobaric hypoxia, and the correcting effects of preconditioning. Neurosci Behav Physiol. 2005 a. 35(4): 383-388.

433. Rybnikova E.A., Mironova V.I., Pivina S.G., Ordyan N.E., Tyulkova E.I., Samoilov M.O. Hypoxic preconditioning prevents development of post-stress depressions in rats. Dokl Biol Sci. 2006 a. 411: 431-433.

434. Rybnikova E.A., Mironova V.I., Pivina S.G., Ordyan N.E., Tulkova E.I., Samoilov M.O. Hormonal mechanisms of neuroprotective effects of the mild hypoxic preconditioning in rats. Dokl Biol Sci. 2008 b. 421: 239-240.

435. Rybnikova E., Mironova V., Pivina S., Tulkova E., Ordyan N., Nalivaeva N., Turner A., Samoilov M. Involvement of the hypothalamic-pituitary-adrenal axis in the antidepressant-like effects of mild hypoxic preconditioning in rats. Psychoneuroendocrinology. 2007 a. 32(7): 813-823.

436. Rybnikova E., Mironova V., Pivina S., Tulkova E., Ordyan N., Vataeva L., Vershinina E., Abritalin E., Kolchev A., Nalivaeva N., Turner A.J., Samoilov M. Antidepressant-like effects of mild hypoxia preconditioning in the learned helplessness model in rats. Neurosci Lett. 2007 b. 417(3): 234-239.

437. Rybnikova E.A., Mironova V.I., Tiul'kova E.I., Samoĭlov M.O. The anxyolytic effect of mild hypobaric hypoxia in a model of post-traumatic stress disorder in rats. Zh Vyssh Nerv Deiat Im I P Pavlova. 2008 c. 58(4): 486-492 (In Russian, summary in English).

438. Rybnikova E.A., Samoilov M.O., Mironova V.I., Tyul'kova E.I., Pivina S.G., Vataeva L.A., Ordyan N.E., Abritalin E.Y., Kolchev A.I. The possible use of hypoxic preconditioning for the prophylaxis of post-stress depressive episodes. Neurosci Behav Physiol. 2008 d. 38(7): 721-726.

439. Rybnikova E., Sitnik N., Gluschenko T., Tjulkova E., Samoilov M.O. The preconditioning modified neuronal expression of apoptosis-related proteins of Bcl-2 superfamily following severe hypobaric hypoxia in rats. Brain Res. 2006 b. 1089(1): 195-202.

440. Rybnikova E., Tulkova E., Pelto-Huikko M., Samoilov M. Mild preconditioning hypoxia modifies nerve growth factor-induced gene A messenger RNA expression in the rat brain induced by severe hypoxia. Neurosci Lett. 2002. 329(1): 49-52.

441. Rybnikova E., Vataeva L., Tyulkova E., Gluschenko T., Otellin V., Pelto-Huikko M., Samoilov M.O. Mild hypoxia preconditioning prevents impairment of passive avoidance learning and suppression of brain NGFI-A expression induced by severe hypoxia. Behav Brain Res. 2005 b. 160(1): 107-114.

442. Rybnikova E., Vorobyev M., Pivina S., Samoilov M. Postconditioning by mild hypoxic exposures reduces rat brain injury caused by severe hypoxia. Neurosci Lett. 2012 a. 513(1): 100-105.

443. Rybnikova E.A., Vorob'ev M.G., Pivina S.G., Samoĭlov M.O. A comparison of a neuroprotective effects of hypoxic postconditioning and cerebrolysin in the experimental model. Zh Nevrol Psikhiatr Im S S Korsakova. 2013. 113(2): 54-58 (in Russian).

444. Rybnikova E.A., Vorob'ev M.G., Samoĭlov M.O. Hypoxic postconditioning corrects behavioral abnormalities in a model of post-traumatic stress disorder in rats. Zh Vyssh Nerv Deiat Im I P Pavlova. 2012 b. 62(3): 364-371 (In Russian, summary in English).

445. Sablina A.A., Budanov A.V., Ilyinskaya G.V., Agapova L.S., Kravchenko J.E., Chumakov P.M. The antioxidant function of the p53 tumor suppressor. Nat Med. 2005. 11(12): 1306-1313.

446. Sachi Y., Hirota K., Masutani H., Toda K., Okamoto T., Takigawa M., Yodoi J. Induction of ADF/TRX by oxidative stress in keratinocytes and lymphoid cells. Immunol Lett. 1995. 44(2-3): 189-193.

447. Sadek C.M., Damdimopoulos A.E., Pelto-Huikko M., Gustafsson J.A., Spyrou G., Miranda-Vizuete A. Sptrx-2, a fusion protein composed of one thioredoxin and three tandemly repeated NDP-kinase domains is expressed in human testis germ cells. Genes Cells. 2001. 6(12): 1077-1090.

448. Sadek C.M., Jiménez A., Damdimopoulos A.E., Kieselbach T., Nord M., Gustafsson J.A., Spyrou G., Davis E.C., Oko R., van der Hoorn F.A., Miranda-Vizuete A. Characterization of human thioredoxin-like 2. A novel microtubule-binding thioredoxin expressed predominantly in the cilia of lung airway epithelium and spermatid manchette and axoneme. J Biol Chem. 2003. 278(15): 13133-13142.

449. Saelens X., Festjens N., Vande Walle L., van Gurp M., van Loo G., Vandenabeele P. Toxic proteins released from mitochondria in cell death. Oncogene. 2004. 23(16): 2861-2874.

450. Saito A., Hayashi T., Okuno S., Ferrand-Drake M., Chan P.H. Overexpression of copper/zinc superoxide dismutase in transgenic mice protects against neuronal cell death after transient focal ischemia by blocking activation of the Bad cell death signaling pathway. J Neurosci. 2003. 23(5): 1710-1718.

451. Saito A., Hayashi T., Okuno S., Nishi T., Chan P.H. Oxidative stress affects the integrin-linked kinase signaling pathway after transient focal cerebral ischemia. Stroke. 2004 a. 35(11): 2560-2565.

452. Saito A., Hayashi T., Okuno S., Nishi T., Chan P.H. Modulation of the Omi/HtrA2 signaling pathway after transient focal cerebral ischemia in mouse brains that overexpress SOD1. Brain Res Mol Brain Res. 2004 b. 127(1-2): 89-95.

453. Saito A., Hayashi T., Okuno S., Nishi T., Chan P.H. Modulation of p53 degradation via MDM2-mediated ubiquitylation and the ubiquitin-proteasome system during reperfusion after stroke: role of oxidative stress. J Cereb Blood Flow Metab. 2005. 25(2): 267-280.

454. Saito A., Hayashi T., Okuno S., Nishi T., Chan P.H. Modulation of proline-rich akt substrate survival signaling pathways by oxidative stress in mouse brains after transient focal cerebral ischemia. Stroke. 2006. 37(2): 513-517.

455. Saitoh M., Nishitoh H., Fujii M., Takeda K., Tobiume K., Sawada Y., Kawabata M., Miyazono K., Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J. 1998. 17(9): 2596-2606.

456. Sakahira H., Enari M., Nagata S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature. 1998. 391(6662): 96-99.

457. Sakata H., Niizuma K., Yoshioka H., Kim G.S., Jung J.E., Katsu M., Narasimhan P., Maier C.M., Nishiyama Y., Chan P.H. Minocycline-preconditioned neural stem cells enhance neuroprotection after ischemic stroke in rats. J Neurosci. 2012. 32(10): 3462-3473.

458. Samoilov M.O. Reactions of brain neurons to the hypoxia. Leningrad. Nauka, 1985. 190 p. (In Russian).

459. Samoilov M.O. Brain and adaptation. Molecular and cellular mechanisms. St. Petersburg, 1999. 272 p. (In Russian).

460. Samoĭlov M.O., Churilova A.V., Glushchenko T.S., Rybnikova E.A. Effects of several modes of hypobaric hypoxia on expression profiles of corticosteroid receptors in rat hippocampus. Ross Fiziol Zh Im I M Sechenova. 2012 a. 98(11): 1380-1395 (In Russian, summary in English).

461. Samoĭlov M.O., Lazarevich E.V., Semenov D.G., Mokrushin A.A., Tiul'kova E.I., Romanovskiĭ D.Iu., Miliakova E.A., Dudkin K.N. Adaptive effects of hypoxic preconditioning in brain neurons. Ross Fiziol Zh Im IM Sechenova. 2001 a. 87(6): 714-729 (In Russian).

462. Samoilov M.O., Lazarevich E.V., Semenov D.G., Mokrushin A.A., Tyul'kova E.I., Romanovskii D.Y., Milyakova E.A., Dudkin K.N. The adaptive effects of hypoxic preconditioning of brain neurons. Neurosci Behav Physiol. 2003 a. 33(1): 1-11.

463. Samoĭlov M.O., Mokrushin A.A. Peptide modulation of synaptic plasticity induced by anoxia. Dokl Akad Nauk. 1997. 357(4): 565-567 (In Russian).

464. Samoĭlov M.O., Rybnikova E.A. Molecular-cellular and hormonal mechanisms of induced brain tolerance of extreme factors. Ross Fiziol Zh Im I M Sechenova. 2012. 98(1): 108-126 (In Russian, summary in English).

465. Samoĭlov M.O., Rybnikova E.A., Churilova A.V. Signal molecular and hormonal mechanisms of formation of the hypoxic preconditioning protective effects. Patol Fiziol Eksp Ter. 2012 b. 3: 3-10 (In Russian).

466. Samoilov M.O., Rybnikova E.A., Tjulkova E.I., Spyrou G., Pelto-Huikko M. The mitochondrial antioxidants thioredoxin-2 and Mn-superoxide dismutase are involved in the mechanisms of brain hypoxic tolerance. Dokl Biol Sci. 2002. 387: 498-500.

467. Samoilov M.O., Rybnikova E.A., Tulkova E.I., Vataeva L.A., Otellin V.A., Hozhai L.I., Pelto-Huikko M. Hypobaric hypoxia affects rat behavior and immediate early gene expression in the brain: the corrective effect of preconditioning. Dokl Biol Sci. 2001 b. 381: 513-515.

468. Samoĭlov M.O., Semenov D.G., Tiul'kova E.I., Bolekhan E.A. The molecular cellular mechanisms of the protective effect of short-term anoxia. Fiziol Zh Im IM Sechenova. 1994. 80(12): 71-75 (In Russian).

469. Samoilov M.O., Semenov D.G., Tulkova E.I., Rybnikova E.A., Vataeva L.A., Glushchenko T.S., Stroev S.A., Miller O.L. Molecular mechanisms of short- and long-time effects of hypoxic precongitioning. In book: Problems of hypoxia: molecular, physiological and medical aspects. Editors – Lukjanova L.D., Ushakov I.B. Moscow, RAMS, Voronezh "Istoki". 2003 b. 585 p. P. 96-101 (In Russian).

470. Samoilov M.O., Sitnik N.A., Rybnikova E.A., Gluschenko T.S., Tjulkova E.I. The expression pattern of pro- and antiapoptotic proteins bax and Bcl-2 in rat brain neurons in response to severe hypobaric hypoxia: the correcting effect of hypoxic preconditioning. Dokl Biol Sci. 2005. 402: 176-178.

471. Sanchis-Gomar F. Sestrins: novel antioxidant and AMPK-modulating functions regulated by exercise? J Cell Physiol. 2013. 228(8): 1647-1650.

472. Sasada T., Iwata S., Sato N., Kitaoka Y., Hirota K., Nakamura K., Nishiyama A., Taniguchi Y., Takabayashi A., Yodoi J. Redox control of resistance to cis-diamminedichloroplatinum (II) (CDDP): protective effect of human thioredoxin against CDDP-induced cytotoxicity. J Clin Invest. 1996. 97(10): 2268-2276.

473. Sasaki H., Matsuno T., Nakagawa K., Matsuoka J., Tanaka N. Superoxide induces hepatocyte apoptosis during the early phase of reperfusion after murine liver ischemia. Transplant Proc. 1998. 30(7): 2958-2959.

474. Schmidt-Kastner R., Aguirre-Chen C., Kietzmann T., Saul I., Busto R., Ginsberg M.D. Nuclear localization of the hypoxia-regulated pro-apoptotic protein BNIP3 after global brain ischemia in the rat hippocampus. Brain Res. 2004. 1001(1-2): 133-142.

475. Schremmer B., Manevich Y., Feinstein S.I., Fisher A.B. Peroxiredoxins in the lung with emphasis on peroxiredoxin VI. Subcell Biochem. 2007. 44: 317-344.

476. Schweizer U., Bräuer A.U., Köhrle J., Nitsch R., Savaskan N.E. Selenium and brain function: a poorly recognized liaison. Brain Res Brain Res Rev. 2004. 45(3): 164-178.

477. Selye H. A Syndrome produced by Diverse Nocuous Agents. Nature. 1936. 138 (3479): 32.

478. Selye H. Experimental evidence supporting the conception of "adaptation energy". Am. J. Physiol. 1938. 123: 758-765.

479. Selye H. Stress and disease. Science. 1955. 122 (3171): 625–631.

480. Selye H. The stress of life. New York: McGraw-Hill. 1956.

481. Selye H. Stress without distress. Philadelphia: J. B. Lippincott Co. 1974.

482. Semenov D.G., Beliakov A.V., Samoĭlov M.O. Comparison of Ca2+ responses to stimulation of glutamate receptors in the rat brain cortex following hypobaric hypoxia of different degree. Ross Fiziol Zh Im I M Sechenova. 2009 a. 95(9): 977-983 (In Russian, summary in English).

483. Semenov D.G., Beliakov A.V., Glushchenko T.S., Samoĭlov M.O. Participation of metabotropic glutamate receptors of the brain in mechanizms of hypoxic signaling. Patol Fiziol Eksp Ter. 2012. 3: 11-19 (In Russian).

484. Semenov D.G., Belyakov A.V., Samoilov M.O. Moderate hypobaric hypoxia modifies Ca2+-mediated glutamatergic signal transduction in rat cerebral cortex. Bull Exp Biol Med. 2009 b. 147(2): 185-188.

485. Semenov D.G., Belyakov A.V., Samoilov M.O. Comparison of $Ca^2$+ responses to stimulation of glutamate receptors in the rat cerebral cortex after hypobaric hypoxia of different severities. Neurosci Behav Physiol. 2010. 40(9): 1012-1016.

486. Semenov D.G., Miller O.L., Samoilov M.O. Effect of in vivo hypoxic preconditioning on changes in intracellular calcium content induced by long-term anoxia in rat brain slices. Bull Exp Biol Med. 2004. 138(4): 338-340.

487. Semenov D.G., Samoilov M.O., Lazarewicz J.W. Calcium transients in the model of rapidly induced anoxic tolerance in rat cortical slices: involvement of NMDA receptors. Neurosignals. 2002. 11(6): 329-335.

488. Semenov D.G., Samoilov M.O., Lazarewicz J.W. Preconditioning reduces hypoxia-evoked alterations in glutamatergic Ca2+ signaling in rat cortex. Acta Neurobiol Exp (Wars). 2008. 68(2): 169-179.

489. Seo M.S., Kang S.W., Kim K., Baines I.C., Lee T.H., Rhee S.G. Identification of a new type of mammalian peroxiredoxin that forms an intramolecular disulfide as a reaction intermediate. J Biol Chem. 2000. 275(27): 20346-20354.

490. Serebrovskaya T.V., Manukhina E.B., Smith M.L., Downey H.F., Mallet R.T. Intermittent hypoxia: cause of or therapy for systemic hypertension? Exp Biol Med (Maywood). 2008. 233(6): 627-650.

491. Serrano J., Encinas J.M., Salas E., Fernandez A.P., Castro-Blanco S., Fernandez-Vizarra P., Bentura M.L., Rodrigo J. Hypobaric hypoxia modifies constitutive nitric oxide synthase activity and protein nitration in the rat cerebellum. Brain Res. 2003. 976(1): 109-119.

492. Sharma A., Singh S.B., Panjwani U., Yadav D.K., Amitabh K., Singh S., Selvamurthy W. Effect of a carbohydrate supplement on feeding behaviour and exercise in rats exposed to hypobaric hypoxia. Appetite. 2002. 39(2): 127-135.

493. Sharp F.R., Ran R., Lu A., Tang Y., Strauss K.I., Glass T., Ardizzone T., Bernaudin M. Hypoxic preconditioning protects against ischemic brain injury. NeuroRx. 2004. 1(1): 26-35.

494. Sherstnev V.V. The role of cellular development and cell death in neurochemical organization and integrative brain functions--normal and pathological. Med Pregl. 2004. 57(3-4): 120-124.

495. Sherstnev V.V., Yurasov V.V., Storozheva Z.I., Gruden' M.A., Yakovleva N.E. Biochemical markers of apoptosis in different parts of the brain during learning. Neurosci Behav Physiol. 2006. 36(9): 915-919.

496. Shi Q., Fu J., Ge D., He Y., Ran J., Liu Z., Wei J., Diao T., Lu Y. Huperzine A ameliorates cognitive deficits and oxidative stress in the hippocampus of rats exposed to acute hypobaric hypoxia. Neurochem Res. 2012. 37(9): 2042-2052.

497. Shi Y. Mechanical aspects of apoptosome assembly. Curr Opin Cell Biol. 2006. 18(6): 677-684.

498. Shukitt-Hale B., Stillman M.J., Lieberman H.R. Tyrosine administration prevents hypoxia-induced decrements in learning and memory. Physiol Behav. 1996. 59(4-5): 867-871.

499. Shukitt-Hale B., Stillman M.J., Welch D.I., Levy A., Devine J.A., Lieberman H.R. Hypobaric hypoxia impairs spatial memory in an elevation-dependent fashion. Behav Neural Biol. 1994. 62(3): 244-252.

500. Siesjö B.K. Brain energy metabolism. Chichester ets., 1978. 607 p.

501. Simonova Z., Sterbova K., Brozek G., Komarek V., Sykova E. Postnatal hypobaric hypoxia in rats impairs water maze learning and the morphology of neurones and macroglia in cortex and hippocampus. Behav Brain Res. 2003. 141(2): 195-205.

502. Sims N.R., Anderson M.F. Mitochondrial contributions to tissue damage in stroke. Neurochem Int. 2002. 40(6): 511-526.

503. Singh S.B., Sharma A., Panjwani U., Yadav D.K., Chandra K., Sharma K.N., Selvamurthy W. Hypobaric hypoxia and hedonic matrix in rats. Jpn J Physiol. 1997. 47(4): 327-333.

504. Sirotinin M.M. Life at highlands and the altitude sicknesses. Kiev. 1939 (In Ukrainian).

505. Siu F.K., Lo S.C., Leung M.C. Electro-acupuncture potentiates the disulphide-reducing activities of thioredoxin system by increasing thioredoxin expression in ischemia-reperfused rat brains. Life Sci. 2005. 77(4): 386-399.

506. Slee E.A., Harte M.T., Kluck R.M., Wolf B.B., Casiano C.A., Newmeyer D.D., Wang H.G., Reed J.C., Nicholson D.W., Alnemri E.S., Green D.R., Martin S.J. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspase-2, -3, -6, -7, -8 and -10 in a caspase-9-dependent manner. J Cell Biol. 1999. 144(2): 281-292.

507. Slot J.W., Geuze H.J., Freeman B.A., Crapo J.D. Intracellular localization of the copper-zinc and manganese superoxide dismutases in rat liver parenchymal cells. Lab Invest. 1986. 55(3): 363–371.

508. Somani S.M., Husain K. Interaction of exercise training and chronic ethanol ingestion on antioxidant system of rat brain regions. J Appl Toxicol. 1997. 17(5): 329-336.

509. Somani S.M., Ravi R., Rybak L.P. Effect of exercise training on antioxidant system in brain regions of rat. Pharmacol Biochem Behav. 1995. 50(4): 635-639.

510. Song Y.S., Lee Y.S., Chan P.H. Oxidative stress transiently decreases the IKK complex (IKKalpha, beta, and gamma), an upstream component of NF-kappaB signaling, after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab. 2005. 25(10): 1301-1311.

511. Song Y.S., Lee Y.S., Narasimhan P., Chan P.H. Reduced oxidative stress promotes NF-kappaB-mediated neuroprotective gene expression after transient focal cerebral ischemia: lymphocytotrophic cytokines and antiapoptotic factors. J Cereb Blood Flow Metab. 2007. 27(4): 764-775.

512. Spierings D., McStay G., Saleh M., Bender C., Chipuk J., Maurer U., Green D.R. Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. Science. 2005. 310(5745): 66-67.

513. Spyrou G., Enmark E., Miranda-Vizuete A., Gustafsson J. Cloning and expression of a novel mammalian thioredoxin. J Biol Chem. 1997. 272(5): 2936-2941.

514. Stenzel-Poore M.P., Stevens S.L., Xiong Z., Lessov N.S., Harrington C.A., Mori M., Meller R., Rosenzweig H.L., Tobar E., Shaw T.E., Chu X., Simon R.P. Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. Lancet. 2003. 362(9389): 1028-1037.

515. Stepanichev M.Yu., Kudryashova I.V., Yakovlev A.A., Onufriev M.V., Khaspekov L.G., Lyzhin A.A., Lazareva N.A., Gulyaeva N.V. Central administration of a caspase inhibitor impairs shuttle-box performance in rats. Neuroscience. 2005. 136(2): 579-591.

516. Stetler R.A., Zhang F., Liu C., Chen J. Ischemic tolerance as an active and intrinsic neuroprotective mechanism. Handb Clin Neurol. 2009. 92: 171-195.

517. Stroev S.A., Gluschenko T.S., Tjulkova E.I., Rybnikova E.A., Samoilov M.O., Pelto-Huikko M. Induction of manganese superoxide dismutase expression after severe hypobaric hypoxia in the hippocampus of preconditioned and non-preconditioned rats. Nejrokhimia 2005. 22(4): 292-298. (In Russian, summary in English).

518. Stroev S.A., Samoilov M.O. Endogenous antioxidants and brain hypoxic tolerance. St. Petersburg: I.P. Pavlov Institute of Physiology of RAS, 2006. 145 p. (In Russian, summary in English).

519. Stroev S.A., Tjulkova E.I., Samoilov M.O., Pelto-Huikko M. Influence of moderate hypobaric hypoxia to the expression of peptide antioxidants in brain. Materials of Russian-Polish working symposium «Hypoxic, ischemic preconditioning of brain». 2008. P. 102-107 (In Russian).

520. Stroev S.A., Tjulkova E.I., Samoilov M.O., Pelto-Huikko M. Expression of endogenous antioxidants in rat hippocampal neurons after four kinds of hypobaric hypoxia modeling high altitude. In: Selected abstracts from the symposium on the effects of chronic hypoxia on diseases at high altitude, august 2008, La Paz, Bolivia. Wilderness Environ Med. 2009. 20(2): 181–191.

521. Stroev S.A., Tyul'kova E.I., Vataeva L.A., Samoilov M.O., Pelto-Huikko M.T. Effects of prenatal hypoxia on expression of thioredoxin-1 in the rat hippocampus at different stages of postnatal ontogeny. Neurochemical Journal. 2011. 5(3): 200–204.

522. Sugawara T., Chan P.H. Reactive oxygen radicals and pathogenesis of neuronal death after cerebral ischemia. Antioxid Redox Signal. 2003. 5(5): 597-607.

523. Sugawara T., Noshita N., Lewen A., Gasche Y., Ferrand-Drake M., Fujimura M., Morita-Fujimura Y., Chan P.H. Overexpression of copper/zinc-superoxide dismutase in transgenic rats protects vulnerable neurons against ischemic damage by blocking the mitochondrial pathway of caspase activation. J. of Neurosci. 2002. 22(1): 209-217.

524. Sugaya K., Chouinard M., Greene R., Robbins M., Personett D., Kent C., Gallagher M., McKinney M. Molecular indices of neuronal and glial plasticity in the hippocampal formation in a rodent model of age-induced spatial learning impairment. J. Neurosci. 1996. 16(10): 3427-3443.

525. Sugino T., Nozaki K., Takagi Y., Hashimoto N. 3-Nitropropionic acid induces ischemic tolerance in gerbil hippocampus in vivo. Neurosci Lett. 1999. 259(1): 9-12.

526. Sun N., Hao J.R., Li X.Y., Yin X.H., Zong Y.Y., Zhang G.Y., Gao C. GluR6-FasL-Trx2 mediates denitrosylation and activation of procaspase-3 in cerebral ischemia/reperfusion in rats. Cell Death Dis. 2013. 4: e771.

527. Sun X.C., Li L., Zhang M., Li W.B., Li Q.J., Zhao L. Division of CA1, CA3 and DG regions of the hippocampus of Wistar rat. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2012. 28(2): 189-192 (In Chinese, summary in English).

528. Sung J.H., Cho E.H., Kim M.O., Koh P.O. Identification of proteins differentially expressed by melatonin treatment in cerebral ischemic injury – a proteomics approach. J Pineal Res. 2009. 46(3): 300-306.

529. Susin S.A., Zamzami N., Castedo M., Daugas E., Wang H.G., Geley S., Fassy F., Reed J.C., Kroemer G. The central executioner of apoptosis: multiple connections between protease activation and mitochondria in Fas/APO-1/CD95- and ceramide-induced apoptosis. J Exp Med. 1997. 186(1): 25-37.

530. Sutherland G., Bose R., Louw D., Pinsky C. Global elevation of brain superoxide dismutase activity following forebrain ischemia in rat. Neurosci Lett. 1991. 128(2): 169-172.

531. Szabo C. The pathophysiological role of peroxinitrite in shock, inflammation, and ischemia-reperfusion injury. Shock. 1996. 6(2): 79–88.

532. Tait S.W., Green D.R. Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol. 2010. 11(9): 621-632.

533. Takagi Y., Horikawa F., Nozaki K., Sugino T., Hashimoto N., Yodoi J. Expression and distribution of redox regulatory protein, thioredoxin during transient focal brain ischemia in the rat. Neurosci Lett. 1998 a. 251(1): 25-28.

534. Takagi Y., Mitsui A., Nishiyama A., Nozaki K., Sono H., Gon Y., Hashimoto N., Yodoi J. Overexpression of thioredoxin in transgenic mice attenuates focal ischemic brain damage. Proc Natl Acad Sci U S A. 1999. 96(7): 4131-4136.

535. Takagi Y., Tokime T., Nozaki K., Gon Y., Kikuchi H., Yodoi J. Redox control of neuronal damage during brain ischemia after middle cerebral artery occlusion in the rat: immunohistochemical and hybridization studies of thioredoxin. J Cereb Blood Flow Metab. 1998 b. 18(2): 206-214.

536. Takeuchi A., Miyaishi O., Kiuchi K., Isobe K. Cu/Zn- and Mn-superoxide dismutases are specifically up-regulated in neurons after focal brain injury. J Neurobiol. 2000. 45(1): 39-46.

537. Tanaka N., Ikeda Y., Ohta Y., Deguchi K., Tian F., Shang J., Matsuura T., Abe K. Expression of Keap1-Nrf2 system and antioxidative proteins in mouse brain after transient middle cerebral artery occlusion. Brain Res. 2011. 1370: 246-253.

538. Tanaka T., Hosoi F., Yamaguchi-Iwai Y., Nakamura H., Masutani H., Ueda S., Nishiyama A., Takeda S., Wada H., Spyrou G., Yodoi J. Thioredoxin-2 (TRX-2) is an essential gene regulating mitochondria-dependent apoptosis. EMBO J. 2002. 21(7): 1695-1703.

539. Tanaka T., Nakamura H., Nishiyama A., Hosoi F., Masutani H., Wada H., Yodoi J. Redox regulation by thioredoxin superfamily; protection against oxidative stress and aging. Free Radic Res. 2000. 33(6): 851-855.

540. Taniguchi Y., Taniguchi-Ueda Y., Mori K., Yodoi J. A novel promoter sequence is involved in the oxidative stress-induced expression of the adult T-cell leukemia-derived factor (ADF)/human thioredoxin (Trx) gene. Nucleic Acids Res. 1996. 24(14): 2746-2752.

541. Tell G., Quadrifoglio F., Tiribelli C., Kelley M.R. The many functions of APE1/Ref-1: not only a DNA repair enzyme. Antioxid Redox Signal. 2009. 11(3): 601-620.

542. Tian L., Nie H., Zhang Y., Chen Y., Peng Z., Cai M., Wei H., Qin P., Dong H., Xiong L. Recombinant human thioredoxin-1 promotes neurogenesis and facilitates cognitive recovery following cerebral ischemia in mice. Neuropharmacology. 2013. pii: S0028-3908(13)00503-0. doi: 10.1016/j.neuropharm.2013.10.027 [Epub ahead of print].

543. Thompson J.A., Hess M.L. The oxygen free radical system: a fundamental mechanism in the production of myocardial necrosis. Prog Cardiovasc Dis. 1986. 28(6): 449-462.

544. Tiul'kova E.I., Vataeva L.A., Samoĭlov M.O. Effect of prenatal hypobaric hypoxia on activity of the rat brain phosphoinositide system. Zh Evol Biokhim Fiziol. 2010. 46(5): 406-410 (In Russian).

545. Tokuda Y., Uozumi T., Kawasaki T. The superoxide dismutase activities of cerebral tissues, assayed by the chemiluminescence method, in the gerbil focal ischemia/reperfusion and global ischemia models. Neurochem Int. 1993. 23(2): 107-114.

546. Tomimoto H., Akiguchi I., Wakita H., Kimura J., Hori K., Yodoi J. Astroglial expression of ATL-derived factor, a human thioredoxin homologue, in the gerbil brain after transient global ischemia. Brain Res. 1993. 625(1): 1-8.

547. Tonissen K.F., Di Trapani G. Thioredoxin system inhibitors as mediators of apoptosis for cancer therapy. Mol Nutr Food Res. 2009. 53(1): 87-103.

548. Toyoda T., Kassell N.F., Lee K.S. Induction of ischemic tolerance and antioxidant activity by brief focal ischemia. 1997. 8(4): 847-851.

549. Toyoda T., Lee K.S. Differential induction of superoxide dismutase in core and penumbra regions after transient focal ischemia in the rat neocortex. Neurosci Lett. 1997. 235(1-2): 29-32.

550. Tretter L., Adam-Vizi V. Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. Philos Trans R Soc Lond B Biol Sci. 2005. 360(1464): 2335-2345.

551. Trofimov S.S., Ostrovskaya R.U., Kravchenko E.V., Smolnikova N.M., Bondarenko N.A., Kutepova O.A., Nemova E.P., Voronina T.A. Behavioral disorders of rats exposed to prenatal hypoxia and their correction by postnatal influences of piracetam. Bull. Exp. Biol. Med. 1993 a. 115(1): 43-45 (In Russian).

552. Trofimov S.S., Ostrovskaya R.U., Smolnikova N.M., Kravchenko E.V., Kutepova O.A., Bondarenko N.A., Nerobkova L.N., Nemova E.P., Voronina T.A. Exp. Clin. Pharmacol. 1993 b. 56(6): 8-11 (In Russian).

553. Tsujimoto Y, Shimizu S. VDAC regulation by the Bcl-2 family of proteins. Cell Death Differ. 2000. 7(12): 1174-1181.

554. Tugoy I.A., Stroev S.A. Morphix – the program for morphological and densitometrical analysis of digital images. Morfologiia. 2006. 129(2): 96 (In Russian).

555. Twiddy D., Brown D.G., Adrain C., Jukes R., Martin S.J., Cohen G.M., MacFarlane M., Cain K. Pro-apoptotic proteins released from the mitochondria regulate the protein composition and caspase-processing activity of the native Apaf-1/caspase-9 apoptosome complex. J Biol Chem. 2004. 279(19): 19665-19682.

556. Tyul'kova E.I., Semenov D.G., Vataeva L.A., Belyakov A.V., Samoilov M.O. Effect of prenatal hypobaric hypoxia on glutamatergic signal transduction in rat brain. Bull Exp Biol Med. 2011. 151(3): 275-277.

557. Ueda S., Masutani H., Nakamura H., Tanaka T., Ueno M., Yodoi J. Redox control of cell death. Antioxid Redox Signal. 2002. 4(3): 405-414.

558. Ueda S., Nakamura H., Masutani H., Sasada T., Yonehara S., Takabayashi A., Yamaoka Y., Yodoi J. Redox regulation of caspase-3(-like) protease activity: regulatory roles of thioredoxin and cytochrome c. J Immunol. 1998. 161(12): 6689-6695.

559. Ueno M., Masutani H., Arai R.J., Yamauchi A., Hirota K., Sakai T., Inamoto T., Yamaoka Y., Yodoi J., Nikaido T. Thioredoxin-dependent redox regulation of p53-mediated p21 activation. J Biol Chem. 1999. 274(50): 35809-35815.

560. Ulukaya E., Acilan C., Yilmaz Y. Apoptosis: why and how does it occur in biology? Cell Biochem Funct. 2011. 29(6): 468-480.

561. Umasuthan N., Revathy K.S., Lee Y., Whang I., Lee J. Mitochondrial thioredoxin-2 from Manila clam (Ruditapes philippinarum) is a potent antioxidant enzyme involved in antibacterial response. Fish Shellfish Immunol. 2012. 32(4): 513-523.

562. Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007. 39(1): 44-84.

563. van Gurp M., Festjens N., van Loo G., Saelens X., Vandenabeele P. Mitochondrial intermembrane proteins in cell death. Biochem Biophys Res Commun. 2003. 304(3): 487-497.

564. Vanden Hoek T.L., Becker L.B., Shao Z., Li C., Schumacker P.T. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. J Biol Chem. 1998. 273(29): 18092-18098.

565. Vataeva L.A., Kostkin V.B., Makukhina G.V., Khozhai L.I., Otellin V.A. Effect of the time of prenatal hypoxia on the open-field behavior in male and female rats. Dokl Biol Sci. 2001. 380: 410-411.

566. Vataeva L.A., Kostkin V.B., Makukhina G.V., Khozhaĭ L.I., Otellin V.A. Conditional reflexive reaction of passive avoidance of rat males and females exposed to hypoxia during different stages of prenatal development. Zh Evol Biokhim Fiziol. 2004 a. 40(3): 250-253 (In Russian).

567. Vataeva L.A., Tulkova E.I., Samoilov M.O. The effect of preceding mild hypoxia on the alteration of the acquisition and retention of passive avoidance behaviour caused by the severe hypobaric hypoxia in rats. Zh Vyssh Nervn Dejat. 2004 b. 54(6): 795-801 (In Russian).

568. Vataeva L.A., Tyul'kova E.I., Khozhai L.I., Samoilov M.O., Otellin V.A. Training in the Morris water maze of female and male rats exposed to hypoxia at various periods of prenatal development. J Evol Biochem Physiol. 2005. 41(6): 660-664.

569. Vataeva L.A., Tyul'kova E.I., Samoilov M.O. Influence of severe hypoxia on rat emotional behavior: the modifying effect of preconditioning. Dokl Biol Sci. 2004 c. 395: 109-111.

570. Verma R., Mishra V., Sasmal D., Raghubir R. Pharmacological evaluation of glutamate transporter 1 (GLT-1) mediated neuroprotection following cerebral ischemia/reperfusion injury. Eur J Pharmacol. 2010. 638(1-3): 65-71.

571. Vieira H.L., Haouzi D., El Hamel C., Jacotot E., Belzacq A.S., Brenner C., Kroemer G. Permeabilization of the mitochondrial inner membrane during apoptosis: impact of the adenine nucleotide translocator. Cell Death Differ. 2000. 7(12): 1146-1154.

572. Vieira H., Kroemer G. Mitochondria as targets of apoptosis regulation by nitric oxide. IUBMB Life. 2003. 55(10-11): 613-616.

573. Vladimirov G.E., Galvjalo M.Ja., Gorjuhina T.A., Dmitriev G.A., Oppel V.V., Raiko Z.A. Using the stay at high-mountainous climate for aims of high-altitude pilot training. In: Oxygen

Starvation and Struggle Against it (Questions of Training and Diet). Leningrad, 1939. p. 43–104 (In Russian).

574. Vorob'ev M.G., Rybnikov E.A., Samoĭlov M.O. Changes in intensity of hypoxic brain damage in rats induced by hypoxic postconditioning. Morfologiia. 2012. 141(1): 12-15 (In Russian).

575. Wacker B.K., Perfater J.L., Gidday J.M. Hypoxic preconditioning induces stroke tolerance in mice via a cascading HIF, sphingosine kinase, and CCL2 signaling pathway. J Neurochem. 2012. 123(6): 954-962.

576. Wang D., Masutani H., Oka S., Tanaka T., Yamaguchi-Iwai Y., Nakamura H., Yodoi J. Control of mitochondrial outer membrane permeabilization and Bcl-xL levels by thioredoxin 2 in DT40 cells. J Biol Chem. 2006 a. 281(11): 7384-7391.

577. Wang L., Jiang D.M. Neuroprotective effect of Buyang Huanwu Decoction on spinal ischemia/reperfusion injury in rats. J Ethnopharmacol. 2009. 124(2): 219-223.

578. Wang P., Chen H., Qin H., Sankarapandi S., Becher M.W., Wong P.C., Zweier J.L. Overexpression of human copper, zinc-superoxide dismutase (SOD1) prevents postischemic injury. Proc Natl Acad Sci U S A. 1998. 95(8): 4556-4560.

579. Wang Q., Tompkins K.D., Simonyi A., Korthuis R.J., Sun A.Y., Sun G.Y. Apocynin protects against global cerebral ischemia-reperfusion-induced oxidative stress and injury in the gerbil hippocampus. Brain Res. 2006 b. 1090(1): 182-189.

580. Wang X., Svedin P., Nie C., Lapatto R., Zhu C., Gustavsson M., Sandberg M., Karlsson J.O., Romero R., Hagberg H., Mallard C. N-acetylcysteine reduces lipopolysaccharide-sensitized hypoxic-ischemic brain injury. Ann Neurol. 2007. 61(3): 263-271.

581. Watanabe R., Nakamura H., Masutani H., Yodoi J. Anti-oxidative, anti-cancer and anti-inflammatory actions by thioredoxin 1 and thioredoxin-binding protein-2. Pharmacol Ther. 2010. 127(3): 261-270.

582. Watson W.H., Yang X., Choi Y.E., Jones D.P., Kehrer J.P. Thioredoxin and its role in toxicology. Toxicol Sci. 2004. 78(1): 3-14.

583. Weber G.F., Menko A.S. The canonical intrinsic mitochondrial death pathway has a non-apoptotic role in signaling lens cell differentiation. J Biol Chem. 2005. 280(23): 22135-22145.

584. Weisiger R.A., Fridovich I. Mitochondrial superoxide simutase. Site of synthesis and intramitochondrial localization. J Biol Chem. 1973. 248(13): 4793-4796.

585. Wellmann S., Buhrer C., Moderegger E., Zelmer A., Kirschner R., Koehne P., Fujita J., Seeger K. Oxygen-regulated expression of the RNA-binding proteins RBM3 and CIRP by a HIF-1-independent mechanism. J Cell Sci. 2004. 117(Pt 9): 1785-1794.

586. Welsh S.J., Williams R.R., Birmingham A., Newman D.J., Kirkpatrick D.L., Powis G. The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1alpha and vascular endothelial growth factor formation. Mol Cancer Ther. 2003. 2(3): 235-243.

587. Westerterp-Plantenga M.S. Effects of extreme environments on food intake in human subjects. Proc Nutr Soc. 1999. 58(4): 791-798.

588. Wiegand F., Liao W., Busch C., Castell S., Knapp F., Lindauer U., Megow D., Meisel A., Redetzky A., Ruscher K., Trendelenburg G., Victorov I., Riepe M., Diener H.C., Dirnagl U. Respiratory chain inhibition induces tolerance to focal cerebral ischemia. J Cereb Blood Flow Metab. 1999. 19(11): 1229-1237.

589. Winterbourn C.C. The biological chemistry of hydrogen peroxide. Methods Enzymol. 2013. 528: 3-25.

590. Woo D., Kaushal R., Kissela B., Sekar P., Wolujewicz M., Pal P., Alwell K., Haverbusch M., Ewing I., Miller R., Kleindorfer D., Flaherty M., Chakraborty R., Deka R., Broderick J. Association of Phosphodiesterase 4D with ischemic stroke: a population-based case-control study. Stroke. 2006. 37(2): 371-376.

591. World C.J., Yamawaki H., Berk B.C. Thioredoxin in the cardiovascular system. J Mol Med (Berl). 2006. 84(12): 997-1003.

592. Wu C., Parrott A.M., Fu C., Liu T., Marino S.M., Gladyshev V.N., Jain M.R., Baykal A.T., Li Q., Oka S., Sadoshima J., Beuve A., Simmons W.J., Li H. Thioredoxin 1-mediated post-translational modifications: reduction, transnitrosylation, denitrosylation, and related proteomics methodologies. Antioxid Redox Signal. 2011. 15(9): 2565-2604.

593. Wu C., Zhan R.Z., Qi S., Fujihara H., Taga K., Shimoji K. A forebrain ischemic preconditioning model established in C57Black/Crj6 mice. J Neurosci Methods. 2001. 107(1-2): 101-106.

594. Würstle M.L., Laussmann M.A., Rehm M. The central role of initiator caspase-9 in apoptosis signal transduction and the regulation of its activation and activity on the apoptosome. Exp Cell Res. 2012. 318(11): 1213-1220.

595. Xia Y., Haddad G.G. Effect of prolonged O2 deprivation on Na+ channels: differential regulation in adult versus fetal rat brain. Neuroscience. 1999. 94(4): 1231-1243.

596. Xu J., Li T., Wu H., Xu T. Role of thioredoxin in lung disease. Pulm Pharmacol Ther. 2012. 25(2): 154-162.

597. Xu Y., Kiningham K.K., Devalaraja M.N., Yeh C.-C., Majima H., Kasarskis E.J., St Clair, D.K. An intronic NF-kappaB element is essential for induction of the human manganese superoxide dismutase gene by tumor necrosis factor-alpha and interleukin-1 beta. DNA Cell Biol. 1999. 18(9): 709–722.

598. Yaginuma H., Sato N., Homma S., Oppenheim R.W. Roles of caspases in the programmed cell death of motoneurons in vivo. Arch Histol Cytol. 2001. 64(5): 461-474.

599. Yamada T., Kawahara K., Kosugi T., Tanaka M. Nitric oxide produced during sublethal ischemia is crucial for the preconditioning-induced down-regulation of glutamate transporter GLT-1 in neuron/astrocyte co-cultures. Neurochem Res. 2006. 31(1): 49-56.

600. Yamagata K., Tagami M., Yamori Y. Neuronal vulnerability of stroke-prone spontaneously hypertensive rats to ischemia and its prevention with antioxidants such as vitamin E. Neuroscience. 2010. 170(1): 1-7.

601. Yamamoto M., Yang G., Hong C., Liu J., Holle E., Yu X., Wagner T., Vatner S.F., Sadoshima J. Inhibition of endogenous thioredoxin in the heart increases oxidative stress and cardiac hypertrophy. J Clin Invest. 2003. 112(9): 1395-1406.

602. Yamaoka Y., Shimohama S., Kimura J., Fukunaga R., Taniguchi T. Neuronal damage in the rat hippocampus induced by in vivo hypoxia. Exp Toxicol Pathol. 1993. 45(4): 205-209.

603. Yamashita N., Hoshida S., Otsu K., Taniguchi N., Kuzuya T., Hori M. The involvement of cytokines in the second window of ischaemic preconditioning. Br J Pharmacol. 2000. 131(3): 415-422.

604. Yamawaki H., Haendeler J., Berk B.C. Thioredoxin: a key regulator of cardiovascular homeostasis. Circ Res. 2003. 93(11): 1029-1033.

605. Yan B.C., Park J.H., Ahn J.H., Lee Y.J., Lee T.H., Lee C.H., Cho J.H., Kim M.J., Kim T.Y., Kang I.J., Won M.H. Comparison of the immunoreactivity of Trx2/Prx3 redox system in the hippocampal CA1 region between the young and adult gerbil induced by transient cerebral ischemia. Neurochem Res. 2012. 37(5): 1019-1030.

606. Yang C., Carrier F. The UV-inducible RNA-binding protein A18 (A18 hnRNP) plays a protective role in the genotoxic stress response. J Biol Chem. 2001. 276(50): 47277-47284.

607. Yang J., Li C., Ding L., Guo Q., You Q, Jin S. Gambogic acid deactivates cytosolic and mitochondrial thioredoxins by covalent binding to the functional domain. J Nat Prod. 2012 a. 75(6): 1108-1116.

608. Yang L., Wu D., Wang X., Cederbaum AI. Depletion of cytosolic or mitochondrial thioredoxin increases CYP2E1-induced oxidative stress via an ASK-1-JNK1 pathway in HepG2 cells. Free Radic Biol Med. 2011. 51(1): 185-196.

609. Yang R., Weber D.J., Carrier F. Post-transcriptional regulation of thioredoxin by the stress inducible heterogenous ribonucleoprotein A18. Nucleic Acids Res. 2006. 34(4): 1224-1236.

610. Yang X.H., Liu H.G., Liu X., Chen J.N. Thioredoxin and impaired spatial learning and memory in the rats exposed to intermittent hypoxia. Chin Med J. (Engl). 2012 b. 125(17): 3074-3080.

611. Yi C.H., Yuan J. The Jekyll and Hyde functions of caspases. Dev Cell. 2009. 16(1): 21-34.

612. Yin F., Sancheti H., Cadenas E. Mitochondrial thiols in the regulation of cell death pathways. Antioxid Redox Signal. 2012. 17(12): 1714-1727.

613. Yodoi J., Nakamura H., Masutani H. Redox regulation of stress signals: possible roles of dendritic stellate TRX producer cells (DST cell types). Biol Chem. 2002. 383(3-4): 585-590.

614. Yoshida T., Kondo N., Oka S., Ahsan M.K., Hara T., Masutani H., Nakamura H., Yodoi J. Thioredoxin-binding protein-2 (TBP-2): its potential roles in the aging process. Biofactors. 2006. 27(1-4): 47-51.

615. Yoshida T., Nakamura H., Masutani H., Yodoi J. The involvement of thioredoxin and thioredoxin binding protein-2 on cellular proliferation and aging process. Ann N Y Acad Sci. 2005. 1055: 1-12.

616. Yoshida T., Oka S., Masutani H., Nakamura H., Yodoi J. The role of thioredoxin in the aging process: involvement of oxidative stress. Antioxid Redox Signal. 2003. 5(5): 563-570.

617. Youn H.D., Kim E.J., Roe J.H., Hah Y.C., Kang S.O. A novel nickel-containing superoxide dismutase from Streptomyces spp. Biochem J. 1996. 318(Pt 3): 889-896.

618. Yu C.H., Moon C.T., Sur J.H., Chun Y.I., Choi W.H., Yhee J.Y. Serial expression of hypoxia inducible factor-1α and neuronal apoptosis in hippocampus of rats with chronic ischemic brain. J Korean Neurosurg Soc. 2011. 50(6): 481-485.

619. Yuan J., Yankner B.A. Apoptosis in the nervous system. Nature. 2000. 407(6805): 802-809.

620. Yuan Q., Hong S., Han S., Zeng L., Liu F., Ding G., Kang Y., Mao J., Cai M., Zhu Y., Wang Q.X. Preconditioning with physiological levels of ethanol protect kidney against ischemia/reperfusion injury by modulating oxidative stress. PLoS One. 2011. 6(10): e25811.

621. Yuan S., Akey C.W. Apoptosome structure, assembly, and procaspase activation. Structure. 2013. 21(4): 501-515.

622. Zamorskii I.I., Pishak V.P. Effect of melatonin on cyclic nucleotide content and intensity of lipid peroxidation in the hippocampus and habenula of rats exposed to acute hypoxia. Bull Exp Biol Med. 2000. 130(8): 756-758.

623. Zenkov N.K., Lankin V.Z., Menshchikova E.B. Oxidative stress. Biochemical and pathophysiological aspects. Moscow: MAIK "Nauka / Interperiodica, 2001. 343 p. (In Russian)

624. Zenkov N.K., Menshchikova E.B., Shergin S.M. Oxidative stress. Diagnosis, treatment, prevention. RAMS: Novosibirsk, 1993. 181 p. (In Russian).

625. Zhang H., Go Y.M., Jones D.P. Mitochondrial thioredoxin-2/peroxiredoxin-3 system functions in parallel with mitochondrial GSH system in protection against oxidative stress. Arch Biochem Biophys. 2007 a. 465(1): 119-126.

626. Zhang K., Peng B.W., Sanchez R.M. Decreased IH in hippocampal area CA1 pyramidal neurons after perinatal seizure-inducing hypoxia. Epilepsia. 2006. 47(6): 1023-1028.

627. Zhang M., Li W.B., Geng J.X., Li Q.J., Sun X.C., Xian X.H., Qi J., Li S.Q. The upregulation of glial glutamate transporter-1 participates in the induction of brain ischemic tolerance in rats. J Cereb Blood Flow Metab. 2007 b. 27(7): 1352-1368.

628. Zhang P., Liu B., Kang S.W., Seo M.S., Rhee S.G., Obeid L.M. Thioredoxin peroxidase is a novel inhibitor of apoptosis with a mechanism distinct from that of Bcl-2. J Biol Chem. 1997. 272(49): 30615-30618.

629. Zhang R., Al-Lamki R., Bai L., Streb J.W., Miano J.M., Bradley J., Min W. Thioredoxin-2 inhibits mitochondria-located ASK1-mediated apoptosis in a JNK-independent manner. Circ Res. 2004. 94(11): 1483-1491.

630. Zhao X., Strong R., Zhang J., Sun G., Tsien J.Z., Cui Z., Grotta J.C., Aronowski J. Neuronal PPARgamma deficiency increases susceptibility to brain damage after cerebral ischemia. J Neurosci. 2009. 29(19): 6186-6195.

631. Zhao Y.D., Cheng S.Y., Ou S., Xiao Z., He W.J., Jian-Cui, Ruan H.Z. Effect of hypobaric hypoxia on the P2X receptors of pyramidal cells in the immature rat hippocampus CA1 sub-field. Brain Inj. 2012. 26(3): 282-290.

632. Zhou F., Gomi M., Fujimoto M., Hayase M., Marumo T., Masutani H., Yodoi J., Hashimoto N., Nozaki K., Takagi Y. Attenuation of neuronal degeneration in thioredoxin-1 overexpressing mice after mild focal ischemia. Brain Res. 2009. 1272: 62-70.

633. Zhou F., Liu P.P., Ying G.Y., Zhu X.D., Shen H., Chen G. Effects of thioredoxin-1 on neurogenesis after brain ischemia/reperfusion injury. CNS Neurosci Ther. 2013. 19(3): 204-205.

634. Zhou J., Yu Q., Chng W.J. TXNIP (VDUP-1, TBP-2): a major redox regulator commonly suppressed in cancer by epigenetic mechanisms. Int J Biochem Cell Biol. 2011. 43(12): 1668-1673.

635. Zhou W., Li S., Wan N., Zhang Z., Guo R., Chen B. Effects of various degrees of oxidative stress induced by intermittent hypoxia in rat myocardial tissues. Respirology. 2012. 17(5): 821-829.

636. Zhu C., Xu F., Fukuda A., Wang X., Fukuda H., Korhonen L., Hagberg H., Lannering B., Nilsson M., Eriksson P.S., Northington F.J., Björk-Eriksson T., Lindholm D., Blomgren K. X chromosome-linked inhibitor of apoptosis protein reduces oxidative stress after cerebral irradiation or hypoxia-ischemia through up-regulation of mitochondrial antioxidants. Eur J Neurosci. 2007. 26(12): 3402-3410.

637. Zhu W.Z., Xie Y., Chen L., Yang H.T., Zhou Z.N. Intermittent high altitude hypoxia inhibits opening of mitochondrial permeability transition pores against reperfusion injury. J Mol Cell Cardiol. 2006. 40(1): 96-106.

638. Zhu X.H., Yan H.C., Zhang J., Qu H.D., Qiu X.S., Chen L., Li S.J., Cao X., Bean J.C., Chen L.H., Qin X.H., Liu J.H., Bai X.C., Mei L., Gao T.M. Intermittent hypoxia promotes hippocampal neurogenesis and produces antidepressant-like effects in adult rats. J Neurosci. 2010. 30(38): 12653-12663.

639. Zhu X.L., Xiong L.Z., Wang Q., Liu Z.G., Ma X., Zhu Z.H., Hu S., Gong G., Chen S.Y. Therapeutic time window and mechanism of tetramethylpyrazine on transient focal cerebral ischemia/reperfusion injury in rats. Neurosci Lett. 2009. 449(1): 24-27.

640. Zhuravin I.A., Tumanova N.L., Potapov D.O. Structural changes in the sensomotor part of the brain in early postnatal ontogenesis of rats, who suffered prenatal hypoxia. Zh Evol Biokhim Fiziol. 2001. 37(6): 518-520 (In Russian).

641. Zou H., Henzel W.J., Liu X., Lutschg A., Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell. 1997. 90(3): 405-413.

642. Zou H., Li Y., Liu X., Wang X. An Apaf-1 cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J Biol Chem. 1999. 274(17): 11549-11556.

643. Zubieta-Calleja G.R., Paulev P.E., Zubieta-Calleja L., Zubieta-Calleja N., Zubieta-Castillo G. Hypoventilation in chronic mountain sickness: a mechanism to preserve energy. J Physiol Pharmacol. 2006. 57 Suppl 4: 425-430.

644. Zubieta-Calleja G., Zubieta-Castillo G., Zubieta-Calleja L., Ardaya-Zubieta G., Paulev P.E. Do over 200 million healthy altitude residents really suffer from chronic Acid-base disorders? Indian J Clin Biochem. 2011. 26(1):.62-65.

645. Zubieta-Castillo G. Sr., Zubieta-Calleja G.R. Jr., Zubieta-Calleja L. Chronic mountain sickness: the reaction of physical disorders to chronic hypoxia. J Physiol Pharmacol. 2006. 57 Suppl 4: 431-442.

# Original publications

**The articles are reproduced with kind permissions and official licenses from John Wiley and Sons (II) and Springer Science and Business Media (IV, V, VI, VIII, IX, X), with kind permissions from Elsevier (I and III), and from Editor-in-Chief of Acta Neurobiologiae Experimentalis Professor Kris Turlejski (VII) and Editor of Neuroscience letters Dr Stephen G. Waxman (I).**

The owners of the copyrights (Springer Science and Business Media, Elsevier, John Wiley and Sons, and Polish Neuroscience Society – PTBUN and Nencki Institute of Experimental Biology) and the original publisher (journal title, volume, year of publication, page, article title, names of authors, figure numbers, original copyright notice) are given to the publications in which the materials were originally published. The publication titles, volume numbers, names of authors, titles of the articles and page numbers are given also both in List of original publications (see pages 12-13) and in the reproduced copies of the articles. The Copyrights (with years) of Springer Science and Business Media, Elsevier, John Wiley and Sons, and Polish Neuroscience Society are indicated in the reproduced copies of the articles.