



KAI LEHTIMÄKI

Seizures and Cytokines

Experimental and Clinical Studies



ACADEMIC DISSERTATION

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS	6
ABBREVIATIONS	7
1. INTRODUCTION	9
2. REVIEW OF THE LITERATURE	10
2.1. Cytokines	10
2.1.1. <i>Interleukin-1 family of cytokines</i>	11
2.1.2. <i>TNF family</i>	12
2.1.3. <i>IL-6 family of cytokines</i>	12
2.1.4. <i>Pro-inflammatory and anti-inflammatory cytokines</i>	13
2.1. Experimental models of seizures, epilepsy and epileptogenesis	13
2.2.1. <i>Chemically induced seizures</i>	14
2.2.2. <i>Focal electrical stimulation and electroconvulsive shocks</i>	14
2.2.3. <i>Kindling</i>	15
2.3. Human epilepsy	16
2.3.1. <i>Seizure Classification</i>	16
2.3.2. <i>Epileptic syndromes</i>	17
2.3.4. <i>Status epilepticus</i>	18
2.3.4. <i>Neuronal damage in experimental and human epilepsy</i>	18
2.3.5. <i>Prognosis of epilepsies: controlled vs. refractory</i>	19
2.4. Electroconvulsive therapy and depressive disorder	20
2.4.1. <i>Depressive disorder</i>	20
2.4.2. <i>Electroconvulsive therapy</i>	20
2.4.3. <i>Neuronal damage after ECT</i>	21

2.5. Cytokine expression after seizures	22
<i>2.5.1. Expression of cytokines after seizures in experimental models</i>	22
<i>2.5.2. Release of cytokines after seizures in humans</i>	23
2.6. Mechanisms of cytokine induction after seizures	23
2.7. Effect of cytokines on seizure susceptibility	24
2.8. Effect of cytokines on seizure related neuronal damage	26
3. PURPOSE OF THE STUDY	27
4. MATERIALS AND METHODS	28
4.1. Expression cytokines and cytokine receptors after SE	28
<i>4.1.1. Kainic acid induced status epilepticus</i>	28
<i>4.1.2. Extraction of mRNA</i>	28
<i>4.1.3. Oligonucleotide probe preparation</i>	29
<i>4.1.4. Northern blot hybridization</i>	30
<i>4.1.5. In situ hybridization histochemistry</i>	30
4.2. Cytokine and cytokine receptor levels after seizures in human patients	31
<i>4.2.1. Patients with acute seizures</i>	31
<i>4.2.2. Patients with chronic localization related epilepsy</i>	31
<i>4.2.3. Patients with depressive disorder undergoing ECT</i>	32
<i>4.2.4. ELISA measurements for human cytokines</i>	32
4.3. Effect of exogenous hrIL-6 on seizure susceptibility in rats	33
<i>4.3.1. PTZ induced seizures and intranasal IL-6 treatment</i>	33
<i>4.3.2. Brain preparation and measurement of hrIL-6 levels</i>	33
4.4. Statistical analysis	34

5. RESULTS	35
5.1. Expression of cytokines and cytokine receptors after SE	35
5.2. Levels of cytokines and cytokine receptors after acute seizures	36
<i>5.2.1 Levels of IL-6 in CSF and serum</i>	36
<i>5.2.2. Levels of sIL-6R and sGp130 in CSF and serum</i>	36
5.3. Plasma levels of cytokines and cytokine receptors: video-EEG study	36
<i>5.3.1. Basal (inter-ictal) levels of cytokines and cytokine receptors</i>	37
<i>5.3.2. Post-ictal levels of cytokines and cytokine receptors</i>	37
5.4. Plasma levels of cytokines: ECT study	37
<i>5.4.1 Basal levels of cytokines</i>	37
<i>5.4.2. Levels of cytokines after seizure during ECT</i>	38
5.5. Effect of intranasal hrIL-6 on seizure susceptibility in rats	38
<i>5.5.1. Effect of hrIL-6 on PTZ induced seizures</i>	38
<i>5.5.2. Levels of hrIL-6 in the rat brain</i>	39
6. DISCUSSION	39
6.1. Origin of cytokines	39
6.2. Time course of cytokine expression	40
6.3. Mechanisms of cytokine release	41
6.4. Effect of cytokines on seizures	42
SUMMARY AND CONCLUSIONS	44
TABLES	47
YHTEENVETO	54
ACKNOWLEDGEMENTS	57
REFERENCES	60
ORIGINAL PUBLICATIONS	

LIST OF ORIGINAL PUBLICATIONS

The original publications will be referred to the text by Roman numerals I-V:

- I Lehtimäki KA, Peltola J, Koskikallio E, Keränen T, Honkaniemi J (2003). Expression of cytokines and cytokine receptors in the rat brain after kainic acid-induced seizures. *Brain Res Mol Brain Res* 110:253-60.
- II Lehtimäki KA, Keränen T, Huhtala H, Hurme M, Ollikainen J, Honkaniemi J, Palmio J, Peltola J (2004). Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration. *J Neuroimmunology* 152:121-5.
- III Kalueff AV, Lehtimäki KA, Ylinen A, Honkaniemi J, Peltola J (2004). Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats. *Neurosci Lett* 365:106-10.
- IV Lehtimäki KA, Keränen T, Palmio J, Mäkinen R, Hurme M, Honkaniemi J, Peltola J (2007). Increased plasma levels of cytokines after seizures in localization-related epilepsy. *Acta Neurol Scand* 116:226-30.
- V Lehtimäki KA, Keränen T, Huuhka M, Palmio J, Hurme M, Leinonen E, Peltola J (2007). Increase in Plasma Pro-inflammatory Cytokines after Electroconvulsive Therapy in Patients with Depressive Disorder. *J ECT* 24:88-91.

ABBREVIATIONS

BDNF, brain derived neurotrophic factor
CNS, central nervous system
cNTF, ciliary neurotrophic factor
CSF, cerebrospinal fluid
CT-1, cardiotrophin-1
dATP, deoxyadenosinetriphosphate
DEPC, diethylpyrocarbonate
DNA, deoxyribonucleic acid
DSM-IV, diagnostic and statistical manual of mental disorders; fourth edition
ECS, electroconvulsive shock
ECT, electroconvulsive therapy
EEG, electroencephalogram
EGF, epidermal growth factor
ELISA, enzyme-linked immunosorbent assay
FasL, Fas ligand
FGF, fibroblast growth factor
GABA, gamma-aminobutyrate
GEPRS, genetically epilepsy prone rat strain
GM-CSF, granulocyte-macrophage colony stimulating factor
GFAP, glial fibrillary acidic protein
Gp130, glycoprotein 130
hrIL-6, human recombinant interleukin-6
ICE, interleukin-1 converting enzyme
ILAE, International League Against Epilepsy
IL-1 β , interleukin-1 β
IL-1 α , interleukin-1 α
IL-1ra, interleukin-1 receptor antagonist
IL-2, interleukin-2
IL-4, interleukin-4
IL-6, interleukin-6
IL-6R, interleukin-6 receptor
IL-10, interleukin-10
IL-11, interleukin-11
IL-18, interleukin-18
IU, international unit
LIF, leukemia inhibitory factor
LIFR, leukemia inhibitory factor receptor
LT- α , Lymphotoxin- α
LT- β , Lymphotoxin- β
LTP, long term potentiation
MECS, maximal electroconvulsive seizures
MgCl, magnesium chloride
MIP-1 α , macrophage inflammatory protein-1 α
MRI, magnetic resonance imaging

mRNA, messenger ribonucleic acid
NaCl, sodium chloride
NGF, nerve growth factor
NMDA, N-methyl-D-aspartate
NTR, neurotrophin receptor
OSM, oncostatin-M
PBS, phosphate buffered saline
PDGF, platelet derived growth factor
PTZ, pentylenetetrazole
RT-PCR, reverse-transcriptase polymerase chain reaction
SDS, sodium dodecyl sulphate
sGp130, soluble glycoprotein 130
SE, status epilepticus
sIL-6R, soluble interleukin-6 receptor
sLIFR, soluble leukemia inhibitory factor receptor
SSC, standard saline citrate
sTNFR, soluble tumor necrosis factor receptor
TLE, temporal lobe epilepsy
TNF α , tumor necrosis factor- α
TNF β , tumor necrosis factor- β
TRAIL, TNF-related apoptosis-inducing ligand

1. INTRODUCTION

Inflammatory cytokines are a group of soluble factors that mediate signals principally between inflammatory system cells during inflammation in the peripheral tissues. They are also expressed in low quantities in the central nervous system (CNS) under normal conditions, where they are thought to be involved in physiological processes such as learning and memory (Schneider et al., 1998; Jankowsky and Patterson 1999). Inflammatory cytokines are also released from the central nervous system cells in various pathological conditions, such as ischemia and nervous tissue injury (Kossmann et al., 1995; Hans et al., 1999; Vila et al., 2000).

Epileptic seizure is characterized as a rhythmic electrical discharge of group of neurons in the brain. Activation of inflammatory cytokines after seizures was first reported by Minami et al, who showed that prolonged seizures induced by injection of pentylenetetrazole (PTZ) in rats caused up-regulation of interleukin-1 β (IL-1 β), which is a key pro-inflammatory cytokine, in the CNS (Minami et al., 1990). By using more severe kainic acid model of status epilepticus (SE) in rat, they showed that in addition to IL-1 β , interleukin-6 (IL-6), tumor necrosis factor- α (TNF α) and leukemia inhibitory factor (LIF) mRNAs were also up-regulated in the CNS (Minami et al., 1991).

Sheng et al. reported in 1994 that in surgical hippocampal specimen of patients with chronic drug resistant temporal lobe epilepsy (TLE) showed immunoreactivity of IL-1 (Sheng et al., 1994). A temporal relationship between cytokines and seizures in humans was first introduced by Peltola et al, where they showed that the levels of interleukin-1 receptor antagonist (IL-1ra) and IL-6 levels were increased in cerebrospinal fluid (CSF) and plasma within 24 hours after single tonic-clonic seizure (Peltola et al., 1998; Peltola et al., 2000). This finding raised the possibility that seizure activity with very short duration may be followed by increased release of cytokines into CSF and plasma. Later cytokines proved also to have powerful modulating effects on neuronal

survival, plasticity as well as electrical properties (Penkowa et al., 2001; Vezzani et al., 1999), indicating that activation of the cytokine network may have relevance in the pathophysiology of epilepsy.

The present studies were conducted to study expression and release of inflammatory cytokines after seizures in more detail, with an emphasis on time course and mechanisms as well as the origin of cytokine release after seizures.

2. REVIEW OF THE LITERATURE

2.1. Cytokines

The term “cytokine” was first used by Cohen et al. in 1974 recognising that these molecules mediating inflammatory signals (originally referred to lymphokines) were in fact produced by a variety of other cell types than activated lymphocytes. Cytokines are currently defined as regulatory proteins secreted by white blood cells and a variety of other cells in the body modulating actions of immune system cells and inflammatory response. It is an umbrella of several groups of growth factors (originally regarded as separate entities), such as lymphokines, monokines, hematopoietic growth factors, connective tissue growth factors and chemotactic chemokines. Today, more than 200 molecules are defined as cytokines. Whether “classical” growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), nerve growth factor (NGF) and fibroblast growth factor (FGF) belong among “true” cytokines is frequently debated. However, in addition to their action in promoting growth, they also exert at least “cytokine like” actions (Vilcek, 1998).

Key features in cytokine biology are their pleiotropic actions and functional redundancy (TABLE 1). Pleiotrophism means that a particular cytokine usually has effects in several different tissues or cell types in the body, i.e. they are not tissue specific, as was initially

assumed. Secondly, the signalling of a group of cytokines may converge on the same or structurally similar receptor molecules or intracellular signalling pathways, thus forming cytokine (TABLE 2) and cytokine receptor families (TABLE 3). The utilisation of common receptor molecules by a group of cytokines leads to functional redundancy, meaning that a group of structurally distinct cytokines may have similar effects on target cells. A third important feature of cytokine actions is the synergism/antagonism principle, meaning that the effect of a particular cytokine may be potentiated or antagonised by other cytokines. Cytokines also regulate the production of other cytokines, i.e. they act as a network (TABLE 1). These features explain the enormous complexity of cytokine biology: a large number of cytokines is usually produced as a cascade within a tissue with simultaneous synergistic and antagonistic interactions (Vilcek, 1998).

2.1.1. Interleukin-1 family of cytokines

The interleukin-1 family of cytokines includes interleukin-1 α (IL-1 α) and interleukin-1 β (IL-1 β), with indistinguishable biological effects. They are highly pro-inflammatory cytokines and affect virtually every cell type in the body (Dinarello, 1998). They both act through IL-1 type I receptor. The actions of these cytokines are antagonized by interleukin-1 receptor antagonist (IL-1ra), which has affinity to the same receptor molecule without activating intracellular signalling pathways. Another type of IL-1 receptor also exists, namely IL-1 receptor type II, which is thought to be a decoy receptor lacking intracellular signalling capability (Slack et al., 1993; Sims et al., 1993), thereby acting as an antagonist of IL-1 α and IL-1 β actions (Dinarello, 1998). IL-1 β is cleaved from precursor protein (pro-IL-1 β) by interleukin-1 β converting enzyme (ICE), also known as caspase-1. This cleavage is essential for the formation of the active form of IL-1 β (Black et al., 1988).

2.1.2. *TNF family*

Tumor necrosis factor- α (TNF α) is also a primary regulator of immune and inflammatory reactions, and has mainly pro-inflammatory and cytotoxic effects (Zhang and Tracey, 1998). The actions of TNF α are mediated via type I and type II receptors, both of which are expressed in virtually all cell types of the body. TNF α is also produced by most cell types of the body as a response to a wide range of stimuli. Several other TNF α related cytokines are also identified (TABLE 2), such as tumor necrosis factor- β (TNF β) and Fas ligand (FasL). The low affinity receptor of nerve growth factor (NTR), which also binds other neurotrophins, shares structural homology with TNF receptors and belong to the same cytokine receptor superfamily (TABLE 3) (Ware et al., 1998).

2.1.3. *IL-6 family of cytokines*

Initially, IL-6 was identified as a cytokine which promotes antibody production by activated B-cells (Kishimoto et al., 1989). Later IL-6 was found to be multifunctional cytokine acting on hematopoiesis, endocrine system and nervous system (Hirano, 1998). Other functionally related cytokines have been discovered, forming a family of cytokines on the basis of structurally similar receptor molecules (TABLE 3). This family of cytokines includes factors such as interleukin-11 (IL-11), ciliary neurotrophic factor (cNTF), leukaemia inhibitory factor (LIF), cardiotrophin-1 (CT-1) and oncostatin-M (OSM) (TABLE 3). This group of cytokines is also called neuropoietic cytokines due to their effects on hematopoietic cells as well as on neural tissue cells (Taga and Kishimoto, 1997).

A key feature of these cytokines is their utilisation of a common signal transducing receptor Glycoprotein 130 (Gp130) (TABLE 3). These cytokines have specific high-affinity receptors (for example IL-6 receptor (IL-6R) and leukaemia inhibitory factor receptor (LIFR)), however, the formation of receptor complex including low affinity receptor Gp130 is needed for intracellular signalling (Hirano, 1998). These IL-6 family receptor molecules also exist in soluble

forms thereby acting as modulators of particular cytokine actions. The soluble receptors may have both antagonistic and agonistic effects, thereby creating further diversity of cytokine action (Hirano, 1998).

2.1.4. Pro-inflammatory and anti-inflammatory cytokines

In previous chapters, groups of cytokines or cytokine families have been introduced mainly on the basis of structural homology of cytokines or their receptors. On functional level, cytokines may be regarded as pro-inflammatory or anti-inflammatory in nature. Pro-inflammatory cytokines mainly have stimulatory effects on other cytokines, i.e. IL-1 stimulates production of TNF, IL-2, IL-6 (Vilcek, 1998). TNF and IL-1 also induce each other's production (Neta et al., 1992). In addition to these stimulatory cytokines, cytokines with inhibitory effects on cytokine production have also been identified, such as IL-4 (Hart et al., 1989), IL-6 (Aderka et al., 1989), IL-10 (Fiorentino et al., 1991), IL-13 (Minty et al., 1993). These anti-inflammatory cytokines are of crucial importance in competing with pro-inflammatory cytokines in order to prevent chronic inflammatory state.

2.2. Experimental models of seizures, epilepsy and epileptogenesis

Seizure is characterized as electric discharge of the aggregate of neurons. Epilepsy means a tendency to spontaneous unprovoked seizures. Several experimental approaches have been introduced in recent decades for studying isolated seizures, prolonged seizures, status epilepticus (SE) and epileptogenesis (TABLE 4) (Fisher, 1989). The most commonly used models are chemoconvulsants (kainic acid, pentylentetrazole (PTZ) or pilocarpine) or electrical stimulation of distinctive brain structures. Depending on the dose of chemoconvulsant or the duration of the electrical stimulation, a condition of continuous seizure activity and convulsions (status epilepticus (SE)) may evolve, lasting up to several hours. These models of convulsive SE are associated with

severe neuronal damage throughout the CNS. After a silent period of 1 to 3 weeks, spontaneous seizures usually occur. This silent period is called epileptogenesis. During this seizure-free latent period, neuronal degeneration, neurogenesis as well as synaptic reorganization occurs (Sperk, 1983).

2.2.1. Chemically induced seizures

Chemoconvulsants act by disturbing the balance between inhibitory and excitatory systems (De Deyn, 1992). The most widely used convulsant is kainic acid, which is a glutamate agonist. Kainic acid causes severe SE with a seizure activity and motor convulsions lasting several hours (Sperk et al., 1983). Pilocarpine is another widely used cholinergic chemoconvulsant which shares many of the properties of kainic acid (Cavalheiro et al., 1996). A common feature to these models is severe widely distributed neuronal damage in the brain and associated adaptive changes (gliosis, neuronal sprouting, neurogenesis) which occur during few weeks after SE (Sperk et al., 1983; Mello et al., 1993). In both models, spontaneous seizures also occur after silent period of 1 to 3 weeks. Seizures may also be induced by blocking of inhibitory GABAergic systems (Fisher, 1989). Most widely used convulsants of this type are pentylenetetrazole (PTZ) or bicuculline methiodide. Importantly, in commonly used doses they seem not to cause severe loss of neurons in the brain (Nehlig et al., 1996) (in contrast to kainic acid and pilocarpine models), neither of these models are associated with spontaneous seizures over time.

2.2.2. Focal electrical stimulation and electroconvulsive shocks

Electrical stimulation of the selected brain structures is an alternative method in inducing seizures. These models enable studying of specific neuronal circuitries associated with seizures and epilepsy. Stimulation is usually targeted at epileptogenic areas of the brain such as hippocampus (Vicedomini and Nadler, 1987), amygdala (Nissinen et al., 2000) or perforant

pathway (Sloviter, 1983). Continuous electrical stimulation (for approximately 20-30 minutes in amygdala) causes SE manifesting as severe seizure activity which continues even after stimulation is stopped (self-sustained status epilepticus) (Nissinen et al., 2000). Electrically induced SE is associated with severe neuronal damage (comparable to chemically induced SE) and spontaneous seizures also occur after a seizure-free period (Nissinen et al., 2000). During this latent period, neuronal degeneration as well as synaptic reorganization occurs (Nissinen et al., 2000). Another electrical seizure induction model used especially in drug development is maximal electroconvulsive shock (MECS) model (Fisher et al., 1989) in which seizure is induced via ear / eye electrodes instead of focal electrical stimulation of the brain. These seizures have not reported to cause detectable neuronal damage (Devanand et al., 1994; Cole et al., 2002).

2.2.3. Kindling

Kindling refers to a phenomenon in which repeated initially non-convulsive stimulations (either chemical or electrical) progress into isolated seizures of increasing severity. In electrical stimulation models, hippocampus and amygdala are the most commonly used targets for stimulation. As a process, kindling is believed to mimic events comparable to epileptogenesis. Kindling evolves during several weeks of repeated stimulation sessions (McNamara, 1980 and 1986). After reaching fully kindled state (i.e. rat shows isolated severe motor generalized seizure and electrographic afterdischarge triggered by stimulus), spontaneous seizures do not occur. However, if stimulations are continued for over a long period of time (overkindling), spontaneous seizures do also eventually occur (Brandt et al., 2004). Progressive loss of specific neuron populations is evident during kindling (Cavazos et al., 1994; Bengzon et al., 1997).

2.3. Human epilepsy

Epilepsy is a neurological disease affecting 0.5 – 1% of the population. Epilepsy manifests as spontaneous seizures, which have been traditionally classified into focal and generalized seizures. Dichotomy of seizures into focal and generalized forms by the International League Against Epilepsy (ILAE) was first introduced in 1964. This dichotomy was based on different pathophysiological features and responses to antiepileptic medication (Wyllie and Lüders, 1996a). In addition to the classification of different types of seizures, epileptic disorders were classified by ILAE in 1970 (followed by several revisions) into different syndromes according to the type of seizures (focal or generalized), seizure etiology (idiopathic, cryptogenic and symptomatic epilepsies), patient's age and EEG findings (Wyllie and Lüders, 1996b). The current seizure and epilepsy classification schema was introduced in 2001 by ILAE (Engel J.Jr, 2001). This classification provides rather diagnostic schema than rigid classification of epileptic seizures and syndromes. The prognosis of epilepsy is diverse, and approximately 25 - 30 % of patients are medically refractory (Brodie and Kwan, 2002). Among epileptic syndromes, focal epilepsy is regarded as more resistant to drug treatments (Semah 1998). Temporal lobe epilepsy with hippocampal structural abnormality (hippocampal sclerosis) especially has poor prognosis (Semah 1998; Stephen et al., 2001).

2.3.1. Seizure classification

In modern ILAE seizure classification (Engel J Jr, 2001), seizures are divided into focal and generalized seizures. In focal seizures, initial seizure activation involves only one hemisphere, whereas in generalized seizures, seizure involves activation of both cerebral hemispheres. Focal seizures are further classified into sensory, motor, gelastic, hemiclonic, secondarily generalized and reflex seizures (TABLE 5). Generalized seizures are divided into tonic-clonic, clonic, absence, tonic, spasms, and myoclonic seizures, as well as several other seizure types

(TABLE 5). Self-limited seizures are distinguished from continuous seizure types (generalized and focal status epilepticus) (TABLE 7). Precipitating stimuli for reflex seizures are recognized as a separate entity.

2.3.2. Epileptic syndromes

Due to the recognition that no single seizure type is specific to an epileptic disorder and that every seizure disorder usually includes more than one type of seizures, additional clinical findings have to be used in order to classify epileptic disorders with similar prognosis and treatment options. In addition to the typical seizures, classification of epilepsies has been based on age at onset, intellectual development, neurological examination, imaging study findings, and electroencephalographic findings. Similarly to the classification of seizures, classification of epileptic syndromes has undergone revisions during years. Traditionally epileptic syndromes were first divided into localization related and generalized epilepsies (Wyllie and Lüders, 1996b). Localization related and generalized epilepsies were further divided on the basis of etiology into idiopathic, cryptogenic and symptomatic epilepsies. Idiopathic epilepsies are a group of disorders with unknown etiology, whereas cryptogenic etiology refers to epilepsy with unknown but probable symptomatic cause. In symptomatic epilepsies, a detectable brain insult is considered as a cause of epileptic seizures (Wyllie and Lüders, 1996b).

In the modern syndrome classification by ILAE in 2001, syndrome dichotomy into idiopathic and symptomatic forms is avoided, although the terms “idiopathic” and “symptomatic” are retained. The former term “cryptogenic” (referring to the unknown but probably symptomatic etiology of epilepsy) is replaced with the term “probably symptomatic”. A list of recognised syndromes is presented in TABLE 6.

2.3.3. Status epilepticus

Status epilepticus is a term used to describe the condition of continuous seizure activity for over 200 years (Lowenstein, 1999). In the modern seizure classification by ILAE (Engel J Jr, 2001), status epilepticus is distinguished from self-limiting seizures and referred to “continuous seizure” (TABLE 7) regardless of seizure duration. Among self-limiting seizures, the duration of simple partial seizure in humans is approximately 28 seconds, whereas complex partial seizure lasts 78 seconds on average (Jenssen et al., 2006). The duration of primarily generalized tonic-clonic seizure is approximately 66 seconds, while secondarily generalized seizure lasts 130 seconds on average (Jenssen et al., 2006). The definition of status epilepticus is not universally accepted, and several context-dependent definitions exist. Operative definition of status epilepticus (which affects choices of treatment options in clinical settings) in human patients has been proposed to be continuous seizure activity of five minutes, or two recurrent seizures within five minute time interval without return of consciousness of a patient (Lowenstein, 1999). However, some centres traditionally regard only continuous seizures lasting more than 30 minutes as status epilepticus. Status epilepticus is associated with high risk of morbidity and mortality, which are strongly associated with etiologic factors such as trauma, neoplasm, vascular accidents, infections etc. as well as patient’s age (mortality and morbidity increases with age) (Gilliam, 1996).

2.3.4. Neuronal damage in experimental and human epilepsy

After experimental SE, severe selective neuronal damage is evident in the hippocampus, amygdala, thalamus and temporal lobe cortex irrespective of the seizure stimulation method (Meldrum 2002). Using traditional histological methods, it was concluded that continuous seizure activity for approximately 30 minutes or more is sufficient to produce selective necrosis within the nervous system (Meldrum 2002). However, using a highly sensitive method of labelling fragmented intranuclear DNA (a marker of irreversible neuronal damage) it was shown that only

very brief seizure activity (even without visible motor convulsions) could produce an increased number of degenerating neurons in the granular cell layer of the hippocampus (Bengzon et al., 1997).

Although the mechanisms and anatomical distribution of neuronal damage after seizures are well characterized in animal models, only limited data is available about neuronal damage in human epileptic patients. Among some generalized epilepsies, patients do not appear to have symptoms of progressive neuronal injury even after SE (Shorvon and Walker 2005). However, in patients with TLE, a progressive cognitive impairment has been well documented (Sutula and Pitänen 2002b). By using MRI volumetric techniques, it has been shown that recurrent seizures may also cause further damage in the temporal lobe structures (Kälviäinen and Salmenperä, 2002; Sutula and Pitkänen, 2002a). These findings indicate that temporal lobe structures are especially vulnerable to prolonged or recurrent seizures.

2.3.5. Prognosis of epilepsies: controlled vs. refractory

Among patients having their first ever epileptic seizure, only 40% develop recurrent seizures in the future requiring anti-epileptic medication (Hauser et al., 1990). Within this population, about 60% of patients have epilepsy which is controlled with one or two anti-epileptic drugs, whereas about 30-40 % remains uncontrolled from the beginning despite of sufficient anti-epileptic medication (Kwan and Brodie, 2000). In such cases, epilepsy is regarded as refractory (Arroy et al., 2002). The risk of refractory epilepsy is especially high in the case of detectable brain lesion, i.e. in temporal lobe epilepsy with hippocampal sclerosis (Semah et al., 1998; Stephen et al., 2001), where approximately 80% of patients are refractory to drug treatment and require consideration of surgical resection.

2.4. Electroconvulsive therapy and depressive disorder

2.4.1. Depressive disorder

Depression is a psychiatric disorder with key symptoms of depressed mood and loss of interest or pleasure. The diagnostic criteria of depression also include weight loss, insomnia, hypersomnia, agitation, fatigue, feelings of worthlessness or guilt, diminished ability to concentrate or suicidal thoughts (American Psychiatric Association, 1994). A depressive episode is reported to occur in 16 % of the population during their lives (Steffens et al., 2000; Kessler et al., 2003). The pathogenesis of depression is not fully understood, however, depletion of monoamine neurotransmitters (norepinephrine, dopamine and serotonin) has been suggested (Stahl 2001, Delgado 2000). Decreased activity of neurotrophic factors such as brain derived neurotrophic factor (BDNF) has been proposed (Gould et al., 2003). Some authors suggest that depression may be triggered by inflammatory signals associated with infections or acute stress reaction (Connor and Leonard, 1998). Treatment of depression includes pharmacotherapy and psychotherapy (cognitive, behavioural interpersonal, psychodynamic) and electroconvulsive therapy (ECT) (American Psychiatric Association, 2000).

2.4.2. Electroconvulsive therapy

ECT is regarded as the most effective therapy for severe and treatment resistant depression (American Psychiatric Association, 2001). ECT is used also in schizophrenia (especially with affective symptoms or catatonia) and mania. ECT was first introduced by the neuropsychiatrist Meduna in 1934 on the realisation that symptoms of schizophrenia were reduced after spontaneous epileptic seizures. Seizures were first induced using camphor, pentylenetetrazole (PTZ) or insulin coma (Meduna, 1936, Fink 1984, Kalinowski 1986). In 1938, the first electrically induced seizures were introduced for catatonic schizophrenia (Fink 1984; Kalinowski 1986). Thereafter, ECT became first line treatment in schizophrenia and affective disorders (Kolb and Vogel 1942). Later it

was recognised that ECT was even more effective in depressive disorder than schizophrenia (Smith et al., 1943). ECT under general anaesthesia and muscle relaxation was introduced in 1950 (Kalinowski 1986).

A generalized epileptic seizure is necessary for a therapeutic response in ECT. The efficacy of treatment depends on electrical dose that exceeds the seizure threshold level (Sackeim, 2000). There is also a gradual increase in seizure threshold level and decrease in seizure duration during ECT (Coffey et al., 1995, Kales et al., 1997). Seizure threshold level also increases as a function of patient's age (Boylan et al., 2000). Due to this effect, ECT has been used in treatment resistant epilepsy in few patients (Lisanby et al., 2001). The mechanism of ECT action in depression is unknown. However, ECT has been reported to modify neurotransmitter metabolism, neurotrophic factor expression, hormones and cerebral blood flow (Huuhka, 2005).

2.4.3. Neuronal damage after ECT

Electroconvulsive therapy has been regarded as a relatively safe form of treatment for severely depressed patients. However, some patients show transient symptoms of cognitive dysfunction after treatment (Sackeim 2000), raising concern whether repeated treatment sessions produce neuronal damage. In clinical studies employing magnetic resonance imaging (MRI), no decrease in hippocampal volume has been reported after ECT (Ende et al., 2000). Levels of S-100 protein (a marker of neuronal damage), has been reported to be normal in CSF after ECT (Zachrisson et al., 2000). In experimental studies, no convincing evidence has emerged suggesting neuronal tissue damage after repeated brief electroconvulsive seizures (ECS) (Devanand et al., 1994; Cole et al., 2002).

2.5. Cytokine expression after seizures

2.5.1. Expression of cytokines and cytokine receptors after seizures in experimental models

Several cytokine mRNAs (including IL-1 β , TNF α , IL-6 and LIF) have been shown to be up-regulated in various brain structures as early as 2 – 4 hours after systemic kainic acid injection (after approximately 1 - 3 hours of seizure activity) using RT-PCR method (Minami et al., 1991). Increased expression of these cytokines was found in hippocampus, cerebral cortex, thalamus, hypothalamus and striatum with varying time courses and anatomical distribution (Minami et al., 1991). Translation of cytokine mRNAs into protein after SE has been reported for IL-1 β , IL-1ra, TNF α and IL-6 using immunohistochemistry (Eriksson et al., 1999; Vezzani et al., 1999; De Simoni et al., 2000). Peak levels of cytokine mRNAs have been reported to occur at 12 h for IL-1 β and at 12 – 24 h for IL-1ra in kainic acid model (Eriksson et al., 2000a), whereas IL-6 and LIF mRNA expression peaked at 12 h in pilocarpine model of SE (Jankowsky and Patterson, 1999). In most studies, expression of cytokines returns to basal level by 24 hours after SE (Eriksson et al., 2000; Jankowsky and Patterson, 1999). After SE, IL-1 β and IL-1ra mRNAs and proteins are expressed by microglial cells (Eriksson et al., 1999), whereas IL-6 and LIF mRNAs are expressed mainly by astrocytes (Jankowsky and Patterson, 1999, Rosell et al., 2003). TNF α immunoreactivity is detected in cells with glial cell morphology in hippocampus after limbic SE (De Simoni et al., 2000).

Expression of cytokines IL-1 β and IL-1ra after seizures has also been reported in models using PTZ and bicuculline (less severe seizure models compared to kainic acid or pilocarpine models) (Minami et al., 1990; Vezzani et al., 1999; Vezzani et al., 2000). Increased levels of IL-1 β and TNF α mRNAs have also been reported after single seizures in cerebral cortex, hippocampus and amygdala using the kindling model (Plata-Salaman et al., 2000). Single ECS administration has also been reported to up-regulate IL-6 mRNA in mice (Sallmann et al., 2000).

After pilocarpine induced SE, induction of IL-6R and LIFR mRNAs was reported in the hippocampus in scattered fashion, whereas neuronal cell layers expressed IL-6R and LIFR under basal conditions (Rosell et al. 2003). Kainic acid induced SE was followed by rapid and short lasting induction of Gp130 in the granule cell layer of the hippocampus, and more delayed and long lasting induction in the glial cells in the hippocampus (Choi et al., 2003).

2.5.2. Release of cytokines after seizures in humans

The first report about increased cytokine activity in epilepsy in humans was published by Sheng et al. in 1994, where they reported that immunoreactivity of IL-1 (including IL-1 α and IL-1 β) was detected in the hippocampus of surgically treated patients with TLE, suggesting that chronically epileptic tissue may show chronic inflammation. Peltola et al. first reported that the release of cytokines may indeed be temporally associated with seizures, i.e. cytokines are released post-ictally (Peltola et al., 1998; Peltola et al., 2000). Increased levels of IL-6 were found only in CSF samples collected during 24 hours after the seizure, whereas samples collected later showed no elevated levels of cytokines. The levels of IL-6 and IL-1ra were increased, whereas IL-1 β , TNF α and NGF were unchanged. The levels of IL-6 (which under normal conditions are low) were markedly increased in CSF and to a lesser extent in plasma suggesting intrathecal production. Finally, after ECT, increased levels of TNF α have been reported at 2 hours after ECS in patients with depressive disorder (Hestad et al., 2003).

2.6. Mechanisms of cytokine induction after seizures

In peripheral tissues, a cascade of inflammatory reaction is triggered by an enormous number of different pathological stimuli. This fact also holds true in CNS, where cytokine induction has been detected after brain injury, ischemia, infection, autoimmune diseases, etc. In fact, after SE induced by kainic acid, immunoreactivity of IL-1 β and IL-1ra are found in a manner closely

resembling areas suffering severe neuronal damage (Eriksson et al., 1999). It is clear that SE lasting for several hours causes excitotoxic neuronal damage with secondary inflammatory reaction (Sperk et al., 1983). However, increased expression of IL-1 β and IL-1ra after kainic acid induced SE has been reported to be inhibited by pre-treatment with NMDA receptor antagonists (Eriksson et al., 2000b), suggesting that cytokine activation is mediated by activation of NMDA receptors. Increased expression of IL-6 has also been reported after LTP stimulation in glial cells near the stimulation site, suggesting that neuronal activation may trigger expression of IL-6 (Jankowsky et al., 2000). Cytokine immunoreactivity (Vezzani et al., 1999) or mRNA expression (Plata-Salaman et al., 2000) has also been reported after relatively brief seizure episodes, which are not associated with acute severe neuronal necrosis, however, delayed apoptotic type of neurodegeneration most likely occur (Bengzon et al., 1997). It is currently unknown whether this delayed neuronal apoptosis is associated with secondary inflammatory like reaction. Interestingly, ECSs (unlike focal limbic seizures) are not associated with evidence of neuronal damage (Devanand et al., 1994; Cole et al., 2002). Increased levels of IL-6 mRNA have been reported after single ECS in mice (Sallmann et al., 2000). Together these studies suggest that severe seizures are associated with excitotoxic neuronal damage and secondary inflammation and cytokine up-regulation. However, in brief seizure episodes not sufficient to cause direct excitotoxic damage, cytokine network may be activated via direct neuronal activation.

2.7. Effect of cytokines on seizure susceptibility

The first experiment in which the effect of inflammatory cytokines on seizure activity was reported by Vezzani et al. in 1999, where they showed that IL-1 β administered intrahippocampally increases the duration of kainic acid induced seizure activity, an effect that was blocked with IL-1ra (Vezzani et al., 1999). IL-1ra instead had powerful anti-convulsive effects in a bicuculline model in mice using both intrahippocampal injection and over-expression in astrocytes

(Vezzani et al., 2000). Mice over-expressing IL-1ra were also more resistant to seizures, where only 57% of mice over-expressing IL-1ra showed seizures after bicuculline injection compared to 100% in wild type mice (Vezzani et al., 2000). Conversely, anticonvulsive and antiepileptogenic effect of intracerebroventricular injection of IL-1 β has been also reported in a kindling model of isolated seizures and epileptogenesis (Sayyah et al., 2005).

Recently, a novel anticonvulsive treatment strategy has been reported by Ravizza et al., where they report that inhibition of IL-1 β production in the brain using interleukin converting enzyme (ICE/caspase-1) inhibitors result in decrease in seizure duration in kainic acid model as well as increased resistance to seizures (Ravizza et al., 2006). The effect of IL-1 system in seizure susceptibility was also supported by recent data, where transgenic mice with IL-1 receptor deficiency were resistant to febrile seizures (Dube et al., 2005).

The effect of IL-6 on seizures was first reported by Penkowa et al., when they found that IL-6 deficient mice were more sensitive to kainic acid induced seizures (Penkowa et al., 2001), suggesting that IL-6 may be an anticonvulsive cytokine. Contradictory results were later reported by Samland et al., where they showed that mice over-expressing IL-6 in astrocytes experienced severe seizures after a small dose of kainic acid (5mg/ml) which was subconvulsive in wild type mice (Samland et al., 2003).

TNF α was recently reported to have anticonvulsive effects. In this study, Balosso et al. showed that transgenic mice over-expressing TNF α had decreased seizure susceptibility (Balosso et al., 2005). This anticonvulsive effect was found to be mediated via p75 receptor component. On the other hand, intraperitoneal injection of TNF α has been reported to increase duration of afterdischarges during kindling (Shandra et al., 2002).

2.8. Effect of cytokines on seizure related neuronal damage

Activation of inflammatory cytokines after seizures has raised the question whether these cytokines influence pathophysiological events such as progressive neuronal damage, glial cell activation, neurogenesis and remodelling of neuronal circuitries after seizures. Several experiments have been able to manipulate cytokine activity while measuring these histopathological parameters. Panegyres and Hughes first reported that intraventricular injection of IL-1ra in kainic acid model of SE reduced subsequent neuronal loss, suggesting that endogenous pro-inflammatory cytokine IL-1 β in fact exacerbated excitotoxic neuronal damage (Panegyres and Hughes 1998). They also found that expression of astrocyte marker, glial fibrillary acidic protein (GFAP), was diminished by IL-1ra treatment, suggesting that IL-1 β activates gliosis after SE. Toulmond and co-workers showed in 1992 that intracerebral IL-6 injection diminished excitotoxic neuronal damage induced by local NMDA injection in rat striatum (Toulmond et al., 1992). Using IL-6 deficient mice, Penkowa et al showed that neuronal damage was exacerbated after SE induced by kainic acid in mice with IL-6 deficiency, suggesting that IL-6 may be neuroprotective after SE. Simultaneously, glial cell activation and brain inflammatory response was reduced in IL-6 deficient mice (Penkowa et al., 2001). In the immature rat brain (which is more resistant to seizure induced damage) the expression of cytokines and pattern of neuronal injury has been assessed by Rizzi et al. using kainic acid model. They found that on postnatal day 9, kainic acid induced seizures were not associated with increase in levels of inflammatory cytokines or neuronal cell damage. On day 15, only increase in IL-1 β expression was found at 4h after seizures, followed by scattered damaged cells in the CA3 and subiculum at 24 hours. On postnatal day 21, increased levels of IL-1 β , TNF α and IL-6 was evident at 4 h after the seizure, followed by more severe damage in the hippocampus CA1 and CA3 regions as well as forebrain areas at 24 hours after the seizure (Rizzi et al., 2003). The authors concluded that delayed neuronal damage after seizures occurring later during postnatal development may be caused by preceding inflammatory cytokine up-regulation after seizures. While it has been

well established that activation of cytokines modulates the extent of neuronal damage after SE, whether modulation of neuronal damage after recurrent brief seizures by cytokines occurs is not known.

3. PURPOSE OF THE STUDY

- i) To study time course and localization of inflammatory cytokine and cytokine receptor expression after SE induced by kainic acid in rats

- ii) To study levels of IL-6 and soluble IL-6 receptors (sIL-6R, sGp130) in CSF and serum after different types of acute seizures in human patients

- iii) To study the effect of exogenous IL-6 administration on seizure susceptibility in rats

- iv) To study levels of IL-1 β , IL-1ra, IL-6, sIL-6R and sGp130 levels in plasma after seizures during video-EEG recording in patients with chronic localization-related epilepsy

- v) To study plasma levels of IL-1 β , IL-1ra and IL-6 after seizures during ECT in patients with depressive disorder

4. MATERIALS AND METHODS

4.1. Expression of cytokines and cytokine receptors after kainic acid induced SE

4.1.1. Kainic acid induced status epilepticus

Adult male Sprague-Dawley rats were used in kainic acid experiment. Kainic acid model was selected because of its practical advances (lack of electrodes potentially interfering with cytokine network, clear seizure behavioural manifestation and severe seizures with acceptable mortality). The neuropathological consequences are also well established. Status epilepticus was induced with subcutaneous injection of kainic acid (10 mg/kg; Sigma, St Louis, MO, USA or Ocean Produce Int. Canada) dissolved in 0.9% saline. After injection, rats showed seizure activity with increasing severity (staring, movement arrest, wet dog shakes, unilateral limb clonus followed by bilateral clonus and loss of balance). Generalized seizure activity was evident approximately an hour later from kainic acid injection, which was defined as zero time point. The rats were killed at 1, 3, 6, 12, 24, 72 hours and 7 days after onset of generalized seizure activity and brains were processed for further analysis of Northern Blot (n=14) and *in situ* hybridization (n=28). Untreated animals were used as controls in both *in situ* hybridization (n=4) and Northern Blot analysis (n=2). For *in situ* hybridization, brains were removed and frozen on dry ice. Coronal sections (14µm thick) were cut with a cryostat and mounted onto Fisherbrand Superfrost Plus slides (Fisher Scientific, Pittsburgh, USA). For Northern Blot analysis, brains were removed and cut in the coronal plane between optic chiasma and anterior pons and stored at -80 °C for mRNA extraction procedure.

4.1.2. Extraction of mRNA

Brains were homogenized in a solution containing 200mM NaCl, 200 mM Tris (pH 7.5), 1.5 mM MgCl₂, 2% SDS and 200 µg/ml proteinase K (Fermentas) in diethylpyrocarbonate (DEPC)-treated water. The lysates were passed four times through a sterile 21-gauge needle and

thereafter incubated at 45 °C in a water bath for 60 min. NaCl was added to each sample to a final concentration of 500 mM. The samples were then passed four times through a 21-gauge needle. Oligo(d)T cellulose (75 mg; Invitrogen) was added to each sample, and the samples were incubated at room temperature for 60 min. The samples were then centrifuged at 8000 rpm for 10 min at 4 °C and the supernatant was removed. The pellets were washed twice in a buffer containing 500 mM NaCl and 10 mM Tris (pH 7.5) in DEPC-treated water and thereafter three times in a buffer containing 250 mM NaCl and 10 mM Tris (pH 7.5) in DEPC-treated water. The samples were then transferred into spin-columns (Invitrogen) and washed four times with buffer containing 250 mM NaCl and 10 mM Tris (pH 7.5) in DEPC-treated water. Messenger RNA was eluted with 400 µl of buffer containing 10 mM Tris (pH 7.5) in DEPC-treated water. Sixty IUs of RNase inhibitor (Fermentas) and 5 IUs of RNase free DNase (Boehringer Mannheim) were added to each sample and the samples were then incubated at 37 °C for 30 min. The mRNA was ethanol precipitated, dissolved in water and stored at -80 °C until used.

4.1.3. Oligonucleotide probe preparation

The oligonucleotide probes used are listed in Table 1 (I). Searches of the Genbank database revealed no significant homology of the oligonucleotide sequences with any other previously characterized gene. The oligonucleotide probes for cytokine receptors were designed to detect exons that are present in both soluble and membrane bound forms of cytokine receptors (Horiuchi et al., 1994; Chambers et al., 1997; Diamant et al., 1997). The oligonucleotides were labelled with ³³P-dATP (DuPont-NEN Research Products, Boston, USA) for in situ hybridization histochemistry and with ³²P-dATP for Northern blot hybridization using terminal deoxynucleotidyltransferase (Fermentas). The labelled oligonucleotides were purified using Qiaex II kit (Qiagen).

4.1.4. Northern blot hybridization

RNA samples (8 µg/ lane for TNF α , 6 µg/ lane for IL-6R and 4 µg/ lane for the other cytokines and their receptors) were run in agarose gel. The samples were transferred onto a nylon membrane (Hybond N+, Amersham) and crosslinked with UV-light (Stratalinker; Stratagene). The membrane was prehybridized at 42 °C for 60 min in a hybridization solution containing 4 x standard saline citrate (SSC), 50% formamide, 5x Denhardt's solution, 1% sodium dodecyl sulphate (SDS), 0.02 M phosphate buffer (pH 7.0), 10% dextran sulphate and 100 µg/ml heat denatured salmon sperm DNA. Thereafter the labelled probe was added to the hybridization solution to a final concentration of up to 5 x 10⁶ cpm/ ml, and the membrane was hybridized at 42 °C overnight. The membrane was washed with increasing stringencies with maximal stringency of 2 x SSC at 65 °C. The membrane was covered with Biomax MR (Kodak) autoradiographic film (Rochester, NY, USA) and exposed for 3–7 days. Northern blots were quantified with UTHSCSA Image Tool 3.00 software (University of Texas Health Science Center, San Antonio). The integrated densities of each transcript were balanced against cyclophilin in the same lane and the values representing control and kainic acid-treated animals were compared.

4.1.5. In situ hybridization histochemistry

At least four sections for each subject were hybridized with each oligonucleotide probe. The sections were air dried at room temperature and hybridized at 42 °C for 12–18 h with a mixture of 4 X SSC, 50% formamide, 1 X Denhardt's solution, 1% sarcosyl, 0.02 M phosphate buffer (pH 7.0), 10% dextran sulphate, 500 µg/ml heat denatured salmon sperm DNA, 200 mM dithiothreitol and 1x10⁷ cpm/ml of the labelled probe. After hybridization, the sections were washed four times (15 min each) in 1 X SSC at 55 °C and thereafter left to cool for 1–3 h at room temperature. The sections were then dipped in distilled water and subsequently in 75 and 90%

ethanol and air dried at room temperature. The sections were covered with Biomax MR (Kodak, Rochester, NY, USA) and exposed for up to 6 months.

4.2. Cytokine and cytokine receptor levels after seizures in human patients

4.2.1. Patients with acute seizures

A total of 33 consecutive patients in the emergency department of the Tampere University Hospital with acute seizures were included in the study for CSF and serum measurements of cytokines and cytokine receptors. Patients did not show signs or symptoms of inflammatory or infectious diseases. The control group consisted of 17 patients in the emergency department who were studied to exclude neurological disease and they yielded normal neurological examination as well as laboratory findings. Patients were divided into three groups according to the seizures: single tonic-clonic seizure (n=16), recurrent tonic-clonic seizures (n=10, two or more recurrent tonic-clonic seizures) and patients with prolonged partial seizures (n=7, partial or complex partial seizures from 10 minutes up to two hours in duration). From these seven patients, CSF sample was taken for diagnostic purposes only from two patients, due to obvious seizure etiology. The etiology of seizures in patients is presented in TABLE 1 (II). Serum and CSF samples were obtained within the first 24 hours after the seizure. All subjects were informed about the risks and benefits of CSF examination and they gave their written consent to participate. The study protocol was approved by Ethics Committee of Tampere University Hospital.

4.2.2. Patients with chronic localization related epilepsy

A total of 11 patients with chronic refractory epilepsy undergoing video-EEG recording for diagnostic or treatment purposes were studied for plasma cytokine and cytokine receptor levels. One patient was studied twice with a six-month interval. Plasma samples were collected at the beginning of the video-EEG recording. After the seizure, samples were obtained at

1, 2, 6, 12 and 24 hours after the seizure. In seven patients, antiepileptic medication was reduced. Eight healthy controls were studied as a control group. All patients and control subjects gave their informed consent to participate. The study protocol was approved by Ethics Committee of Tampere University Hospital. Clinical data on patients is presented in TABLE 1 (IV).

4.2.3. Patients with depressive disorder undergoing ECT

Nine patients suffering from severe depression undergoing ECT were included in the study. All patients fulfilled the diagnostic criteria of DSM-IV major depressive disorder. Patients received psychotropic medication, which was unchanged during ECT. Seizures were induced using Thymatron DGx (Somatics, Inc., Lake Bluff, IL, USA) brief-pulse device with bilateral electrodes. The initial stimulus dosage was evaluated according to patient's age, gender and medication, and was around five times patient's age. Anaesthesia method has been described in detail in a recent study (Palmio et al., 2005). Plasma samples were obtained prior to seizure, and at 1 h, 3 h, 6h and 24 h after the seizure. It should be noted that patients were in different stages of their ECT course during sample collection. Eight healthy controls were included in the study. Clinical information and ECT parameters are presented in TABLE 1 (V). All patients and control subjects gave their informed written consent to participate. The study protocol was approved by the Ethics Committee of the Tampere University Hospital.

4.2.4. ELISA measurements for human cytokines

We used enzyme-linked immunosorbent assay (ELISA) for the measurement of IL-6, sIL-6R and sGp130 in CSF and serum of patients and controls in acute seizure study. Plasma samples of chronically epileptic patients undergoing video-EEG study and control subjects were also examined using ELISA for levels of IL-1 β , IL-1ra, IL-6, sIL-6R and sGp130. Plasma samples of patients with depressive disorder undergoing ECT and control subjects were also studied using

ELISA for levels of IL-1 β , IL-1ra and IL-6. Commercially available kits were used in all studies according to manufacturer's instructions. Pelikine Compact (CLB, Amsterdam, Netherlands) kit was used for IL-6 measurements in CSF and serum/plasma samples from patients and control subjects. Kits for IL-1 β , IL-1ra, sIL-6R and sGp130 measurements in CSF and serum/plasma were purchased from R&D Systems (Abingdon, UK). The specificity of cytokine measurements (both between structurally similar cytokines as well as different species) was tested by manufacturers of ELISA kits.

4.3. Effect of exogenous hrIL-6 on seizure susceptibility in rats

4.3.1. PTZ induced seizures and intranasal IL-6 treatment

In the second part of the experimental studies, adult male Wistar rats were used. The rats received a total 40 μ l of human recombinant IL-6 (hrIL-6) diluted in sterile isotonic saline (10 μ g/ml; R&D Systems, USA) or vehicle in both nostrils as four 10 μ l injections at 3 minute time intervals. After one hour, the rats were injected intraperitoneally with pentylenetetrazole (PTZ) (75mg/kg; Sigma, UK). Thereafter rats were behaviourally observed for 30 minutes by a trained examiner. The following parameters were recorded: onset of the first twitch, the duration of oro-facial seizures, duration of clonic and tonic components of seizures, rats having motor seizures and mortality. The PTZ model was selected since PTZ induced seizures have clear behavioural manifestation enabling observation of seizure duration.

4.3.2. Brain preparation and measurement of hrIL-6 levels

The rats were killed and brains were removed and dissected into frontal (anterior to chiasma), temporoparietal and cerebellar/brain stem block and stored in -70 °C. Thereafter, tissue samples from IL-6 treated and control rats were weighed and homogenized using a Potter homogenizer in 50 mM PBS (pH = 7,5). Samples were centrifuged for 15 minutes (5000 rpm) and

supernatant was removed and stored at -70 °C. An ELISA kit from R&D Systems (USA) was used for the measurement of human recombinant IL-6 in tissue samples according to manufacturer's instructions.

4.4. Statistical analysis

Statistical analysis was performed using Windows SPSS 9.0 software. In the acute seizure study, statistical difference in cytokine and cytokine receptor levels between different seizure groups was tested using non-parametric Mann-Whitney test. In video-EEG and ECT studies, statistical difference in basal cytokine and cytokine receptor levels between patients and healthy controls were tested using Mann-Whitney test. Differences in cytokine and cytokine receptor levels at different time points after seizures during video-EEG recording or after ECT were tested as non-parametric related samples using Wilcoxon signed ranks test and Bonferroni's correction. The level of correlation between seizure parameters and cytokine levels in ECT study was tested using Spearman's test. In the experimental study using intranasal IL-6, differences in seizure parameters and human IL-6 levels were tested using Student's t-test. A p value of 0.05 was considered significant in all studies.

5. RESULTS

5.1. Expression of cytokines and cytokine receptors after kainic acid induced SE

Northern blot analysis revealed that IL-1 β expression was increased at 1 – 24 h after the beginning of generalized seizure activity and peaking at 12 h. Induction of IL-1 β was localized mainly in the thalamus and temporal lobe cortical areas. TNF α expression was detectable at 3 – 12 time points in Northern blot analysis, originating mainly from the hippocampus according to *in situ* hybridization (Figure 2 (I)).

Increased expression of IL-6 was found at 3 – 24 h time points, with highest expression at 12 h time point. *In situ* hybridization revealed scattered expression in the hippocampus, amygdala and temporal lobe cortex. Expression was also detected in the ependymal layer of the lateral ventricles. The expression of LIF was increased at 3 – 24 h time points in Northern blot analysis, peaking at 12 h time point. Expression was evident in the hippocampus in a scattered manner, cerebral cortex and amygdala also showed increased expression. Also LIF was induced in the ependymal layer of the lateral ventricles (Figure 1 (I)).

Northern Blot analysis revealed that IL-6R expression increased after SE at 3 – 12 h time points and peaking at 6 h. Induction was detectable only in the hippocampal pyramidal cell layer with *in situ* hybridization. LIFR expression was evident during 3 h – 3 d after SE, mainly in the gyrus dentatus and pyramidal cells of the CA3 region of the hippocampus, and in the cortical areas of the temporal and parietal lobes (Figure 1 (I)). Gp130 was expressed throughout the brain under basal conditions. Increased expression was, however, detected in the meningeal layer of the temporal lobe and in the ependymal layer of the lateral ventricles, as well as thalamus at 12 h after SE (Figure 2 (I)). Delayed scattered increase was found in the hippocampus, temporal lobe cortical areas and amygdala, and in the thalamus. In the hippocampus, delayed expression of Gp130 correlates to distribution of proliferated glial cells (not shown).

5.2. Levels of cytokines and cytokine receptors after acute seizures

5.2.1 Levels of IL-6 in CSF and serum

IL-6 levels were significantly increased in both CSF and serum samples after seizures compared to control patients (Figure 1 (II)). In patients with recurrent tonic-clonic seizures, IL-6 levels were significantly higher compared to patients with single tonic-clonic seizure both in CSF ($p = 0.05$, Mann-Whitney test) and serum ($p < 0.001$, Mann-Whitney test). After recurrent tonic-clonic seizures, serum IL-6 level was also significantly higher compared to prolonged partial seizures ($p < 0.01$, Mann-Whitney test). Serum levels of IL-6 in patients with prolonged partial seizures were significantly increased when compared to control patients ($p = 0.001$, Mann-Whitney test).

5.2.2. Levels of sIL-6R and sGp130 in CSF and serum

A significant decrease in CSF sIL-6R level was found only in a subgroup of patients with single tonic-clonic seizure compared to control patients ($p = 0.05$, Mann-Whitney test) (Figure 2 (II)), probably due to greater number of patients in this group. Significantly decreased level of sIL-6 in serum samples was found only in patients with prolonged partial seizures ($p < 0.05$, Mann-Whitney test). The levels of sGp130 (Figure 3 (II)) were not significantly altered after seizures, with the exception of increased serum levels in patients with single tonic-clonic seizures compared with control patients ($p < 0.05$, Mann-Whitney test).

5.3. Plasma levels of cytokines and cytokine receptors: video-EEG study

5.3.1. Basal (inter-ictal) levels of cytokines and cytokine receptors

Between basal (inter-ictal) samples of patients and healthy controls, no significant difference was found in IL-1 β or IL-1ra or sGp130 levels. A slight increase in plasma IL-6 levels was found in patients (1.75 ± 1.51 pg/ml) compared to healthy controls (0.99 ± 1.02 pg/ml),

however the difference did not quite reach statistical significance ($p = 0.06$, Mann-Whitney test). The basal plasma levels of sIL-6R in patients showed a trend to decreased levels compared with healthy controls (22.0 ± 5.57 ng/ml and 30.0 ± 2.96 ng/ml respectively; $p = 0.09$, Mann-Whitney test).

5.3.2. Post-ictal levels of cytokines and cytokine receptors

After seizures during video-EEG recordings, IL-1 β plasma levels were not significantly changed post-ictally compared to pre-ictal levels. On the other hand, plasma levels of IL-1ra (Figure 1a (IV)) were elevated at 2 h and 12 h time points ($p < 0.01$ and $p < 0.05$ respectively, Wilcoxon signed ranks test). The levels of IL-1ra appeared higher after secondarily generalized seizures, but the difference was not statistically significant. The plasma levels of IL-6 (Figure 1b (IV)) were increased post-ictally at 6 h time point ($p < 0.05$, Wilcoxon signed ranks test). Significantly higher levels of IL-6 were evident after secondarily generalized seizures compared to partial seizures ($p < 0.05$, Mann-Whitney test). No significant differences between inter-ictal and post-ictal plasma levels of sIL-6R or sGp130 were found.

5.4. Plasma levels of cytokines: ECT study

5.4.1 Basal levels of cytokines

The basal levels of IL-1 β were significantly lower in patients (median 0,08 pg/ml) compared to control subjects (median 0.33 pg/ml) ($p < 0.01$, Mann-Whitney test), whereas elevated basal levels of IL-6 were observed in patients (median 1.47 pg/ml) compared to control subjects (median 0.99 pg/ml) ($p < 0.05$, Mann-Whitney test). This difference in basal IL-6 levels was found to be caused by elevated IL-6 levels in a subgroup of patients who were in the later stage of their ECT course. Statistically, the basal levels of IL-6 correlated positively with the number of ECT

treatments ($r = 0.80$, $p = 0.01$, Spearman's test). The levels of IL-1ra showed no significant differences between patients and control subjects.

5.4.2. Levels of cytokines after seizure during ECT

We found that after electrographic generalized seizure during ECT, the levels of IL-1 β were transiently elevated ($p < 0.05$, Wilcoxon signed ranks test) (Figure 1a (V)). The levels of IL-6 also showed significant ($p < 0.05$, Wilcoxon signed ranks test) increase after ECT (Figure 1b (V)). On the other hand, no significant change was observed in IL-1ra levels (data not shown). The levels of both cytokines were elevated at 3h time point and falling towards basal levels at 24 h. Interestingly, the rise in IL-6 levels after ECT was significantly correlated to the charge (stimulus dose) used to induce seizure ($r = 0.98$, $p < 0.001$, Spearman's test), i.e. more robust IL-6 release was observed in patients with high charge used in seizure induction. IL-1 β or IL-1ra levels were not clearly associated with the seizure parameters.

5.5. Effect of intranasal hrIL-6 on seizure susceptibility in rats

5.5.1. Effect of IL-6 on PTZ induced seizures

We found that IL-6 markedly exacerbates the severity of PTZ induced seizures in rats (TABLE 1 (III)). The latency period for seizure after PTZ injection was significantly shorter in IL-6 treated rats (260 ± 56 sec) compared with vehicle treated rats (417 ± 59 sec; $p < 0.05$, Student's t-test). The duration of the oro-facial phase of seizures was similar in both IL-6 treated and vehicle treated rats, however, the duration of more severe clonic/tonic components was significantly longer in IL-6 treated rats (51 ± 8 sec) compared to vehicle treated rats (27 ± 6 sec, $p < 0.05$, Student's t-test). Total seizure duration was prolonged by IL-6 treatment (59 ± 10 sec) compared with vehicle treated (36 ± 7 sec; $p < 0.05$, Student's t-test). IL-6 treated rats also had a high mortality rate (55%) compared with vehicle treated rats (18%), but the difference was not statistically significant.

5.5.2. Levels of hrIL-6 in the rat brain

To assess the efficacy of intranasal recombinant human IL-6 treatment, we measured the levels of human IL-6 in rat brain after intranasal treatment and PTZ induced seizures. We found that in vehicle treated rat brain only low concentration of human IL-6 was detected, probably due to cross reactivity between human and rat IL-6 molecules. In frontal lobe samples high concentrations of IL-6 were detected only in IL-6 treated rats (18.6 ± 5.4 pg/g) compared to vehicle treated rats (2.2 ± 0.6 pg/g, $p < 0.05$, Student's t-test). In other brain tissue samples (temporoparietal and cerebellar block) hrIL-6 concentrations were comparable to saline treated rats.

6. DISCUSSION

The present dissertation provides evidence that inflammatory cytokines are induced widely in the brain as a response to SE, with concomitant induction of cytokine receptors, especially in the limbic structures such as hippocampus. The present dissertation also provides evidence from human patients that inflammatory cytokines are released after seizures, most likely originating from the CNS, as a response to seizure activity. We also found that IL-6 is a cytokine activated most dramatically after seizures, reflecting their severity. We were also able to demonstrate that IL-6 may act as a pro-convulsant cytokine.

6.1. Origin of cytokines

According to earlier experimental studies, it is clear that inflammatory cytokines are produced in the brain after SE (Vezzani et al., 1999; Jankowsky et al., 1999; De Simoni et al., 2000), and that less severe but still prolonged seizure activity is also able to induce cytokine

production in the brain (Vezzani et al., 1999; Vezzani et al., 2000). Single isolated seizures result also with increased expression of cytokines (Plata-Salaman et al., 2000; Sallmann et al., 2000). Cytokine mRNA expression or immunoreactivity has been localized almost exclusively into astrocytes (IL-6 and LIF) and microglial cells (IL-1 β and IL-1ra). However, whether cytokines measured from peripheral blood are released from the brain is not known. Studies by Peltola et al. showed that IL-6 levels are ten times higher in CSF than plasma after tonic-clonic seizures (Peltola et al., 1998; Peltola et al., 2000), suggesting intrathecal production. Production of cytokines from peripheral blood mononuclear cells in epileptic patients was excluded by Hulkkonen et al. (Hulkkonen et al., 2004). Muscle contractions associated with seizures (especially tonic-clonic seizures) or cerebral ischemia must also be regarded as potential mechanisms of plasma cytokine release. The present dissertation provides further evidence supporting cytokine release from the brain. The levels of cytokines were correlated with parameters of cerebral function, i.e. seizure activity: (1) In patients with acute seizures, the levels of IL-6 correlated to seizure severity; (2) After seizures during video-EEG recording, the plasma levels of IL-6 and IL-1ra were higher after generalized seizures; and (3) In patients undergoing ECT, the levels of IL-6 correlated to the charge used in seizure induction (which is comparable to the volume of seizure focus (Swartz, 2006)). In this approach, muscle contractions are absent due to anaesthesia, excluding peripheral cytokine release due to muscle injury. These patients are also treated in order to prevent disturbances in oxygen delivery and cerebral ischemia.

6.2. Time course of cytokine expression

The present data clearly show that cytokine activation after seizures was a rapid and transient phenomenon. It already begins at 1 h when increased mRNA expression of IL-1 β is detectable, followed by increased expression of TNF α , IL-6 and LIF at 3 h after the onset of generalized seizure activity of SE. In patients with chronic epilepsy, increased peripheral levels of

IL-1ra and IL-6 were evident at 2 h after the seizure according to the video-EEG data. After seizures during ECT, IL-1 β and IL-6 levels were elevated at 3 h after the seizure. Cytokine mRNA expression was found to peak 12 h after SE, present at 24 h but absent 3 days after SE. In patients with chronic epilepsy, the peak of IL-6 and IL-1ra levels was found at 6 h after the seizure in plasma, returning to basal levels by 24 h. This clearly shorter time course when compared to experimental SE is most likely explained by secondary inflammation associated with tissue necrosis after SE. Interestingly, the time course of cytokine release after seizures during ECT was of even shorter duration, with peak levels of IL-1 β and IL-6 were found at 3 h after the seizure.

6.3. Mechanisms of cytokine release

The present data from human patients suggest that seizure activity *per se* may cause release of cytokines from the brain which is detectable in CSF and peripheral blood after seizures: Cytokine release was detected in conditions which are not associated with acute neuronal tissue necrosis (and secondary inflammation) as in SE lasting 30 minutes or more (Meldrum, 2002). After single generalized seizures, we detected increased levels of IL-6 in CSF and serum. These seizures are not associated with release of markers of neuronal damage in an earlier report (Palmio et al., 2001). Even in the group of recurrent tonic-clonic seizures, the duration of seizure activity is much less than activity causing neuronal tissue necrosis (Meldrum et al., 2002). Interestingly, we found that IL-6 levels in CSF and serum correlated with seizure severity. However, in this study focusing on CSF and serum levels of cytokines after acute seizures, interfering factors must be noted: The patient groups were heterogeneous including several symptomatic seizures as well as cryptogenic seizures with unknown seizure etiology, thus limiting the data interpretation.

Some of these limitations were excluded in a study using a video-EEG recording approach. We were able to study patients with well-established seizure syndrome of localization related epilepsy. We were also able to determine the duration and type of seizure exactly. This study

shows that seizures, even partial seizures, may be followed by detectable increase in plasma cytokine levels. The levels of cytokines also correlated with seizure severity. However, this study approach still has some limitations. The possibility of neuronal injury still exists (especially in patients with TLE) although the form of neuronal damage is most likely apoptotic mechanism without secondary inflammation (Benzon et al., 1997). We therefore conducted a study in which release of cytokines was tested after seizures during ECT of patients with severe depressive disorder. This approach allowed us to observe pure electrographic seizures in the absence of neuronal damage, muscle contractions, and disturbances in oxygen delivery. We found that cytokines IL-1 β and IL-6 were transiently released after electrographic seizure activity. Increase in IL-6 levels correlated to the charge used in ECT, which reflects the volume of seizure foci (Swartz, 2006).

6.4. Effect of cytokines on seizures

A large body of experimental studies have shown that cytokines modulate both susceptibility to seizures and duration of seizure activity. The effect of IL-1 system on seizures is relatively well established: Inhibition of IL- β activity by inhibitor of ICE/caspase-1, over-expression of IL-1ra or deficiency of IL-1 receptor results in decreased seizure susceptibility (Ravizza et al., 2006; Vezzani et al., 2000, Dube et al., 2005). The data on the actions of IL-6 on seizures appears to be more complex. Transgenic mice with IL-6 deficiency showed more severe seizures after kainic acid injection, suggesting that IL-6 may have an anti-convulsive effect (Penkowa et al., 2001). On the other hand, transgenic mice over-expressing IL-6 were also more sensitive to kainic acid induced seizures, thus suggesting pro-convulsive action (Samland et al., 2003). In the present dissertation, we administered IL-6 intranasally, which resulted in exacerbation of PTZ induced seizures.

Several factors may explain such contradictory results. First, transgenic mice with IL-6 deficiency and IL-6 over-expression may not be simply compared, due to the fact that IL-6 may have functions during neuronal development. Therefore, differences may (at least in theory) be due to secondary developmental differences in the brain neurochemical or structural features. A more likely explanation is that IL-6 action may be concentration as well as time dependent. Under basal levels, IL-6 may have the effect inhibiting seizure activity in wild type mice, and when absent due to IL-6 deficiency, increased susceptibility to seizures results (Penkowa et al., 2001). However, when chronically over-expressed, IL-6 may cause structural changes as well as neurochemical changes that result in increased susceptibility to seizures when compared with wild type mice (Samland et al., 2003). Spontaneous seizures as well as structural changes in the nervous system have previously been reported in mice over-expressing IL-6 (Cambell et al., 1993). The approach used in present study presents still alternative condition, in which exogenous IL-6 was administered to rats, resulting in acute increase in seizure susceptibility to PTZ. These experiments may be summarised as follows: basal IL-6 expression is essential to maintain normal seizure threshold, however, chronic over-expression causes structural changes associated with increased susceptibility to glutamatergic agents. Presumably exogenous IL-6 also causes shortlived and rapid increase in seizure susceptibility.

SUMMARY AND CONCLUSIONS

Earlier experimental and clinical studies have suggested that inflammatory cytokines are released in the brain after seizures, which may have functional significance in epilepsy. The mechanisms as well as detailed information about the release of cytokines especially in human patients are not well understood. In this dissertation, we studied the production of pro-inflammatory cytokines after seizures using both experimental and clinical approaches. We concentrated on the time course of cytokine production as well as the mechanisms of cytokine release. We have also assessed the effect of IL-6 on seizure threshold in rats.

In the first experiment, we studied the expression of cytokines (IL-1 β , TNF- α , IL-6 and LIF) as well as IL-6 cytokine receptors (IL-6R, LIFR and Gp130) in the rat brain using Northern blot analysis and *in situ* hybridization histochemistry after SE induced by chemoconvulsant kainic acid. We found that all cytokines were up-regulated within hours after SE, returning to basal levels within 24 hours. We also found that IL-6R and LIFR were up-regulated in the hippocampus in structures representing most likely pyramidal cell neurons. Gp130 was expressed constitutively in the brain, however, delayed scattered up-regulation was found throughout the limbic system at 3 – 7 days after SE.

In the second part of the study, we studied the levels of IL-6 and its soluble receptors (sIL-6R and sGp130) in CSF and serum using ELISA in patients with different types of acute seizures and in control subjects. We found that IL-6 levels were increased after seizures both in CSF and serum, with higher levels in CSF. The levels of IL-6 were higher in patients with more severe seizures. We also found some indications that sIL-6R levels may be lower in CSF and serum and that sGp130 levels are higher in serum after seizures, however, the results were not consistent.

The levels of cytokines IL-1 β , IL-1ra, IL-6 and sIL-6R and sGp130 were also studied in plasma in patients with chronic localization-related epilepsy undergoing video-EEG study. In this

experiment, post-ictal cytokine levels were compared to patient's basal (inter-ictal) levels. We found that IL-6 and IL-1ra were increased post-ictally within hours after the seizure, returning to basal levels by 24 hours. IL-6 was increased in a manner reflecting seizure severity: secondarily generalized seizures were followed by clear increase in IL-6 levels whereas complex partial seizures caused only minor increase. During the inter-ictal period there was some indication of increased IL-6 levels and decreased sIL-6 levels in plasma when levels were compared with those of control subjects.

Earlier experiments strongly suggested that cytokines and especially IL-6 is released from the CNS after seizures via a mechanism involving increased neuronal activity and seizure spread. Hypothesis was further tested in patients with severe depression undergoing electroconvulsive therapy. The levels of IL-1 β , IL-1ra and IL-6 were measured using ELISA in plasma after electrographic seizures under general anaesthesia. We found that IL-1 β and IL-6 are strongly increased within the first hours after induced seizure, returning to basal levels within 24 hours. The levels of IL-6 correlated with the charge used in seizure induction, which represents the volume of seizure focus.

An earlier series of experiments suggested a crucial role for IL-6 after seizures as a seizure related multifunctional cytokine. We therefore tested the effect of IL-6 on seizures using the PTZ model of seizures. Human recombinant IL-6 was administered intranasally, which has been shown to be a possible route for drug delivery into CNS. We found that IL-6 strongly exacerbated the severity of PTZ induced seizures when applied prior to seizure induction. We were also able to control delivery of hrIL-6 into the brain: high levels of human IL-6 were measured in frontal lobe block using ELISA only in rats receiving hrIL-6 supporting successful delivery of hrIL-6 into CNS.

As a conclusion, we have here demonstrated that cytokine cascade is activated in the CNS after seizures. Very consistent results were obtained in experimental and different clinical settings, suggesting that the cytokine network is activated during the first 24 hours after the seizure.

It may also be concluded that IL-6 has a special role as a seizure-induced cytokine: the levels of IL-6 were strongly associated with severity of seizures in different experiments. We also found that IL-6 may, in fact, act as a pro-convulsant cytokine. Together with earlier studies, our results suggest that cytokine cascade has clinically relevant effects in the context of seizures and epilepsy. Cytokines are able to modulate neuronal excitability and may therefore be potential targets for future drug therapy.

In addition to spontaneous epileptic seizures, the present studies explore the release of cytokines related to seizures associated not only with epilepsy but also conditions like status epilepticus, various types of symptomatic seizures as well as electroconvulsive therapy. Therefore, activation of cytokine cascade by seizures may also have relevance for other diseases, especially in depression. Our results concerning pro-convulsant effects of IL-6 also suggest that in various inflammatory diseases like infections or autoimmune diseases, activation of cytokines may be associated with decreased seizure threshold resulting in symptomatic seizures. In the context of epilepsy, our results indicate that cytokine cascade activation after recurrent seizures may be of significance in neuropathological changes (like gliosis, neuronal damage, structural plasticity), which are important determinants of prognosis in epilepsy.

TABLE 1. Key features cytokine actions (Modified from Vilcek, 1998).

Pleiotrophy	A cytokine tends to have multiple target cells and multiple actions.
Redundancy	Different cytokines may have similar actions.
Synergism / antagonism	Exposure of cells to two or more cytokines at a time may lead to qualitatively different responses
Cytokine cascade	A cytokine may increase (or decrease) the production of another cytokine.
Receptor transmodulation	A cytokine may increase (or decrease) the expression of receptors for another cytokine or growth factor
Receptor transsignalling	A cytokine may increase (or decrease) signalling by receptors for another cytokine or growth factor

TABLE 2. Structural features of cytokines permit their grouping into families (modified from Vilcek, 1998).

Family	Representative members
Interleukin-2 / interleukin-4	IL-2 IL-4 IL-5 GM-CSF
Interleukin-6/ interleukin-12	IL-6 IL-12
Interferons- α/β	IFN- α IFN- β IFN- ω IFN- τ
Tumor necrosis factors	TNF- α TNF- β / (Lymphotoxin- α (LT- α)) LT- β Fas ligand CD40 ligand TNF-related apoptosis-inducing ligand (TRAIL)
Interleukin-1	IL-1 α IL-1 β IL-1ra IL-18
Transforming growth factor- β	TGF- β Bone morphogenetic proteins Inhibins Activins
Chemokines	C-X-C subfamily (IL-8, many others) C-C subfamily (MIP-1 α , many others) C subfamily (lymphotactin) CX ₃ C subfamily

TABLE 3. Structural features of some cytokine receptors permit their grouping into families (modified from Vilcek, 1998).

Receptor family	receptor subfamilies	representative ligands
Class I cytokine receptors	IL-6 (sharing Gp130)	IL-6
		IL-11
	GM-CSF (common β chain)	cNTF
		LIF
		OSM
		CT-1
	IL-2 (common γ chain)	GM-CSF
		IL-3
		IL-5
		IL-2
IL-13 (common α chain)	IL-4	
	IL-7	
	IL-9	
	IL-15	
	IL-13	
	IL-14	
Class II cytokine receptors (interferon family receptors)	none	IFN- α/β
		IFN- γ
TNF receptor family	none	IL-10
		TNF- α
		TNF- β
		LT- α /LT- β heteromer
		NGF
		Fas ligand
		CD40 ligand
		TRAIL
IL-1 receptor family	none	IL-1 α
		IL-1 β
		IL-1ra
TGF- β receptors	none	TGF- β
		Bone morphogenetic proteins

TABLE 4. Animal models of seizures / epilepsy (Modified from Cole et al., 2002).

Electrical stimulation	Maximal electroconvulsive seizures (MECS) Perforant-path stimulation (PPs) Amygdala stimulation Hippocampal stimulation Electrical kindling (amygdala/ hippocampus)
Chemoconvulsants	Systemic: kainic acid, pilocarpine, picrotoxin, bicuculline, PTZ, penicillin Intracerebral: kainic acid, pilocarpine, picrotoxin, bicuculline, tetanus toxin, pertussis toxin Topical: Alumina cream, penicillin
Physical models	Hyperthermia Freeze lesions Auditory stimulation (Swiss DBA2 mice)
Genetic models (spontaneous seizures)	Genetically epilepsy prone rat strain (GEPRS) Strasbourg rats (absence) Epileptic Beagles
Spontaneous seizure models	Post-kainic acid Post-pilocarpine Post-amygdala stimulation Post-kindling (overkindling)

TABLE 5. The International League Against Epilepsy (ILAE) classification of seizure types (2001).

EPILEPTIC SEIZURE TYPES

Focal seizures

- Focal sensory seizures
 - With elementary sensory symptoms (e.g., occipital and parietal lobe seizures)
 - With experiential sensory symptoms (e.g., temporo parieto occipital junction seizures)
- Focal motor seizures
 - With elementary clonic motor signs
 - With asymmetrical tonic motor seizures (e.g., supplementary motor seizures)
 - With typical (temporal lobe) automatisms (e.g., mesial temporal lobe seizures)
 - With hyperkinetic automatisms
 - With focal negative myoclonus
 - With inhibitory motor seizures
- Gelastic seizures
- Hemiclonic seizures
- Secondarily generalized seizures
- Reflex seizures in focal epilepsy syndromes

Generalized seizures

- Tonic-clonic seizures (includes variations beginning with a clonic or myoclonic phase)
 - Clonic seizures
 - Without tonic features
 - With tonic features
 - Typical absence seizures
 - Atypical absence seizures
 - Myoclonic absence seizures
 - Tonic seizures
 - Spasms
 - Myoclonic seizures
 - Massive bilateral myoclonus
 - Eyelid myoclonia
 - Without absences
 - With absences
 - Myoclonic atonic seizures
 - Negative myoclonus
 - Atonic seizures
 - Reflex seizures in generalized epilepsy syndromes
 - Seizures of the posterior neocortex
 - Neocortical temporal lobe seizures
-

TABLE 6. The International League Against Epilepsy (ILAE) Classification of Epilepsies and Epilepsy related syndromes (2001).

EPILEPSY SYNDROMES AND RELATED CONDITIONS

- Benign familial neonatal seizures
- Early myoclonic encephalopathy
- Ohtahara syndrome
- * Migrating partial seizures of infancy
- West syndrome
- Benign myoclonic epilepsy in infancy
- Benign familial and non-familial infantile seizures
- Dravet's syndrome
- HH syndrome
- * Myoclonic status in nonprogressive encephalopathies
- Benign childhood epilepsy with centrotemporal spikes
- Early onset benign childhood occipital epilepsy (Panayiotopoulos type)
- Late onset childhood occipital epilepsy (Gastaut type)
- Epilepsy with myoclonic absences
- Epilepsy with myoclonic-astatic seizures
- Lennox-Gastaut syndrome
- Landau-Kleffner syndrome
- Epilepsy with continuous spike-and-waves during slow-wave sleep (other than LKS)
- Childhood absence epilepsy
- Progressive myoclonus epilepsies
- Idiopathic generalized epilepsies with variable phenotypes
 - Juvenile absence epilepsy
 - Juvenile myoclonic epilepsy
 - Epilepsy with generalized tonic-clonic seizures only
- Reflex epilepsies
 - Idiopathic photosensitive occipital lobe epilepsy
 - Visual sensitive epilepsies
 - Primary reading epilepsy
 - Startle epilepsy
- Autosomal dominant nocturnal frontal lobe epilepsy
- Familial temporal lobe epilepsies
- * Generalized epilepsies with febrile seizures plus
- * Familial focal epilepsy with variable foci
- Symptomatic (or probably symptomatic) focal epilepsies
 - Limbic epilepsies
 - Mesial temporal lobe epilepsy with hippocampal sclerosis
 - Mesial temporal lobe epilepsy defined by specific etiologies
 - Other types defined by location and etiology
 - Neocortical epilepsies
 - Rasmussen syndrome
 - Other types defined by location and etiology

* Syndromes in development

TABLE 7. The International League Against Epilepsy (ILAE) classification of continuous seizure types (2001).

CONTINUOUS SEIZURE TYPES

Generalized status epilepticus

- Generalized tonic-clonic status epilepticus
- Clonic status epilepticus
- Absence status epilepticus
- Tonic status epilepticus
- Myoclonic status epilepticus

Focal status epilepticus

- Epilepsia partialis continua of Kojevnikov
 - Aura continua
 - Limbic status epilepticus (psychomotor status)
 - Hemiconvulsive status with hemiparesis
-

YHTEENVETO

Sytokiinit ovat peptidirakenteisia välittäjäaineita, jotka tunnetaan parhaiten valkosolujen erittäminä tulehdusreaktion säätelijöinä. Useilla sytokiineilla on osoitettu olevan myös merkitystä keskushermoston normaalissa toiminnassa. Samoin useimmissa hermostoa vaurioittavissa sairauksissa, kuten aivovammoissa, aivoiskemiassa ja tulehduksellisissa sairauksissa, todetaan sytokiinien erittyminen selkäydinnesteeseen ja verenkiertoon. Myös pitkittyneisiin epileptisiin kohtauksiin liittyy lisääntynyt sytokiinien ilmeneminen koe-eläinmalleissa. Myös ihmisillä on osoitettu erityisesti interleukiini-6 (IL-6) pitoisuuksien kohonneen vuorokauden kuluessa yksittäisestä yleistyneestä epileptisestä kohtauksesta sekä aivo-selkäydinnesteessä että veressä.

Tämän tutkimuksen tarkoituksena oli tutkia tarkemmin kohtauksiin liittyvää tulehdusta välittävien sytokiinien erittymistä koe-eläinmallissa sekä potilasaineistoissa. Tavoitteena oli tutkia erityisesti sytokiinien erittymisen mekanismeja ja ajallista yhteyttä kohtauksiin. Tutkimuksen yhtenä keskeisenä kysymyksenä oli: Voiko hermosolujen yliaktivaatio (kuten epileptisen purkauksen yhteydessä) aiheuttaa tulehdusta muistuttavan tilanteen hermostossa? Lisäksi tarkoituksena oli tutkia IL-6:n vaikutusta kohtausalttiuteen koe-eläinmallissa.

Ensimmäisessä osatyössä tutkittiin sytokiinien ja eräiden sytokiinireseptoreiden lähetti-RNA:n ilmenemistä Northern Blot sekä *in situ* hybridisaatiota käyttäen epileptisten sarjakohtauksen (kainihappo) mallia rotalla. Totesimme sytokiinien (interleukiini-1 β (IL-1 β), tuumorinekroositekijä- α (TNF α), interleukiini-6 (IL-6) ja leukemiaa estävän tekijän (LIF)) ilmenemisen lisääntyvän muutaman tunnin kuluessa sarjakohtauksen alkamisesta, ilmeneminen palasi perustasolle vuorokauden kuluessa. Lähes kaikkien sytokiinien osalta ilmeneminen oli voimakkainta ohimolohkon alueella sekä aivokammioiden seinämissä. Myös interleukiini-6

reseptorin (IL-6R) sekä leukemiaa estävän tekijän reseptorin (LIFR) ilmeneminen lisääntyi hippokampuksen alueella lähinnä hermosoluissa.

Seuraavassa työssä selvitettiin IL-6:n ja sen liukoisten reseptorien (sIL-6R ja sGp130) pitoisuuksia aivoselkäydinnesteessä sekä seerumissa erilaisten akuuttien kohtausten jälkeen. Totesimme että IL-6:n pitoisuudet ovat kohonneet merkittävästi kohtausten jälkeen. Interleukiini-6:n pitoisuudet olivat aivoselkäydinnesteessä selkeästi seerumia korkeammat. Myös kohtausten luonne vaikutti tuloksiin siten että IL-6:n pitoisuudet olivat korkeammat pitkittyneiden kohtausten jälkeen verrattuna yksittäiseen kohtaukseen sekä veressä että aivoselkäydinnesteessä.

Seuraavassa osatyössä tutkittiin sytokiiniinien (IL-1 β , interleukiini-1 reseptori antagonisti (IL-1ra), IL-6) ja liukoisten IL-6 sytokiinireseptorien (sIL-6R ja sGp130) pitoisuuksia plasmassa potilailla joilla on todettu krooninen paikallisalkuinen epilepsia, ja jotka olivat tulleet video-EEG tutkimukseen kliinisin perustein. Tutkimuksessa määritettiin sytokiiniinien ja liukoisten IL-6 reseptorien pitoisuudet tutkimukseen tullessa, sekä eri aikapisteissä seurannassa todetun kohtauksen jälkeen. Totesimme että IL-6:n ja IL-1ra:n pitoisuudet kohosivat merkittävästi muutaman tunnin aikana lähtöpitoisuudesta kohtauksen jälkeen. Interleukiini-6:n pitoisuudet olivat selkeästi korkeammat yleistyneiden kohtausten jälkeen verrattuna monimuotoiseen paikallisalkuiseen kohtaukseen.

Seuraavassa tutkimuksessa tutkittiin sytokiiniinien pitoisuuksia plasmassa potilailla joilla oli todettu vaikeahoitoinen masennus ja joilla oli päädytty sähköhoitoon sen hoitamiseksi. Sähköhoidossa aiheutetaan yleisanestesiassa sähköinen yleistynyttä epileptistä kohtausta muistuttava kohtaus jolla on toistettuna tehokas masennusta vähentävä vaikutus. Asetelma mahdollisti sytokiiniinien tutkimisen sähköiseen purkaukseen liittyen siten, että monet aiemmissä tutkimuksissa esiintyneet sekoittavat tekijät oli kyetty eliminoimaan (kohtauksen aiheuttava tekijä, kohtauksen aikainen hapenpuute, kouristukset, jne). Tutkimuksessa todettiin että IL-6:n ja IL-1 β :n pitoisuudet kohosivat jo muutaman tunnin kuluessa lähtötasolta merkittävästi plasmassa ja palasivat

normaalitasolle vuorokauden kuluessa. Interleukiini-6:n pitoisuudet olivat merkittävästi yhteydessä kohtauksen aiheuttamisessa käytettyyn virran suuruuteen, jonka on puolestaan ajateltu kuvaavan aiheutetun kohtauksen pesäkkeen tilavuutta.

Viimeisessä tutkimuksessa selvitettiin aiemmissa töissä merkittäväksi osoittautuneen IL-6:n vaikutusta kohtausalttiuteen pentyleenitetrazoli (PTZ) mallissa rotalla. Kokeessa annosteltiin rotalle nenän kautta ihmisen keinotekoista IL-6:a, jonka jälkeen rotat altistettiin PTZ:lle. Nenän kautta on aiemmin osoitettu kyttävän annostelevaan erilaisia peptidejä jotka kulkeutuvat keskushermostoon. Kokeessa havaittiin että IL-6 alentaa kohtauskynnystä merkittävästi: aikaviive kohtauksen alkuun lyhentyy, samoin kohtauksen kesto pitenee merkittävästi. Lisäksi osoitimme että nenän kautta annosteltu ihmisperäinen IL-6 kulkeutuu aivoihin: ihmisperäisen IL-6:n pitoisuus määritettiin ELISA menetelmällä, ja ainoastaan ihmisperäistä IL-6:a saaneilla rotilla todettiin kohonneet ihmisperäisen IL-6:n pitoisuudet aivojen otsalohkon alueella.

Tehdyt tutkimukset osoittavat että epileptisiin kohtauksiin liittyy sytokiinijärjestelmän aktivoituminen. Aktivoituminen liittyy spontaanien epileptisten kohtausten lisäksi erilaisiin symptomaattisiin kohtauksiin sekä vaikean masennuksen hoidossa käytettävään sähköhoitoon. Siten saaduilla tutkimustuloksilla voi olla laajempaa merkitystä myös muissa aivosairauksissa. Toisaalta todettiin että ulkoisesti annosteltu IL-6 voi laskea kohtauskynnystä. Tuloksen perusteella voidaan ajatella että monissa yleissairauksissa joihin liittyy sytokiinien vapautuminen sekä epileptisiä kohtauksia (infektiot, autoimmuunitaudit), voi kohtauksen syntymekanismi välittyä sytokiinien vaikutuksella. Kroonisessa epilepsiassa puolestaan toistuvat kohtaukset ja niihin liittyvä sytokiiniaktivaatio voi aiheuttaa rakenteellisia muutoksia aivoissa, jotka voivat vaikuttaa epilepsian ennusteeseen ja hoitovaihtoehtoihin. Sytokiinijärjestelmää säätelevät lääkehoidot voivat siten tulevaisuudessa tarjota uusia hoitovaihtoehtoja esimerkiksi vaikeahoitoisessa epilepsiassa ja masennuksessa.

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Research report

Expression of cytokines and cytokine receptors in the rat brain after kainic acid-induced seizures

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Abstract

We have previously shown that IL-6 protein levels are increased in cerebrospinal fluid in humans after recent tonic–clonic seizures with unchanged levels of IL-1 β and TNF α . Here we studied the expression of cytokines IL-6, LIF, IL-1 β and TNF α and cytokine receptors IL-6R, LIFR and Gp130 in the rat brain after kainic acid-induced status epilepticus using Northern blot analysis and in situ hybridization histochemistry. After seizures, IL-6 mRNA was induced in the hippocampus, cortex, amygdala and meninges, and IL-6R was up-regulated in the hippocampus. LIF was up-regulated in the hippocampus, cortex and meninges after seizures, and LIFR mRNA was induced in the hippocampus and cortex. Gp130 was constitutively expressed in the brain. After seizures, Gp130 transcription was rapidly induced in the meninges. In thalamus, cortex, amygdala and hippocampus Gp130 mRNA was induced in a delayed fashion. IL-1 β transcription was induced in the temporal lobe cortex and thalamus, and TNF α in the hippocampus. In general, the cytokine and their receptor mRNA levels were low in intact rat brain, but were induced by seizures. Since IL-6 and LIF transcripts were induced in the meninges after seizures, the protein products of these transcripts may be more readily released in cerebrospinal fluid after seizures. In addition, the activity of IL-6 and LIF signaling pathways may be influenced by increased expression of their receptors after seizures. © 2002 Elsevier Science B.V. All rights reserved.

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Topic: Epilepsy: basic mechanisms

Keywords: Seizure; Interleukin-6; Leukemia inhibitory factor; Cytokine receptor

1. Introduction

Cytokines are generally known as mediators of inflammatory and immune reactions. Recently, cytokines have been shown to regulate also differentiation of nervous system cells during neural development [24], and to regulate physiological functions such as learning and memory in adult rats [19,36]. In the central nervous system (CNS), increased production of cytokines has been detected after pathological processes, such as stroke [46] and trauma [16,22]. Increased secretion of cytokines into the cerebrospinal fluid (CSF) has been reported also after epileptic seizures [31,32].

Neuropoietic family cytokine interleukin-6 (IL-6) is a

cytokine with pro-inflammatory and anti-inflammatory properties [20], and it has been reported to contribute to neuroprotection after status epilepticus [33], and inhibit spread of excitation in vitro [6]. Pro-inflammatory cytokine interleukin-1 β (IL-1 β) has been shown to exert neurotoxic [30] and proconvulsant effects [43,44] in experimental models of status epilepticus, whereas another pro-inflammatory cytokine, tumor necrosis factor- α (TNF α), has neuroprotective [4] and anticonvulsive effects [45]. Leukemia inhibitory factor (LIF) is another member of neuropoietic cytokine family, which has been shown to be up-regulated by experimental status epilepticus [19,26], but no data is available about the functional role of this cytokine after seizures.

The biological activity of cytokines is influenced by changes in the level of cytokine receptor expression. Receptor components of IL-6 and LIF include interleukin-6 receptor (IL-6R) and leukemia inhibitory factor receptor

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(LIFR), and a common signal transducer Gp130 [40]. Alternative splicing of these receptor mRNAs results in formation of soluble receptors [5,9,17], which modulate particular cytokine actions. Soluble form of IL-6R (sIL-6R) has a powerful agonistic effect on IL-6 signaling, a phenomenon called transsignaling [15]. On the other hand, this sIL-6R mediated transsignaling is inhibited by soluble Gp130 (sGp130) [21]. Soluble form of leukemia inhibitory factor receptor (sLIFR) has also been identified, and it serves as an antagonist of LIF actions [10].

We have previously reported increased levels of IL-6 in CSF and plasma in patients with recent tonic-clonic seizures, with unchanged levels of IL-1 β and TNF α [31,32]. However, in experimental studies, increased production of IL-1 β and TNF α has also been reported after seizures [8,12]. This differential release of cytokines into CSF may be due to different anatomical distribution or time course after seizures. Here we studied the expression of IL-6, LIF, IL-1 β and TNF α after kainic acid-induced status epilepticus. Since only IL-6 levels were increased after seizures in humans, we concentrated on the expression of neuropoietic cytokine receptors IL-6R, LIFR and Gp130 after seizures.

2. Materials and methods

2.1. Animal treatments and tissue preparations

Adult male Sprague–Dawley rats were injected subcutaneously with kainic acid (10 mg/kg; Sigma, St Louis, MO, USA or Ocean Produce Int., Canada) in 0.9% saline. After injection of kainic acid, rats first showed behavior such as staring and movement arrest. Thereafter, rats showed wet dog shakes eventually leading to unilateral limb and/or facial clonus. Finally, after on average 60 min, rats had the first generalized tonic-clonic seizure with bilateral limb clonus (Stage 3 seizure in Racine scale), which was considered as zero time point. Thereafter behavioral seizure activity slowly changed continuous, lasting for several hours. The kainic acid-injected animals were killed 1, 3, 6, 12, 24, 72 h or 7 days after the onset of the first typical tonic-clonic seizure. For *in situ* hybridization histochemistry, the brains ($n=4$ for each time point) were

removed and frozen on dry ice. Coronal sections (14- μ m-thick) were cut through the hippocampus with a cryostat and mounted onto Fisherbrand Superfrost Plus slides (Fisher Scientific, Pittsburgh, USA). Untreated animals ($n=4$) were used as controls. For Northern blot analysis, brains (at least two for each time point) were cut between optic chiasma and anterior pons and stored at -80°C . Untreated animals ($n=2$) were used as controls.

2.2. Extraction of mRNA

Brains were homogenized in a solution containing 200 mM NaCl, 200 mM Tris (pH 7.5), 1.5 mM MgCl $_2$, 2% SDS and 200 μ g/ml proteinase K (Fermentas) in diethylpyrocarbonate (DEPC)-treated water. The lysates were passed four times through a sterile 21-gauge needle and thereafter incubated at 45°C in a water bath for 60 min. NaCl was added to each sample to a final concentration of 500 mM. The samples were then passed four times through a 21-gauge needle. Oligo(d)T cellulose (75 mg; Invitrogen) was added to each sample, and the samples were incubated at room temperature for 60 min. The samples were then centrifuged at 8000 rpm for 10 min at 4°C and the supernatant was removed. The pellets were washed twice in a buffer containing 500 mM NaCl and 10 mM Tris (pH 7.5) in DEPC-treated water and thereafter three times in a buffer containing 250 mM NaCl and 10 mM Tris (pH 7.5) in DEPC-treated water. The samples were then transferred into spin-columns (Invitrogen) and washed four times with buffer containing 250 mM NaCl and 10 mM Tris (pH 7.5) in DEPC-treated water. Messenger RNA was eluted with 400 μ l of buffer containing 10 mM Tris (pH 7.5) in DEPC-treated water. Sixty IUs of RNase inhibitor (Fermentas) and 5 IUs of RNase free DNase (Boehringer Mannheim) were added to each sample and the samples were then incubated at 37°C for 30 min. The mRNA was ethanol precipitated, dissolved in water and stored at -80°C until used.

2.3. Oligonucleotide probe preparation

The oligonucleotide probes used are listed in Table 1. Searches of the Genbank database revealed no significant homology of the oligonucleotide sequences with any other

Table 1
List of the oligonucleotide probes used

Gene	5'-oligonucleotide sequence-3'	Bases	Species	Accession number	Ref.
IL-6	gtggtatcctctgtgaagctcctctccggactgtgaag	144–183	Rat	M26744	[27]
LIF	tcggtcatgttggcgccacatagattccacgttgg	224–263	Rat	AB010275	[41]
IL-1 β	ccacgggcaagacatagtagctccacagcttccacgccac	278–322	Rat	M98820	Unpublished observations
TNF α	atgagaagaggctgaggcacagaccgctggagtctggaggc	68–112	Rat	X66539	[14]
IL-6R	ggctgataccagaaggtggcaggtggatccggctgcacc	738–777	Rat	M58587	[2]
LIFR	ggtgatagcctgcgaagacgaccttcatacacatcgt	1038–1077	Rat	D86345	[1]
Gp130	cagctgcaggtcctctggcgcctcctcggagtctagcagg	2352–2391	Rat	M92340	[47]
Cyclophilin	gagttgtccacagtcggagatggtgatcttctgctgtctgcc	490–543	Rat	M19533	[7]

previously characterized gene. The oligonucleotide probes for cytokine receptors were designed to detect exons that are present in both soluble and membrane bound forms of cytokine receptors [5,9,17]. The oligonucleotides were labeled with ^{33}P -dATP (DuPont-NEN Research Products, Boston, USA) for in situ hybridization histochemistry and with ^{32}P -dATP for Northern blot hybridization using terminal deoxynucleotidyltransferase (Fermentas). The labeled oligonucleotides were purified using Qiaex II kit (Qiagen).

2.4. Northern blot hybridization

RNA samples (8 μg /lane for $\text{TNF}\alpha$, 6 μg /lane for IL-6R and 4 μg /lane for the other cytokines and their receptors) were run in agarose gel. The samples were transferred onto a nylon membrane (Hybond N+, Amersham) and crosslinked with UV-light (Stratalinker; Stratagene). The membrane was prehybridized at 42 °C for 60 min in a hybridization solution containing 4 \times standard saline citrate (SSC), 50% formamide, 5 \times Denhardt's solution, 1% sodium dodecyl sulfate (SDS), 0.02 M phosphate buffer (pH 7.0), 10% dextran sulfate and 100 $\mu\text{g}/\text{ml}$ heat denatured salmon sperm DNA. Thereafter the labeled probe was added to the hybridization solution to a final concentration of up to 5 $\times 10^6$ cpm/ml, and the membrane was hybridized at 42 °C overnight. The membrane was washed with increasing stringencies with maximal stringency of 2 \times SSC at 65 °C. The membrane was covered with Biomax MR (Kodak) autoradiographic film (Rochester, NY, USA) and exposed for 3–7 days. Northern blots were quantified with UTHSCSA Image Tool 3.00 software (University of Texas Health Science Center, San Antonio). The integrated densities of each transcript were balanced against cyclophilin in the same lane and the values representing control and kainic acid-treated animals were compared.

2.5. In situ hybridization histochemistry

At least four sections for each subject were hybridized with each oligonucleotide probe. The sections were air dried at room temperature and hybridized at 42 °C for 12–18 h with a mixture of 4 \times SSC, 50% formamide, 1 \times Denhardt's solution, 1% sarcosyl, 0.02 M phosphate buffer (pH 7.0), 10% dextran sulfate, 500 $\mu\text{g}/\text{ml}$ heat denatured salmon sperm DNA, 200 mM dithiothreitol and 1 $\times 10^7$ cpm/ml of the labeled probe. After hybridization, the sections were washed four times (15 min each) in 1 \times SSC at 55 °C and thereafter left to cool for 1–3 h at room temperature. The sections were then dipped in distilled water and subsequently in 75 and 90% ethanol and air dried at room temperature. The sections were covered with Biomax MR (Kodak, Rochester, NY, USA) and exposed for up to 6 months. We have previously

shown that hybridization with sense probes gives a negligible background [50].

3. Results

No IL-6 expression was detected in control brains with Northern blot analysis, but two mRNA species of about 1.2 kb and 2.4 kb were evident in kainic acid-injected rats at 3–24 h. The highest expression of IL-6 was at 12 h with about a 2.2-fold increase in band density (Fig. 1). With in situ hybridization, weak IL-6 expression was detected in the hippocampus, also in the control rat brains. After seizures, IL-6 expression was increased at 6 h in the hippocampus and amygdala, in the piriform, perirhinal and parietal cortices; and in the meninges adjacent to piriform and perirhinal cortices and at the dorsal border of the thalamus. Similar expression was still detected at 12 h, with slightly increased expression in the meninges.

No IL-6R expression was detected in the control rat brains with Northern blot analysis, but a single transcript of approximately 13 kb was detected after seizures. The expression of IL-6R was first detected at 3 h, but still detectable at 12 h after the first seizure (Fig. 1). By 6 h the mRNA expression peaked reaching a 4-fold increase in band density as compared to control animal. With in situ hybridization, IL-6R expression was absent in control rats, but a slight increase was observed in the hippocampus, mainly in the CA1 region, at 12 h in kainic-injected rats.

In the control rat brains, no LIF expression was detected with Northern blot analysis, but a single 4.5 kb transcript was detected after seizures. The increased expression of LIF was detected at 3 h–1 day (Fig. 1) peaking at 12 h when it reached a 4.5-fold increase in Northern blot analysis. With in situ hybridization, LIF expression was undetectable in control rats, but increased expression was detected at 12 h in the hippocampus, in the piriform, perirhinal and parietal cortices; and in the meninges adjacent to temporal lobe and dorsal part of the thalamus.

The expression of LIFR was absent in control rats, but a single approximately 12 kb transcript was detected at 3 h–3 days after seizures with Northern blot analysis (Fig. 1). Densitometric analysis revealed a mild increase in band density ratios at 3 h–1 day (1.5–2.0-fold increase) with the highest, a 3.0-fold-induction at 3 days. With in situ hybridization, the expression of LIFR was undetectable in control rats, but was detected at 12–24 h after seizures in the CA3 field and gyrus dentatus of the hippocampus, and in the piriform, perirhinal and parietal cortices.

With Northern blot analysis, Gp130 was constantly expressed both in control and kainic acid-injected animals with no evident change after seizures. Two transcripts of about 6 kb and 10 kb were detected (Fig. 2). With in situ hybridization, Gp130 was evenly expressed throughout the brain in control rats with the most prominent hybridization signal detected in the pyramidal and granular cell layers of

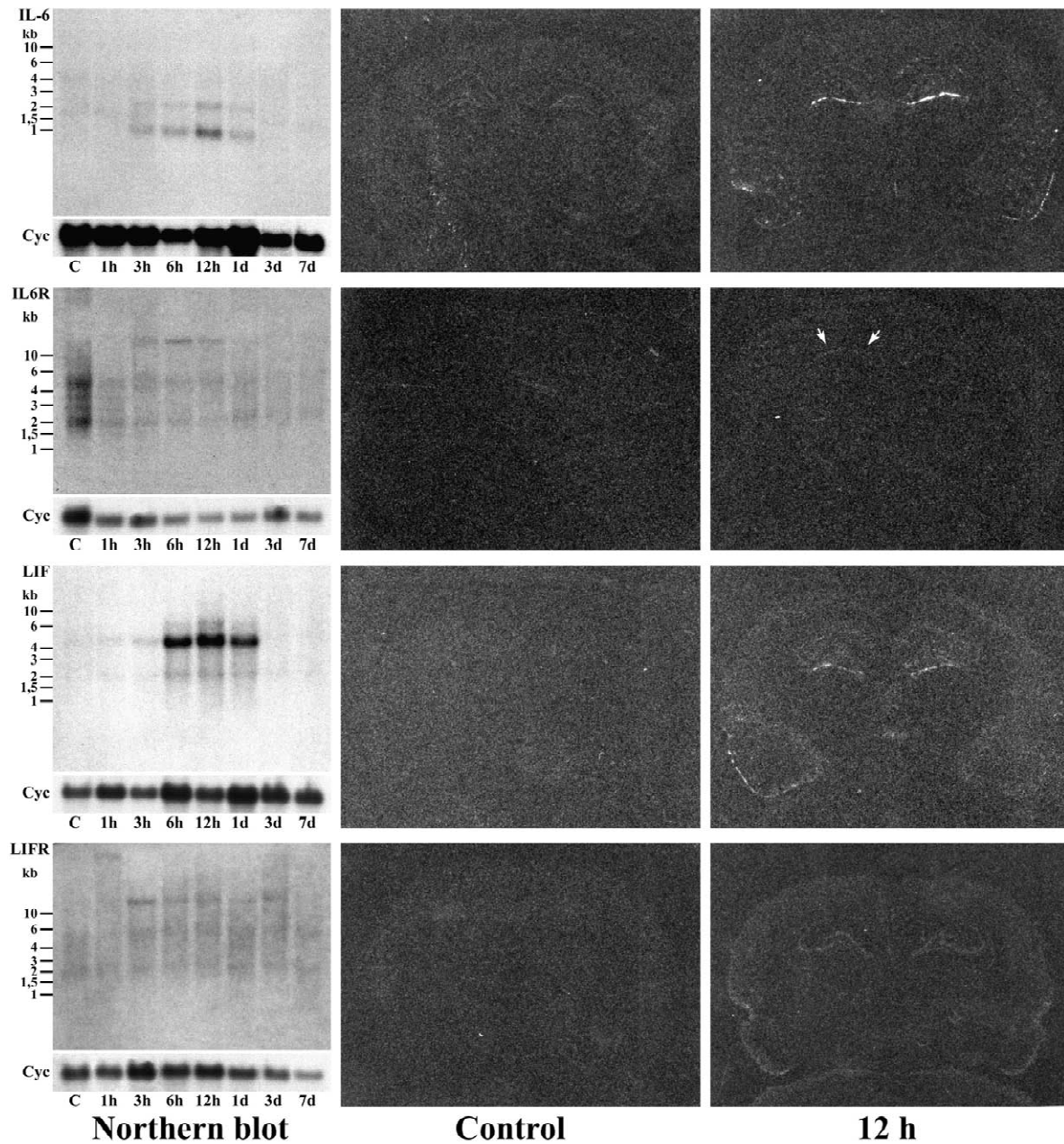


Fig. 1. The expression of IL-6, IL-6R, LIF and LIFR as detected by Northern blot analysis and in situ hybridization histochemistry in the control rat brains and at 12 h after the first generalized seizure. The arrows indicate the induced expression of IL-6R in the CA1 region of the hippocampus. For details, see text.

the hippocampus. A restricted up-regulation of Gp130 was detected at 12 h after seizures in the meninges adjacent to the temporal lobe, basal midbrain and dorsal regions of the thalamus. At 7 days, Gp130 was up-regulated in the hippocampus, piriform and perirhinal cortices, amygdala and thalamus (not shown).

No expression of IL-1 β was detected in control rat brains with Northern blot analysis, but a single transcript of about 1.8 kb was detected after seizures. Increased expression of IL-1 β was detected at 1–24 h, with the highest (4.9-fold) expression at 12 h (Fig. 2). With in situ

hybridization, no expression of IL-1 β was detected in control brains, but it was slightly expressed in the piriform and perirhinal cortices and in the thalamus at 12 h after seizures.

In the control rat brains, a weak basal TNF α expression was detected with Northern blot analysis. This 2.2 kb transcript was induced by 3–12 h after which it gradually decreased to control levels by 3 days (Fig. 2). The highest induction of TNF α was observed at 3 h, when densitometric analysis showed a 2.5-fold increase in band density. With in situ hybridization, no TNF α expression was

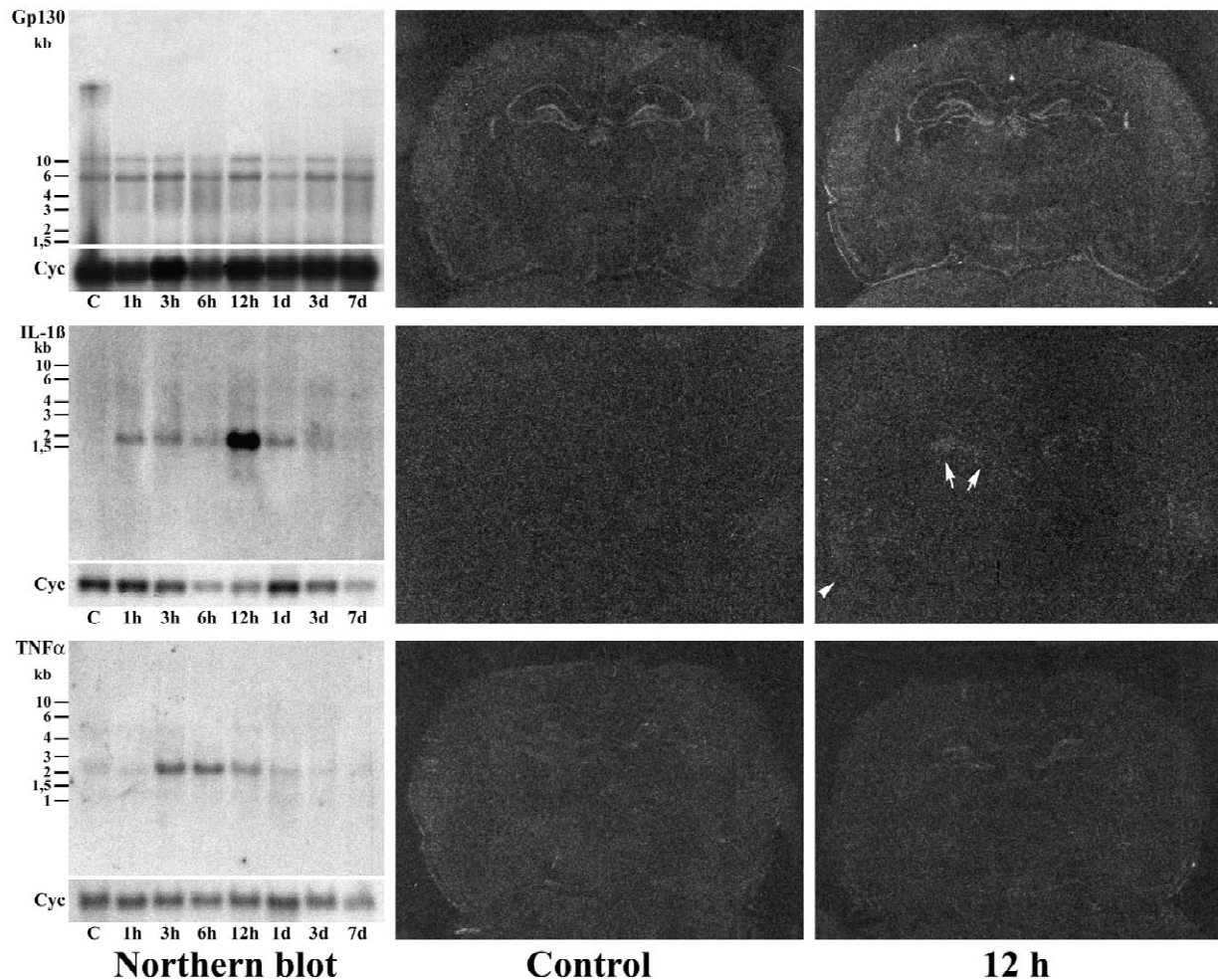


Fig. 2. The expression of Gp130, IL-1 β and TNF α as detected by Northern blot analysis and in situ hybridization. After seizures, the expression of IL-1 β was slightly induced in the thalamus (arrows) and temporal lobe (arrowhead). For details, see text.

detected in control brains, whereas slightly increased expression was detectable in the hippocampus at 12 h after seizures.

4. Discussion

Results of the present study indicate that a cascade of cytokines is induced in distinctive brain areas after kainic acid-induced seizures. Concomitant induction of neuro-poietic cytokine receptors suggest that they modulate the effects of neuropoietic cytokines. Interestingly, IL-6, LIF and Gp130 mRNA were also induced in the meninges by seizures, indicating that these molecules may be secreted into the CSF after seizures.

In agreement with a previous study [8], induction of IL-6 in the hippocampus after seizures was observed. However, our finding about increased IL-6 expression at 6–12 h in the temporal and parietal cortices, in the amygdaloid complex and in the meninges, has not been reported previously. Also, as a novel finding, an IL-6R

transcript of approximately 13 kb was detected after seizures in Northern blot analysis. So far, IL-6R transcript of 5.1 kb in size has been reported in the rat brain [37]. Similar large transcripts have, however, been characterized for other neuropoietic cytokine receptors [5,28,47]. In addition, the observed size of the IL-6R transcript is comparable to the size of the LIFR shown in the present study.

With in situ hybridization histochemistry, LIF mRNA was detected in the hippocampus, temporal and parietal cortices and meninges. Similar results have been reported previously in the pilocarpine model of status epilepticus [19]. However, the induction and distribution of LIFR in the hippocampus and cortex after seizures has not been reported previously. A single LIFR transcript of approximately 12 kb was detected after seizures with Northern blot analysis. The size of LIFR transcript has not been reported in the rat brain, but it is comparable with a LIFR transcript detected in the mouse brain [28].

Gp130 mRNA species of 6 and 10 kb in size were detected both in control and kainic acid injected rats in

Northern blot analysis. The size of Gp130 transcripts has not been reported previously in the rat brain, but our findings are comparable with the size of Gp130 transcripts in rat hepatocytes [47]. In the control rat brain, Gp130 mRNA expression is comparable with a immunohistochemical data by Watanabe et al. [48], but the distribution of Gp130 mRNA after seizures has not been previously documented.

Previously, IL-1 β expression after kainic acid-induced status epilepticus has been detected in the hippocampus, amygdala, temporal lobe cortex and thalamus [12]. In the present study, IL-1 β expression was evident in the temporal lobe cortex and thalamus, but was undetectable in other brain regions. Tumor necrosis factor- α was up-regulated in the hippocampus in a manner comparable with previous studies [4,8], and no expression was detected outside hippocampus.

We have previously reported increased IL-6 levels with unchanged IL-1 β and TNF α levels in CSF in patients with recent tonic-clonic seizures [32]. After kainic acid-induced seizures, IL-6, LIF and Gp130 expression were evident in the meninges, whereas no such expression of IL-1 β or TNF α was detected. Therefore, the expression in the meninges may cause increased IL-6 levels with normal IL-1 β and TNF α levels in the CSF after seizures. Interestingly, IL-6, LIF and Gp130 transcription was induced in the meninges adjacent to the temporal lobe and dorsal portion of the thalamus, areas showing concomitant IL-1 β induction. Previously, intraventricular IL-1 β injection has been reported to increase IL-6 levels in the primate CSF [34]. Interleukin-1 β also increases LIF expression in the peripheral nerve fibroblasts in vitro [39]. Therefore, the seizure-induced IL-1 β transcription in the brain parenchyma may stimulate the expression of IL-6 and LIF in the adjacent meninges.

Up-regulation of IL-6, LIF, IL-1 β and TNF α were detected also in the brain parenchyma after seizures. However, the mechanism causing this cytokine up-regulation after status epilepticus is controversial. Some authors have considered this phenomenon mainly as secondary to neuronal tissue damage [13]. However, considerable evidence has emerged indicating that seizure activity per se may cause cytokine up-regulation. Increased immunoreactivity of IL-1 β has been detected in the hippocampus after bicuculline methiodide administration, in the absence of neuronal cell loss [43]. Increased levels of IL-6 mRNA has also been detected in mice after single electroconvulsive shock administration, and neuronal membrane depolarization and Ca²⁺ influx was reported to cause IL-6 induction [35]. Interestingly, also LTP stimulations have been reported to increase IL-1 β and IL-6 mRNA in the hippocampus [18,36]. We have previously reported increased levels of IL-6 in CSF in patients with single tonic-clonic seizures [32], with unchanged levels of markers of neuronal tissue damage, such as neuron-specific enolase (NSE) and S-100 protein [29]. In the present

study, increased expression of cytokines was observed already at 1–3 h after the first seizure, whereas first degenerating neurons with DNA fragmentation have been detected at 5 h from the kainic acid injection [13]. In addition, cytokines were down-regulated within the first 24 h, whereas neurodegeneration continues for several days [13]. Therefore, our results support the hypothesis that cytokines may be induced by seizure activity.

Recent experimental studies have elucidated the function of cytokines in regulation of neuronal tissue excitability. Interleukin-1 β has been shown to increase the duration of status epilepticus, and this effect is blocked by IL-1ra [43,45]. Blocking of endogenous IL-1 β with IL-1ra also suppresses seizure generalization [8,45]. On the other hand, TNF α has been reported to exert anticonvulsive effects [8]. In addition, IL-6 has been reported to decrease glutamate release and inhibit spread of excitation in the cerebral cortex in vitro [6]. In the present study, kainic acid-induced behavioral seizures lasting for up to 6 h, and increased expression of cytokines was detected during convulsions. Therefore, these cytokines potentially regulate the evolution and duration of status epilepticus. Interestingly, increase in proconvulsive cytokine IL-1 β expression was rapidly counteracted by an increase in anticonvulsive cytokines IL-6 and TNF α , suggesting that these cytokines may serve as a control mechanism of seizure spread.

Cytokines are also involved in regulation of seizure-induced neuronal tissue damage. Exogenous IL-1ra administration has a neuroprotective effect in kainic acid-induced neurodegeneration, suggesting that endogenous IL-1 β has neurotoxic effects after status epilepticus [30]. On the other hand, TNF α knockout mice show exacerbated neuronal damage after kainic acid administration, suggesting neuroprotective effects of TNF α after status epilepticus [4]. Interleukin-6 has been shown to protect cholinergic neurons from excitotoxic neurodegeneration in vivo [42], and in IL-6 knockout mice, increased loss of hippocampal neuron has been reported after kainic acid administration [33]. The neurotoxic effect of IL-1 β is supported by the present data, since the expression of IL-1 β closely matches areas known to undergo massive and rapid neuronal death [38].

Simultaneous increase in LIF and LIFR expression was detected in the hippocampus and cortex after seizures. Expression of LIFR was increased in dentate gyrus granule cells, which are known to contribute mossy fiber sprouting into the molecular layer of the dentate gyrus after seizures [49]. Interestingly, increased LIFR expression was observed also in the CA3 pyramidal neurons, which are also reported to be able to generate axon collaterals after hippocampal damage [23]. In addition, LIFR expression increased in the temporal lobe cortex, and re-organization of neuronal structures are also suggested to occur in these areas after seizures [11,25]. Co-localization of the seizure-induced increase in LIF and LIFR transcription in these brain structures, that share ability to structural plasticity

after injury, indicates a potential involvement of LIF in neural repair. This role of LIF is also supported by reports describing the effect of LIF in sprouting of CNS neurons after injury [3].

In the present study, increased expression was evident in all cytokine receptors studied, suggesting that the activity of neuropoietic cytokine signaling pathways are affected by a changed level of cytokine receptor expression. However, the net effect of increased cytokine receptor expression depends on whether soluble or membrane-bound receptors are formed, which may not be discriminated from the membrane-bound receptor mRNA with Northern blot analysis [17]. Therefore, further studies are needed to assess this seizure-induced regulation of neuropoietic cytokine action by increased cytokine receptor expression.

As a conclusion, this study demonstrates that cytokines are widely expressed in the brain after status epilepticus, probably as a result of sustained seizure activity. Both meningeal and parenchymal distribution was observed, and especially the expression in the meninges may also be reflected into CSF. The activity of these cytokines may be regulated also by increased expression of cytokine receptors. Cytokines were expressed in a manner suggesting involvement of cytokines in controlling seizure activity, neuronal damage and neural repair.

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Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration

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Abstract

Experimental studies suggest that cytokine production may be triggered by seizure activity. Here we determined the levels of interleukin-6 (IL-6) and its soluble receptor components (sIL-6R and sGp130) in CSF and serum from control subjects and patients after different types of seizures. IL-6 levels were increased after seizures, whereas sIL-6R levels were decreased. Interestingly, the levels of IL-6 were strongly increased after recurrent generalized tonic-clonic seizures (GTCS), whereas after single tonic-clonic or prolonged partial seizures IL-6 levels were increased to lesser extent. These results provide further support for a hypothesis of cytokine production induced by seizure activity per se. © 2004 Elsevier B.V. All rights reserved.

Keywords: Cytokine; GTCS; Partial seizure; CSF; Epilepsy

1. Introduction

In the central nervous system (CNS), cytokines are produced as a response to various inflammatory stimuli. Recently, experimental studies have revealed that cytokine production may be induced also by seizure activity (Jankowsky et al., 2000; Vezzani et al., 1999). Interestingly, modulation of cytokine network has been shown to affect duration and spread of seizure activity (D'Arcangelo et al., 2000; De Simoni et al., 2000; Vezzani et al., 1999), as well as seizure susceptibility (Vezzani et al., 2000). In addition, seizure-induced production of cytokines may contribute to formation of structural changes after sustained seizure activity, such as neuronal damage and gliosis (Panegyres and Hughes, 1998; Penkowa et al., 2001).

Interleukin-6 (IL-6) is a cytokine with multiple effects on various cell types and tissues throughout the body. In the CNS, IL-6 has been revealed to have both neuroprotective (Penkowa et al., 2001, 2003) and neurotoxic effects (Campbell et al., 1993). IL-6 receptor complex has two components, interleukin-6 receptor (IL-6R) and glycoprotein 130

(Gp130), both of which are also released in soluble forms. Soluble IL-6R (sIL-6R) has mainly agonistic effects on IL-6 signalling (Peters et al., 1996), whereas soluble Gp130 (sGp130) acts as an antagonist (Muller-Newen et al., 1998; Jostock et al., 2001). Due to the modulating role of these soluble receptors, the levels of these receptor components should be evaluated in order to assess IL-6 activity.

In our previous studies (Peltola et al., 1998, 2000a), increased levels of IL-6 were measured in CSF and plasma of patients with recent generalized tonic-clonic seizures (GTCS). Only patients with single GTCS were included in these studies. However, whether this observed increase in IL-6 levels is related directly to the seizure activity or some other seizure-related phenomenon is not fully understood. In the present study, we examined patients with different seizure patterns in order to evaluate the effect of seizure activity per se on IL-6 system.

2. Patients and methods

Thirty-three consecutive patients coming to the emergency department of Tampere University Hospital with acute seizures were included in the study. Patients with any signs of inflammatory or infectious disease were excluded from the study. Three groups of patients were

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Table 1
Etiology of seizures in different patient groups

	sGTCS	rGTCSs	PPSs
Spontaneous seizure			
symptomatic epilepsy			
vascular		1 (10%)	4 (57.1%)
trauma		1 (10%)	
tumor	2 (12.5%)		1 (14.3%)
cryptogenic epilepsy	11 (68.8%)	1 (10%)	2 (28.6%)
Provoked seizures			
alcohol withdrawal	3 (18.8%)	4 (40.0%)	
acute trauma		1 (10%)	
electrolyte disturbances (hyponatremia)		2 (20.0%)	
Total	16	10	7

examined in this study. The first patient group consisted of 16 patients with single GTCS. The second group included 10 patients with recurrent GTCS, who had experienced two or more recurrent GTCS before admission to the hospital. The third patient group consisted of seven patients with prolonged partial seizures (PPS). In this group, five patients had prolonged simple partial and two patients had prolonged complex partial seizures. The duration of seizures in this group varied from 10 min up to 2 h. Serum samples were obtained from all patients, but CSF sample was unavailable from three patients with recurrent GTCS and from five patients with PPS in whom there was no clinical indication for a CSF sample. The control samples were obtained from 17 patients who were studied to exclude neurological disease, and who yielded normal neurological examination and laboratory findings. The etiology of seizures in different patient groups is listed in Table 1. All subjects were fully informed of the risks and potential benefits of the CSF examination and they gave their written informed consent to participate. The study protocol was approved by the Ethics Committee of Tampere University Hospital.

All samples were obtained within 24 h after the seizure. Commercially available enzyme linked immunosorbent assay (ELISA) kits were used for IL-6 (Pelikine Compact, CLB, Amsterdam, The Netherlands), sIL-6R and sGp130 (R&D Systems, Abingdon, UK) measurements. The sensitivities for assays for IL-6, sIL-6R and sGp130 were 0.6 pg/ml, 6.5 pg/ml and 0.08 ng/ml, respectively. Variation of levels was minimal between different plates as well as within a plate (for details, see manufacturer's information). Samples of patient groups and controls were equally distributed under several plates to minimize potential differences between plates. The assays were performed according to manufacturers' instructions.

Statistical significance of differences between groups was assessed using Mann–Whitney test. A p value of 0.05 or less was considered statistically significant. All analyses were performed using Windows SPSS 9.0 software.

3. Results

3.1. Levels of IL-6 in CSF and serum

When compared to the control subjects, the levels of IL-6 in CSF were increased both in patients with single GTCS and in patients with recurrent GTCS ($p < 0.001$) (Fig. 1). Abnormal CSF levels (± 2 SD of control levels) were detected in 11/16 patients with single GTCS and in 6/7 patients with recurrent GTCS. Furthermore, the CSF levels of IL-6 were significantly higher in patients with recurrent GTCS when compared to those with single GTCS ($p = 0.05$). The serum levels of IL-6 were increased in all seizure groups when compared to control subjects ($p < 0.001$).

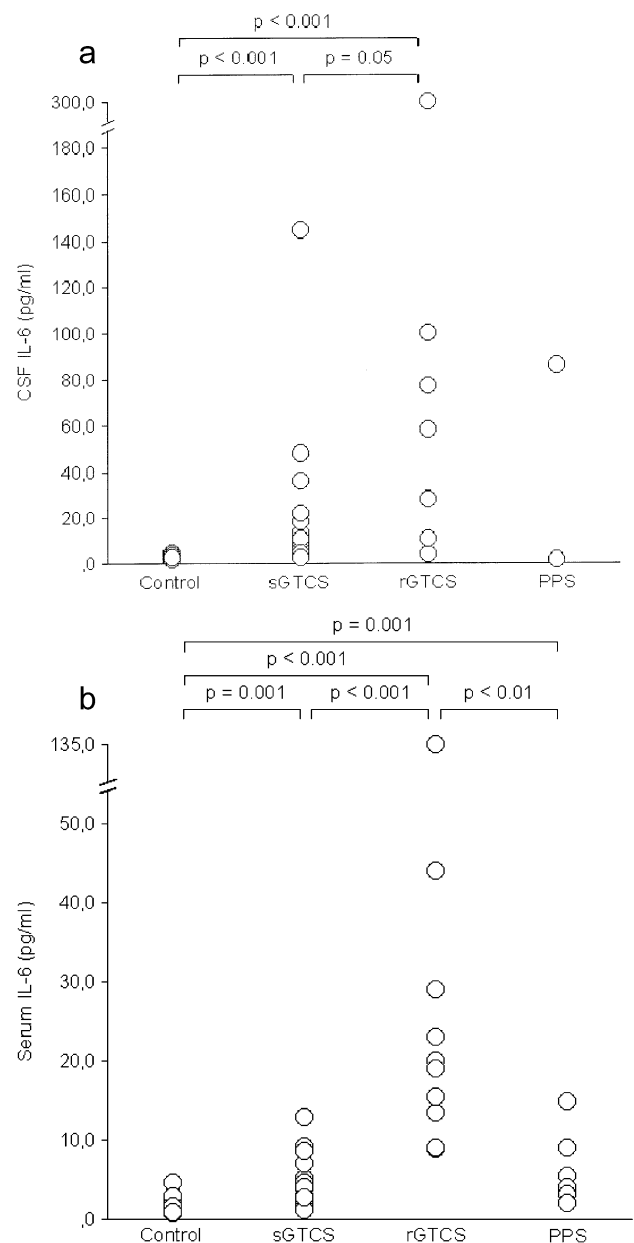


Fig. 1. (a) The levels of IL-6 in CSF. (b) The levels of IL-6 in serum.

Abnormal serum levels were found in 12/16 patients with single GTCS, in 10/10 patients with recurrent GTCS, and in 6/7 patients with PPS. Within the seizure groups, the serum levels of IL-6 were significantly higher ($p < 0.001$) in patients with recurrent GTCS when compared to those with single GTCS and PPS.

3.2. Levels of sIL-6R in CSF and serum

Patients with single GTCS showed a statistically significant decrease in the CSF levels of sIL-6R when compared with control subjects ($p = 0.05$) (Fig. 2). No significant differences were found from other patient groups when compared to controls, probably due to the limited number of patients in these groups. However, when all patients were pooled in a single group, the CSF levels of sIL-6R were significantly decreased ($p < 0.05$). Abnormal CSF levels (± 2 SD of control levels) were evident in 12/16 patients with

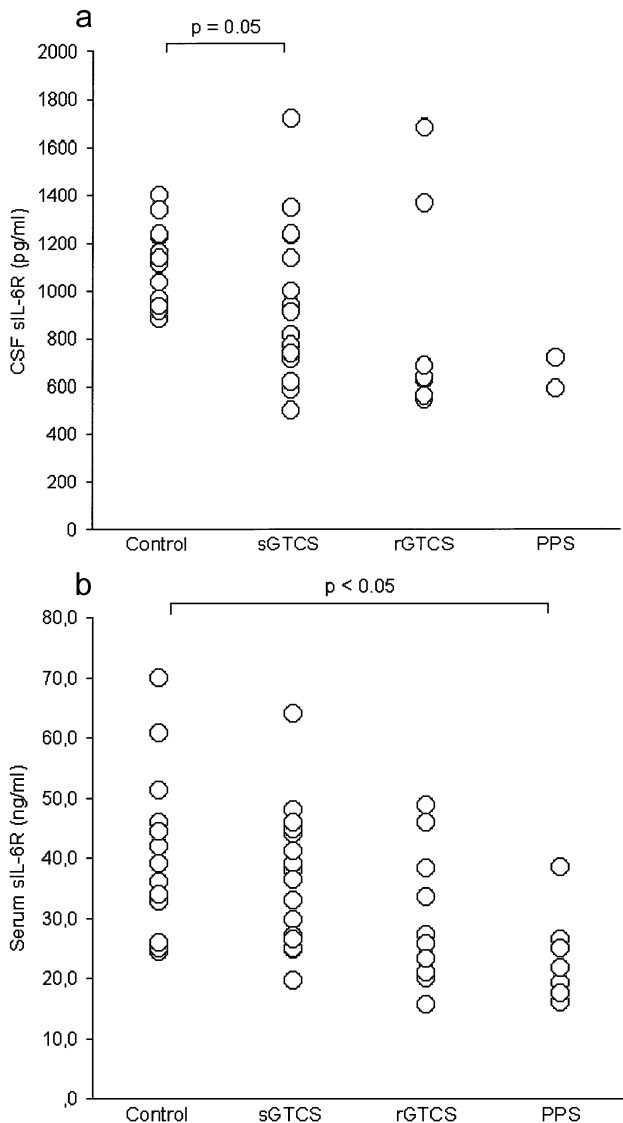


Fig. 2. (a) The levels of sIL-6R in CSF. (b) The levels of sIL-6R in serum.

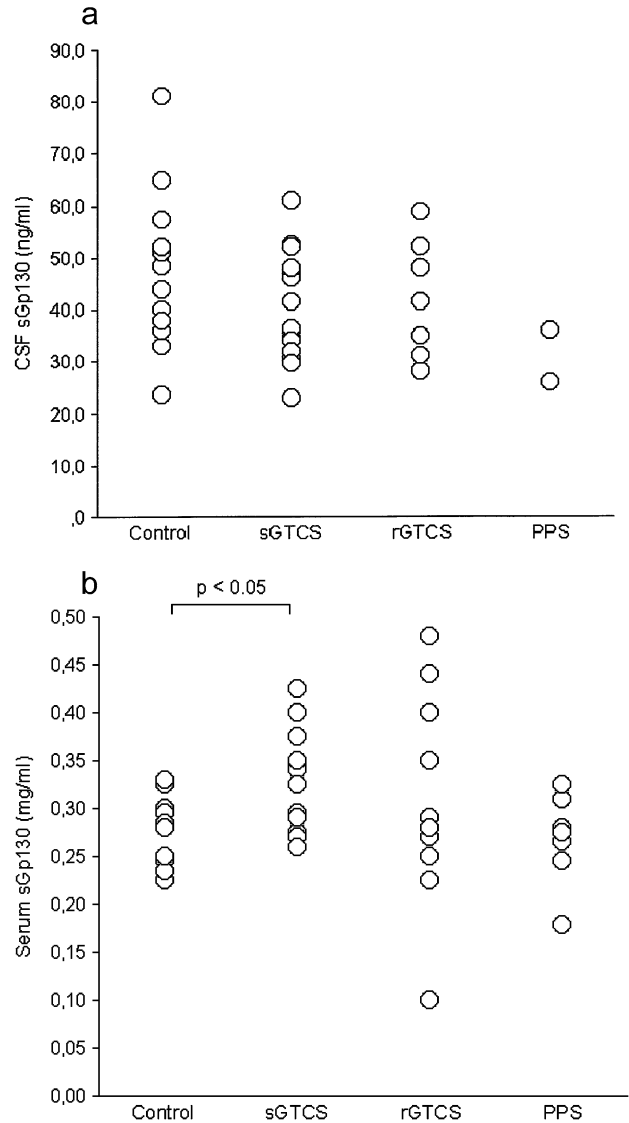


Fig. 3. (a) The levels of sGp130 in CSF. (b) The levels of sGp130 in serum.

single GTCS, in 7/7 patients with recurrent GTCS. In serum, sIL-6R levels were significantly lower in patients with PPS when compared to control subjects ($p < 0.01$), whereas other patient groups showed no significant differences. When all patients were analysed as a single group, a trend to decreased levels of serum sIL-6R was found ($p = 0.092$). Changed levels were found in 2/16 patients with single GTCS, in 4/10 patients with recurrent GTCS and in 4/7 patients with PPS.

3.3. Levels of sGp130 in CSF and serum

The levels of sGp130 showed no significant differences between the seizure groups and control subjects either in CSF or in serum, with the exception of an increase in serum levels of sGp130 in patients with single GTCS when compared to controls ($p < 0.05$) (Fig. 3). CSF values differing from control levels were detected in 4/16 patients with

single GTCS, and in 2/7 patients with recurrent GTCS. In serum, levels were abnormal in 8/16 patients with single GTCS, in 6/10 patients with recurrent GTCS and in 3/7 patients with PPS.

3.4. The effect of time from seizure onset to sampling to IL-6 levels

The mean time from seizure onset to the sample collection was approximately 16 h in single GTCS group, 11 h in recurrent GTCS group and 10 h in PPS group. The effect of time in IL-6 concentration was studied further in the single GTCS group, since this was the largest and most homogeneous group of patients. However, no correlations were found between the delay from the seizure to sample collection (data not shown).

4. Discussion

In patients with recent GTCS, we have previously found a rather selective increase in the levels of IL-6 and interleukin-1 receptor antagonist (IL-1ra) with unchanged interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF α) levels (Peltola et al., 1998, 2000a). The present study confirms and further extends these findings. The levels of IL-6 both in CSF and in serum were increased in all seizure groups (except in two CSF samples in PPS group). Furthermore, the magnitude of the IL-6 activation was most pronounced in patients with recurrent GTCS, i.e. in the case of most severe cerebral epileptic activity. These human data seem to be consistent with experimental studies suggesting that the secretion of IL-6 may be induced by seizure activity per se (Jankowsky et al., 2000, Sallmann et al., 2000). A decrease in sIL-6R levels was found in the CSF when all patients were pooled in a single group, however, this decrease was most clear in patients with single GTCS. Serum levels of sIL-6R were decreased only in patients with PPS. The levels of sGp130 were increased only in serum of patients with single GTCS. These findings indicate that the levels of these soluble receptors are also affected by seizures, although no correlation to the seizure severity was found.

Experimental studies have shown that seizure activity increases expression of several cytokines in the brain parenchyma (Vezzani et al., 1999; Jankowsky et al., 2000). However, only IL-6 release into CSF seems to be clearly increased after seizures (Peltola et al., 1998, 2000a). We have found previously that after kainic acid induced status epilepticus (SE) IL-6 is up-regulated in the meninges in addition to the expression in the brain parenchyma (Lehtimäki et al., 2003). This finding indicates that meninges may also serve as a potential site of IL-6 origin in CSF. After pilocarpine-induced SE, IL-6 positive cells have been detected both in the borders and within the brain vessels, representing most likely endothelial cells and ad-

herent white blood cells (Jankowsky et al., 2000). Interleukin-6 production is also increased in the brain endothelial cells by various noxious stimuli (Reyes et al., 1999). These results suggest that seizure induced release of IL-6 into the serum compartment occurs mainly from the brain vessels. In addition, IL-6 may be partly originated from CSF compartment via venous drainage.

In assessing our results on IL-6 system, causes of seizures as well as possible seizure-related neuronal damage must be considered. Most of our patients had either remote symptomatic or cryptogenic localisation-related epilepsy, i.e. seizures of unidentifiable but probably remote symptomatic cause (Table 1). To our knowledge, there is no data suggesting that these remote symptomatic etiologies per se might be associated with permanent or long-term activation of IL-6 system. However, in the case of acute symptomatic seizures, contribution of the etiology (alcohol withdrawal, electrolyte disturbances, trauma or neoplasia) cannot be ruled out. In addition, in 7 of the 10 patients with acute symptomatic seizures the cause was alcohol withdrawal. Chronic alcoholic patients have been reported to show elevated serum levels of IL-6 due to production from monocytes (Song et al., 1999). However, whether CSF levels are affected by alcohol abuse, is currently unknown.

The potential significance of IL-6 system in epilepsy is unknown. At the systemic level, the levels of IL-6 have been shown to be associated with the parameters of systemic inflammatory response (Peltola et al., 2002). The activation of IL-6 system via stimulation of B-cells could also partly explain the increased prevalence of autoantibodies observed in patients with therapy resistant epilepsy (Peltola et al., 2000b,c). Experimental studies suggest that IL-6 may act as a protective factor against seizure induced neuronal damage (Penkowa et al., 2001). IL-6 has been also reported to inhibit spread of excitation in the rat cerebral cortex (D'Arcangelo et al., 2000). On the other hand, when chronically overproduced, IL-6 has been reported to result in seizures and accompanying histological changes such as neuronal loss and gliosis (Campbell et al., 1993). These changes, commonly referred as temporal or mesial sclerosis, are frequently seen in temporal lobe structures of patients with chronic refractory epilepsy. Therefore, it can be hypothesised that IL-6 response after acute seizures may have beneficial effects. However, when chronically produced due to recurrent seizures or some other pathological condition, IL-6 may contribute to formation of structural changes in the neuronal tissue eventually leading to refractory seizures.

In conclusion, our results demonstrate that seizures in humans cause changes in IL-6 levels in CSF as well as in peripheral blood. The magnitude of these changes is related to the severity of seizures. Seizures may also cause changes in the levels of soluble IL-6 receptor components, resulting in complex modulation of IL-6 activity in CSF and serum compartments. More generally, activation of cytokine network by seizures may serve as a link between excessive

neuronal activity and various immunological changes in patients suffering from seizure disorders.

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Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats

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Abstract

Here we study the role of a pleiotropic pro-inflammatory cytokine, interleukin-6 (IL-6), in epilepsy. To examine this problem, we used human recombinant IL-6 applied intranasally (400 ng/40 µl) to rats 1 h before seizures induced by systemic injection of pentylenetetrazole (PTZ, 75 mg/kg). Overall, compared to the saline-treated control animals ($n = 11$ in each group), IL-6-treated rats demonstrated elevated levels of IL-6 in the frontal lobe (measured by ELISA) and increased severity of PTZ-induced seizures (shorter latency, longer duration and higher mortality). Our findings show that IL-6 plays a pro-convulsant role in the brain and suggest that the IL-6 system may be a novel target for the development of anticonvulsant drugs.

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Keywords: IL-6; Epilepsy; Intranasal administration; Chemically induced seizures; Rat

A pleiotropic cytokine interleukin-6 (IL-6) is involved in inflammatory processes as a systemic mediator of the acute phase response in infection [10,18]. IL-6 has been found in the brain, including neuronal, glial and microglial cells [18,27], where it plays an important modulatory and neuroprotective role [5,27]. IL-6 influences mesocorticolimbic dopamine and serotonin neurotransmission and also has activating effects on locomotion [35–37]. Recently IL-6 was reported to have neurotoxic effects and has also been suggested to play a significant role in various diseases of the central nervous system [5,26]. There is a growing body of evidence linking IL-6 to epilepsy [26,27]. Seizures are a common complication in patients with infectious diseases accompanied by elevated plasma and brain IL-6 levels [10,12–14]. Elevated levels of IL-6 occur in the plasma and cerebrospinal fluid of patients with recent epileptic seizures, where the levels of IL-6 generally correlate with the severity of the seizures [16,17,21–23]. These elevated IL-6 levels were observed in patients without any infection or brain trauma, and are most likely a consequence of neuronal epileptic activity associated with seizures [21–23]. In

rodents, limbic seizures rapidly and transiently enhanced IL-6 in the hippocampus [6,33]. Elevated IL-6 levels are seen after *in vivo* administration of excitatory amino acids [20]. Neuronal depolarisation and Ca^{2+} influx have been shown to directly activate production of IL-6 in the neuronal cells *in vitro* [26]. Finally, transgenic mice over-expressing IL-6 in the brain develop spontaneous behavioural seizures, increased hippocampal excitatory activity and increased sensitivity to glutamatergic-induced seizures [4,27,31].

However, the role of IL-6 in epilepsy is still unclear and thus needs further experimental investigation. In the present study, we directly examined the effects of IL-6 on chemically induced seizures. In order to minimise pyrogenic and other unwanted side effects of IL-6, and since this cytokine penetrates the blood–brain barrier poorly, we used intranasal administration shown to be effective in by-passing the blood–brain barrier for several other cytokines and similar polypeptides [7,9,15,19]. Since rat and human IL-6 possess a high degree of homology, we chose to use human recombinant IL-6 (hIL-6) for our experiments. Since our work is the first study to use IL-6 intranasally, we used 400 ng IL-6 for our studies, basing our choice on recent data [36,37] showing that at this dose IL-6 is able to cause central effects in rats following systemic administration. Choosing the pretreatment time for our studies, we

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considered recent findings showing that (i) cytokine-like molecules may appear in the brain within 20–30 min following their intranasal administration [7,19] and (ii) IL-6 can cause its central effects 30–60 min following its systemic administration [36,37]. As such, 1 h pretreatment time seemed appropriate for IL-6 at this dose to reach the brain and affect seizures. Here we demonstrate that intranasally administered hIL-6 appears in the brain and leads to increased severity of seizures and higher mortality in the model of pentylenetetrazole (PTZ)-induced seizures in rats.

Twenty two adult male Wistar rats (220–240 g; University of Tampere, Finland) were maintained in a standard virus/parasite-free facility and exposed to a 12 h/12 h light–dark cycle. Lights were turned off at 18:00 and on at 06:00 h. The animals were experimentally naïve and housed in pairs, with food and water freely available. The testing was always conducted between 14:00 and 18:00 h. On the days of the experiments animals were brought individually into the experiment room and immobilised in a vertical position, head up, for 5 min in a specially designed plastic restraint box. hIL-6 purchased from R&D Systems (USA; 10 µg diluted in 1 ml of sterile isotonic saline) was used in this study. Intranasal administration was performed in immobilised unanaesthetised animals using a 40 µl micropipette (Labsystems, Finland). Each animal received 10 µl of hIL-6 or isotonic saline at once in each nostril. This treatment was repeated after a 3-min interval (total amount administered was 400 ng/40 µl/rat). After the intranasal administration procedure the animals were returned to their cages for recovery. One hour later, each animal received a bolus of intraperitoneal injection of PTZ (Sigma, UK; 75 mg/kg) and was placed in a new clean cage for observation of seizure profile. Seizures and seizure latency times were observed visually over a 30-min observation period by a trained experimenter blind to the treatment groups. The following parameters were recorded: the time to onset of the first twitch; the number of rats having motor seizures; the duration of the oro-facial, clonic and tonic components of seizures; the mortality rate. The intensity of seizures was registered using a modified Racine's scoring system [25]: 0 (no response), 1 (freezing), 2 (head nodding or isolated twitches), 3 (oro-facial seizure), 4 (clonic seizure), 5 (tonic seizure), 6 (death). Clonic seizures consisted of rhythmic contractions of forelimbs and/or hindlimbs. Tonic seizures

consisted of rigid extension of the fore- and/or hindlimbs with or without posture loss. An animal was considered dead if the heart was not beating upon manual check. Following decapitation, brains were removed and dissected on a cold plate. Frontal lobes (anterior to optic chiasma) were isolated and stored at -70°C until the time of assay. Samples were weighted and homogenised in 50 mM PBS (pH = 7.5) using a Potter homogeniser. Thereafter, they were centrifuged for 15 min (5000 rpm) and supernatant was removed and stored. Commercial ELISA kit (R&D Systems, USA) was used to measure the level of hIL-6 in the frontal lobe of the brain. Animal care and experimental procedures were conducted in accordance with the European legislation and the guidelines of the National Institutes of Health. All animal experiments reported here were approved by the Ethical Committee of the University of Tampere. All results are expressed as means \pm S.E.M. Data were analysed by Student's *t*-test for independent samples. A probability of less than 0.05 was considered statistically significant.

The results of the present study are summarised in Table 1. Overall, hIL-6 produces a dramatic increase in seizure severity in PTZ-treated rats. While the duration of relatively mild oro-facial seizures was similar in both groups (Table 1), the duration of more severe clonic/tonic components and the total duration of seizures were almost double in rats treated with hIL-6. Also, there was a significantly shorter latency to the first twitch (Table 1). Mortality rate (55%) was almost 3-fold higher in the hIL-6-treated group (18%), although not significantly. Analysis of ELISA data shows that intranasal administration of hIL-6 results in a significant increase in IL-6 levels in frontal lobe (18.6 ± 5.4) compared to (2.2 ± 0.6) in saline-treated controls ($P < 0.05$), thus, correlating with an increased severity of seizures. Together, these data clearly indicate a pro-convulsant effect of exogenously applied hIL-6 in the brain following intranasal administration in a model of PTZ-induced seizures.

In general, our findings are the first data that directly link exogenous IL-6 to increased seizure severity in rats. The chemoconvulsant drug PTZ used in the present study to induce seizures, is known to act in the brain through inhibition of gamma-aminobutyric acid (GABA) [29]. Many drugs that act on the GABAergic system are able to affect IL-6. For example, the antiepileptic drug sodium valproate, a stimulator of GABAergic transmission, inhibits IL-6

Table 1
Increased susceptibility to PTZ-induced seizures in rats treated with intranasal human IL-6

Group	Number of rats with seizures					Onset of the first twitch (s)	Seizure duration (s)		
	TW	O	C	T	D		O	C/T	Total
Saline	10/11	11/11	7/11	7/11	2/11	417 \pm 59	9 \pm 3	27 \pm 6	36 \pm 7
IL-6	9/11	10/11	10/11	9/11	6/11	260 \pm 56*	8 \pm 3	51 \pm 8*	59 \pm 10*

TW, twitches; O, oro-facial seizures; C, clonus; T, tonus; C/T, clonic or tonic seizures; D, death. Data are the means \pm S.E.M. Fractions represent the number of rats showing different stages of seizures of the total number of rats. The onset of the first twitch was reckoned as 1800 s (total observation time) in the rats not showing this behaviour.

* $P < 0.05$ by *t*-test vs. saline control rats.

synthesis in several human cell lines [1,12]. The anticonvulsant GABA-A agonist muscimol, injected into the brain, inhibits stress-induced elevation of plasma IL-6 [30]. Anti-convulsant benzodiazepines, known to stimulate GABA-A receptors, inhibit the ability of peripheral blood monocytes to synthesise IL-6 both in humans and mice [8] and decrease the secretion of IL-6 in human prostate cancer cells [1]. In contrast, GABA-A antagonists, such as SR-95531, increase both the basal and the restraint stress-induced plasma IL-6 levels [30]. This therefore demonstrates a clear negative correlation between GABAergic activity and IL-6 production. Although the biological meaning of this correlation has yet to be investigated, its existence confirms the interplay between the GABAergic and IL-6 systems. In line with this, a preferential neuronal localisation of IL-6 in GABAergic neurons of the basal forebrain—an area actively involved in epilepsy pathogenesis—has recently been demonstrated [18]. Clearly, some other brain neurotransmitters, including glutamatergic and mesocortical dopaminergic systems, may also be involved in the modulation of IL-6 action and epileptogenesis [27,35]. However, the important role of the GABAergic system in controlling seizure [29], and the link between GABA- and IL-6-related brain mechanisms may explain why IL-6 exacerbates PTZ-induced seizures.

Considering the existing data linking the IL-6 system to epilepsy, one can argue that IL-6 synthesis may represent a compensatory pathway activated after seizures in order to protect the brain, as does, for example, a well-studied anticonvulsant cytokine IL-1Ra [6,16,32]. However, two other cytokines, IL-1 β and TNF α , are also elevated after seizures along with IL-6 and IL-1Ra [11,16]. These cytokines both possess inflammatory and pro-convulsant properties [28,32–34] and are known to stimulate the expression of IL-6 [26]. Furthermore, analysis of the time course of cytokine release after seizures shows that increase was first seen in pro-inflammatory convulsant cytokines IL-6, IL-1 β and TNF α , while anti-inflammatory anticonvulsant cytokine IL-1Ra peaked much later [6,11,33]. Taken together, these observations indicate that IL-6 release after seizures resembles more that of IL-1 β and TNF α , than of IL-1Ra, and are in line with the hypothesis linking pro-inflammatory cytokines to epilepsy. It is therefore no surprise that many anti-inflammatory drugs generally have anticonvulsant effects. Thus, these findings give additional support to the pro-convulsant mode of action of IL-6 in the brain suggested in our study.

However, there are also data in the literature showing some anticonvulsant potential of IL-6. For example, IL-6 enhances expression of brain adenosine A1 receptors known to inhibit neuronal excitation [2] and inhibits the spread of excitation and glutamate release in the somatosensory cortex in rats [5]. Moreover, IL-6 knockout mice were reported to have increased susceptibility to kainic acid-induced seizures [25]. Although these findings may seem to contradict the potential pro-convulsant role of IL-6, their exact mechanisms

and biological implication remain unclear. In general, several mechanisms may underlie these conflicting results. First, IL-6 may activate cascades of secondary mechanisms in the brain, some of which may be anticonvulsant. Furthermore, IL-6 may have different actions depending on different experimental models of epilepsy. For example, pro-convulsant effects of IL-6 hyper-production are seen in glutamatergic but not in cholinergic-induced seizures [27]. In addition, the difference between central versus peripheral site(s) of action for IL-6 may contribute to such conflicting results as suggested by Bluthé et al. [3]. Clearly, such duality in the mechanisms of actions during epilepsy cannot be excluded for IL-6, which already shows dual effects in the brain with both neuroprotective and neurotoxic actions [5,24,26,31]. Furthermore, it will be of interest to examine the effects of intranasally applied IL-6 in epilepsy in more detail, using different doses and pretreatment times, especially (considering the pleotropic nature of IL-6 and multiplicity of its mechanisms of actions) since, like other cytokines [34], IL-6 may have different and even opposite effects at different doses and check-points. It will also be interesting to analyse CSF and plasma levels of IL-6 after intranasal administration, as well as its levels in other parts of the brain (in addition to frontal lobe). Finally, it may be necessary to further assess the role of IL-6 in different models of epilepsy by influencing its physiological effects (for example, blocking them using inhibitors of IL-6 signal transduction or neutralising antiserum). We suggest that such studies may represent very important directions for future research focusing on the role of IL-6 in epilepsy.

In summary, here we have demonstrated a clear pro-convulsant action of exogenously applied IL-6 in rats. Using the intranasal route for IL-6 delivery to the brain, we minimised possible peripheral side effects of IL-6, resolved the problem of by-passing the blood–brain barrier, and assessed the central effects of IL-6 during seizures induced by inhibition of the central GABAergic system. Our data show that intranasally administered hIL-6 (400 ng) leads to a rise in IL-6 levels in the frontal lobe and increases the susceptibility of rats to PTZ-induced seizures. IL-6 affected predominantly the more severe stages of seizures (Table 1), showing the pro-convulsant profile which is clinically relevant and may be of interest for potential application. Importantly, since behavioural activation and epilepsy are based on the same arousal-related neural pathways, the pro-convulsant profile of IL-6 in our study is consistent with earlier data on the general pro-activatory action of IL-6 on behaviour in rats [35–37]. The results of our studies also confirm recent data showing increased sensitivity to glutamatergic-induced seizures in mice with elevated IL-6 levels in the brain [27]. Taken together, these data and those reported here prove that IL-6 has a marked pro-convulsant effect in several experimental models of epilepsy, and may therefore act in the brain as a pro-convulsant cytokine. These findings may point to a new area for the search of novel anti-epileptic drugs based on targeting the central IL-6 system.

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Increased plasma levels of cytokines after seizures in localization-related epilepsy

Lehtimäki KA, Keränen T, Palmio J, Mäkinen R, Hurme M, Honkaniemi J, Peltola J. Increased plasma levels of cytokines after seizures in localization-related epilepsy.

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Objectives – Experimental studies suggest increased cerebral production of inflammatory cytokines after prolonged seizures.

Whether a single non-prolonged seizure in human patients is associated with activation of cytokine network is still unknown. **Materials and methods** – We studied the levels of interleukin-1 β (IL-1 β), interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6) and soluble IL-6 receptors (sIL-6R and Gp130) in plasma after single seizures during video-EEG recordings in patients with chronic localization-related epilepsy. **Results** – The levels of IL-1ra and IL-6 were increased after seizures, whereas IL-1 β and IL-6 cytokine receptors remained unchanged. **Conclusions** – These results show that only single seizures cause activation of cytokine cascade and associated inflammatory signals. In the case of recurrent seizures, these signals may result in structural changes in the nervous tissue, which are generally associated with drug refractory epilepsy.

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Key words: cytokine; hippocampal sclerosis; inflammation; seizure; temporal lobe epilepsy

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Introduction

Cytokines act principally as mediators of inflammatory signals in peripheral tissues. However, recent experimental studies showed that prolonged epileptic seizures resulted in increased cytokine production in the central nervous system (1, 2). In humans, we have reported increased levels of interleukin-6 (IL-6) and IL-1 receptor antagonist (IL1ra) in cerebrospinal fluid (CSF) and plasma after single generalized or prolonged (generalized or partial) seizures (3–5). In addition, we found that CSF and serum IL-6 levels correlated with the severity of the seizure (4), suggesting that seizures caused release of cytokines. The level of IL-6 after seizure was also correlated with inflammatory parameters of acute phase reaction (6).

Results of our previous studies may be confounded by factors such as the effects of the cause of acute symptomatic seizures (trauma, inflammation, infection, etc.). In order to reduce the role of such factors we recruited a series of patients with chronic localization-related epilepsy undergoing a video-EEG study. It has still remained speculative,

whether increased levels of cytokines indeed occur post-ictally. This approach allowed us to determine both pre- and post-ictal cytokine and soluble cytokine receptor levels as well as accurately assesses the seizure type and duration.

Materials and methods

We included a total of 11 patients who underwent a 4-day video-EEG recording as a part of routine diagnostic or treatment procedures. The clinical characteristics of the patients are presented in Table 1. One of the patients was studied twice at two different sessions, 6 months apart. All the patients gave their written informed consent and the study was approved by the Ethics Committee of Tampere University Hospital. Basal plasma samples were collected at the start of the inter-ictal EEG-recording, and at 1, 2, 12 and 24 h after the seizure. For patients with relatively rare seizures, reduction in antiepileptic medication was done to increase the probability of seizures and thereby the diagnostic value of the recording period. Patients participating did not show clinical symptoms of

Table 1 Clinical characteristics of patients

	Age (years)/gender (M/F)	Characteristics of epilepsy					Recorded seizure	
		Duration (years)	Seizure frequency (seizures/month)	Antiepileptic medication	Epilepsy syndrome	Imaging study findings	Duration (s)	Type
1	45/M	30	8	CBZ/VGB	TLE	Hippocampal sclerosis	83	CPS
2	32/F	11	5	OXC*	TLE	Hippocampal volume loss	50	CPS
3	26/F	8	10	CBZ*	TLE	Hippocampal sclerosis	100	GTCS
4	25/F	24	10	CBZ*	TLE	Normal	56	CPS
5	26/F	24	10	VAL	FLE	Normal	60	CPS
6	34/F	34	14	CBZ/LTG	MFE	Normal	115	GTCS
7	50/M	33	1	CBZ*	TLE	Hippocampal sclerosis	78	CPS
8	21/F	9	4	VAL*	TLE	Signal increase in temporal lobes	70	CPS
9	57/M	39	4	CBZ	FLE	Normal	50	CPS
10	23/F	10	4	OXC*	TLE	Normal	57	CPS
11	48/F	38	4	CBZ/LTG	TLE	Tumor	120	GTCS
12	48/F	38	4	CBZ/LTG*	TLE	Tumor	170	GTCS

CBZ, carbamazepine; VGB, vigabatrin; OXC, oxcarbazepine; VAL, valproate; LTG, lamotrigine; TLE, temporal lobe epilepsy; FLE, frontal lobe epilepsy; MFE, multi-focal epilepsy; CPS, complex partial seizure; GTCS, generalized tonic-clonic seizure.

*Reduction in AED was done (50–75% reduction in daily dose of at least one AED).

infections. The first observed seizure was followed by plasma-sampling and cytokine and cytokine-receptor measurements. Eight healthy controls were also included in the study. Commercial ELISA kits were used according to the manufacturer’s instructions to detect interleukin-1 β (IL-1 β), IL-1ra, IL-6, soluble IL-6 receptor (sIL-6R) and soluble Gp130 (sGp130) levels (Pelikine Compact, CLB Amsterdam, The Netherlands for IL-6 assay; R&D Systems, Abingdon, UK for other assays). Statistical significance of differences between basal levels and different time points was tested as non-parametric related samples using Wilcoxon signed ranks test and Bonferroni’s correction. Statistical significance between cytokine levels in different seizure types was tested using the Mann–Whitney test. Statistical differences between patients and healthy controls were tested using the Mann–Whitney test.

Results

All patients had epileptic seizures during the recording period (reduction of anti-epileptic medication was done in seven patients). All seizures were characterized as single non-prolonged seizures (average duration 84 s). In four patients, samples were collected after secondarily generalized seizure, and in eight patients, following complex partial seizure.

The levels of IL-1 β were not significantly changed post-ictally at any time point when compared with pre-ictal levels (basal 0.21 pg/ml, peaking at 2 h 0.25 pg/ml). However, significant increase in IL-1ra levels was found after seizures at 2 h ($P < 0.05$, Wilcoxon signed ranks test) and

12 h time points ($P < 0.05$, Wilcoxon signed ranks test) (Fig. 1A). Increase in IL-1ra levels was somewhat higher after generalized seizures, although the difference was not statistically significant (Fig. 1A). The levels of IL-6 significantly increased after seizures at 6 h time point ($P < 0.05$, Wilcoxon signed ranks test) (Fig. 1B). A robust increase in IL-6 levels was seen in all patients with secondarily generalized seizures, whereas complex partial seizures caused increase in only two out of eight patients ($P < 0.05$, Mann–Whitney test). The levels of sIL-6R showed a trend towards decreased levels (basal 22.0 ng/ml, reaching its minimum 20.4 ng/ml at 2 h), but the differences were not significant. The sGp130 levels slightly decreased at 24 h time point (basal 0.25 mg/ml, reaching its minimum 0.22 mg/ml at 24 h), but the difference was not statistically significant.

With an exception of two patients (patients 7 and 8), no seizures were evident during 24 h before basal cytokine sampling and EEG recording. In these two patients (partial seizures 12 and 7.5 hours before sampling), basal and post-ictal levels of cytokines were comparable to results from other patients (both had complex partial seizures during recording followed by only a slight increase in IL-1ra and no increase in IL-6 levels). No correlation was found between cytokine or cytokine receptor levels and imaging study findings.

The levels of IL-1 β and IL-1ra showed no statistically significant difference between patient basal levels and healthy controls. Interleukin-6 showed a trend to increased basal levels in patients when compared with control subjects (median 1.75 ± 1.51 and 0.99 ± 1.02 pg/ml, $P = 0.06$, Mann–Whitney test). In addition, sIL-6R showed

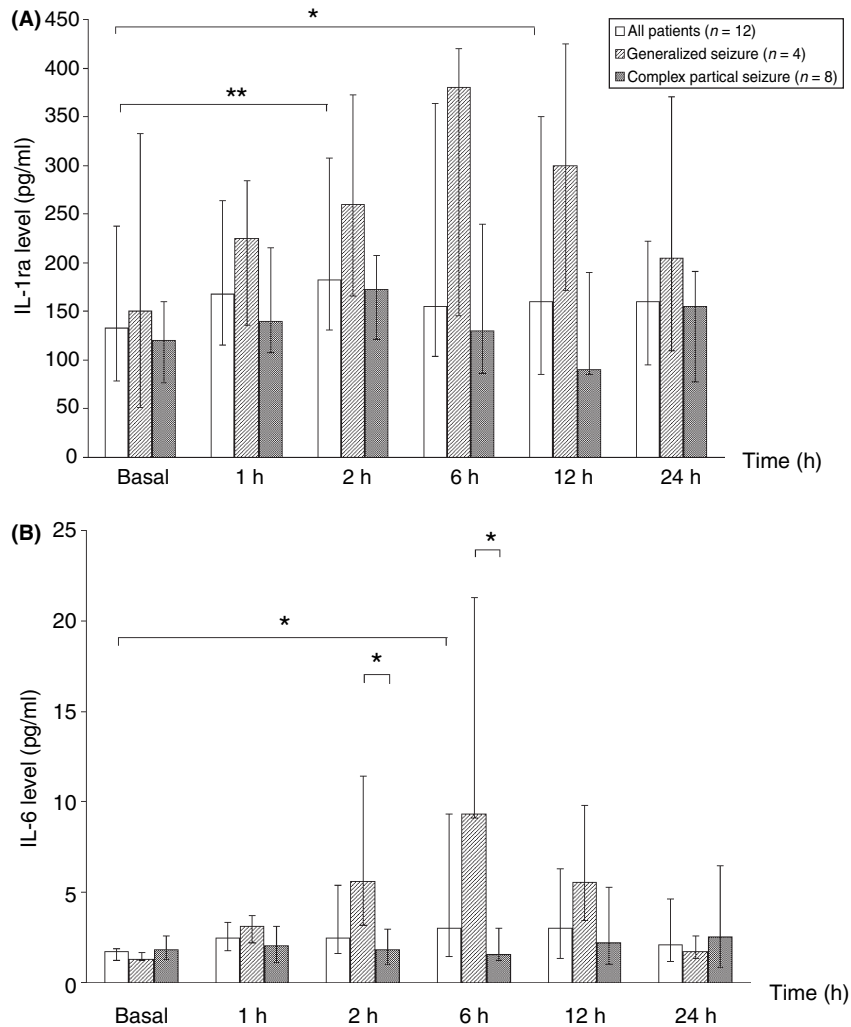


Figure 1. Median plasma levels of IL-1ra (A) and IL-6 (B) in different time points are presented in different seizure groups. Error bars represent upper and lower quartiles. Wilcoxon signed ranks test was used for testing statistical significance of difference between different time points. Statistical difference between different seizure groups at a single time point was tested using the Mann–Whitney test ($*P < 0.05$).

a trend to decreased values in epileptic patients when compared with control subjects (median 22.00 ± 5.57 and 30.00 ± 2.98 ng/ml; $P = 0.09$, Mann–Whitney test). No difference in basal sGp130 was found between patients and control subjects.

Discussion

To the best of our knowledge, the present study is the first to assess both inter-ictal and post-ictal plasma levels of cytokines in patients with epilepsy. The strength of our study is that by the use of video-EEG methodology, accurate evaluation of the seizure diagnosis and the duration of seizures was made possible. By recruiting chronic epilepsy patients, it was also made possible to exclude acute causes of seizures (such as trauma and infections)

which may also induce cytokine production. Previous studies showed that plasma and CSF levels of IL-6 and IL-1ra in patients who had experienced a recent epileptic seizure were higher than in control subjects (3, 4). The present study confirms that the increased plasma levels of IL-6 and IL-1ra are indeed temporally related to seizures. In secondarily generalized seizure, rhythmic epileptic discharges spread to both hemispheres, whereas in complex partial seizure epileptic activity was restricted in the seizure focus and associated structures without spreading to the other hemisphere. In agreement with our previous study (4) we found that the IL-6 production correlated with the severity of the seizure; only minor changes were observed in the plasma levels of IL-6 patients with complex partial seizures compared with those in patients with secondarily generalized tonic–clonic

seizures. Similar to a previous study (3), we could not show any changes in the plasma levels of IL-1 β . In contrast, IL-1ra levels increased after both seizure types, although after generalized seizures levels were higher than after complex partial seizures, suggesting that IL-1ra also may reflect the severity of the seizure. Thus, IL-1ra may be the most sensitive peripheral cytokine marker of seizures. There were no statistically significant seizure-related changes in the levels of the two soluble IL-6 receptor complex components, IL-6 receptor (sIL-6R) and glycoprotein 130 (sGp130). However, a trend towards decreased plasma levels of sIL-6R was observed.

In the present study, we also found that IL-6 levels may be increased slightly during inter-ictal period in patients with chronic epilepsy when compared with healthy controls. This was accompanied by decreased levels of sIL-6R. These changes are similar to post-ictal changes reported in the present and also in a previous study (4). Basal (inter-ictal) IL-1 β and IL-1ra levels did not increase in patients when compared with healthy controls. This suggests that cytokine levels may be disturbed also at the chronic epileptic state during the inter-ictal period. However, an effect resulting from recurring seizures cannot be excluded in explaining these slight differences between patient basal levels and healthy controls.

The origin of post-ictal increase in peripheral cytokine levels reported previously (3, 4) is not fully understood. Increased IL-6 expression has been detected after pilocarpine-induced status epilepticus in presumably glial and perivascular cells (7). IL-1 β and IL-1ra immunoreactivity is increased mainly in microglial cells after seizures induced by kainic acid (1). A recent study in patients with therapy-resistant epilepsy, a population quite similar to that in the present study, found no evidence of increased production of cytokines from peripheral blood mononuclear cells (8). The present study further supports the hypothesis of cerebral production of cytokines triggered by seizure activity. At first, increase in cytokine levels occurs within a few hours after the seizure and returns towards the basal level by 24 h, thus revealing important temporal relationship between seizures and increased cytokine levels. Secondly, increase in cytokine levels was higher after more severe seizures (generalized), suggesting that increased levels of cytokines are causally related to the seizure activity. It seems likely that seizures cause changes in nervous tissue micro-environment causing the glial cell release of cytokines. In the case of generalized seizures, these changes are more widespread (when compared with partial seizures)

throughout the nervous system resulting more robust increase in cytokine levels in peripheral blood and CSF. Therefore, the current knowledge suggests that the seizure-related increase of cytokines in blood reflects preferably central, than peripheral, production of cytokines.

The potential relevance of seizure-induced production of IL-6 and IL-1ra in epilepsy is supported by experimental data. IL-1ra has neuroprotective effects in experimental status epilepticus (9), and has a clear anticonvulsive effect (1). IL-6 is reported to have neuroprotective and anticonvulsive effects after kainic acid-induced status epilepticus (10). In contrast, proconvulsive effects have been reported for IL-6 (11, 12). These data suggest that seizure-induced production of IL-6 and IL-1ra may serve as a mechanism protecting the nervous system from seizure-induced damage. Although IL-1ra has probably a clear anticonvulsive function, the effect of seizure-induced production of IL-6 remains to be determined.

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Increase in Plasma Proinflammatory Cytokines After Electroconvulsive Therapy in Patients With Depressive Disorder

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Objectives: Electroconvulsive therapy (ECT) is regarded as an effective treatment of drug-resistant depression, but its mechanism of action is mostly unknown. We have previously reported that epileptic seizures result in cerebral production of cytokines, which are also reflected in the plasma. In this study, we tested whether ECT is associated with similar acute release of cytokines.

Methods: The plasma levels of cytokines interleukin (IL) 1 β , IL-1 receptor antagonist, and IL-6 were measured using enzyme-linked immunosorbent assay at several time points after ECT. The study included 9 patients who met the diagnostic criteria of major depression (mean age, 55 years; mean Montgomery-Åsberg Depression Rating Scale score, 30).

Results: Our results demonstrate that cytokines IL-1 β and IL-6 are increased at 3- and 6-hour time points after ECT. IL-6 release also correlated to the stimulus dose used, suggesting neuronal depolarization as a mechanism of cytokine release.

Conclusions: These results indicate that ECT is associated with rapid induction of inflammatory cytokines most likely in the central nervous system, which are also measurable in the peripheral blood.

Key Words: seizure, cytokines, depressive disorder, growth factors, electroconvulsive therapy, epilepsy, neuronal plasticity

(*J ECT* 2008;24:88–91)

Depression is a common psychiatric condition, but its pathogenesis is still poorly understood. Inflammatory system activation has been suggested to contribute in the pathogenesis of major depression.^{1–3} Electroconvulsive therapy (ECT) is considered the most effective therapy for severe depression.⁴ The neurobiological action of ECT is not fully understood, but it is acknowledged that ECT has effects on several neurotransmitters and their receptors, neuropeptides, hormones, and neurotrophic factors.⁵ There are only limited data on the immunological effects of ECT.^{6–8}

Recent experimental and clinical studies suggest that epileptic seizures are associated with an acute increase in the production of cytokines both in the central nervous system and peripheral blood compartments.^{9–13} We hypothesized that ECT is associated with an acute increase in the production of cytokines. To test this hypothesis, we studied the plasma levels of interleukin (IL) 1 β , IL-1 receptor antagonist (IL-1ra), and IL-6 at several time points before and after ECT in a series of patients with major depressive disorder.

PATIENTS AND METHODS

Nine patients and 8 healthy controls were included in the study (demographic data are presented in Table 1). All patients gave their informed written consent to obtainment of blood samples. All patients fulfilled the diagnostic criteria of major depressive disorder. The patients received psychotropic medication (summarized in Table 1), which was unchanged during ECT. Electroconvulsive therapy was induced using Thymatron DGx (Somatics, Inc, Lake Bluff, Ill) brief-pulse device with bilateral electrodes. A preselected dosing strategy was used for initial stimulus intensity. This was based on patient's age, gender, and medication (eg, benzodiazepine doses) and was around 5 times the patient's age. During the course of ECT, this dose was adjusted according to the previous responses to reach a seizure with sufficient intensity to obtain adequate therapy response. Anesthesia method is described in detail in the recent study.¹⁴ Plasma samples were obtained immediately before anesthesia induction and at 1, 3, 6, and 24 hours after the seizure. The main scope of the study was to detect possible acute changes in cytokine levels after seizure during ECT. It should be noted that patients were in different stages of their ECT course during sample collection (Table 1).

The levels of IL-1 β , IL-1ra, and IL-6 were measured using commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Abingdon, UK, for IL-1 β and IL-1ra; Pelikine Compact, CLB, Amsterdam, The Netherlands, for IL-6). The severity of depression was scored with Montgomery-Åsberg Depression Rating Scale (MADRS) before and after the ECT series. For statistical analyses, SPSS Windows 9.0 was used. Statistical difference between patients and control group was tested using the Mann-Whitney *U* test and difference between time points using the Wilcoxon signed ranks test and Bonferroni correction. The level of correlation was tested using the Spearman test. A *P* value of 0.05 was considered significant. The study protocol was

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TABLE 1. ECT Parameters and Basal and Post-ECT Levels of Cytokines

Patient	Age, yr/ (F/M)	No. ECTs	Medication*	MADRS Before/After ECT Series	Duration of Seizure (EEG/EMG), s	Charge, mC	Basal/Peak IL-1 β Levels, pg/mL	Basal/Peak IL-1ra Levels, pg/mL	Basal/Peak IL-6 Levels, pg/mL
1	70/F	7	1, 3	25/0	19/18	428.4	0.15/0.36	340/850	6.5/85.0
2	64/M	1	1, 3	39/1	38/25	302.4	0.01/0.05	125/180	1.5/9.1
3	33/F	1	2, 3	29/3	54/42	151.2	0.23/0.50	160/215	1.2/2.0
4	73/F	1	1, 2, 3	32/9	60/34	302.4	0.01/0.22	175/500	1.3/38.5
5	57/F	5	1, 2	18/6	33/21	302.4	0.12/0.67	1000/980	2.3/12.6
6	28/F	4	1, 2	31/11	49/38	201.6	0.01/2.20	130/245	1.5/7.5
7	62/F	3	1, 3	34/9	43/36	327.6	0.08/0.43	320/380	1.0/11.5
8	41/M	6	2	29/33	50/35	151.2	0.16/0.65	310/335	2.9/4.0
9	66/M	5	1, 2, 3	32/10	43/26	252.0	0.06/0.17	570/550	5.0/11.5

*Medication: 1 = neuroleptics, 2 = antidepressants, 3 = benzodiazepines.
F indicates female; M, male; EEG, electroencephalogram; EMG, electromyogram.

approved by the ethics committee of the Tampere University Hospital.

RESULTS

A total of 9 patients were treated with ECT and followed by cytokine measurements. Clinical information (age, gender, severity of depression, and medication) and ECT parameters are presented in Table 1. Basal levels of cytokines (before seizure induction) and peak values after seizure are also presented in Table 1. We found that after ECT, the levels of IL-1 β were increased transiently ($P < 0.05$, Wilcoxon signed ranks test) (Fig. 1A). The levels of IL-6 showed also a significant increase ($P < 0.05$, Wilcoxon signed ranks test) after ECT (Fig. 1B). On the other hand, no significant change was observed in IL-1ra levels (data not shown). The levels of both cytokines were increased at the 3-hour time point, and IL-6 was still increased at the 6-hour time point (Figs. 1A and B), both decreasing toward basal levels at 24 hours. Interestingly, the increase in IL-6 levels after ECT was significantly correlated to the charge (stimulus dose) used to induce a

seizure ($r = 0.945$; $P < 0.01$, Spearman test); that is, more robust IL-6 release was observed in patients with high charge used in seizure induction. IL-1 β or IL-1ra levels were not clearly associated with the seizure parameters. We found no correlation between cytokine levels after ECT and MADRS score before or after ECT series.

The basal levels of IL-1 β were significantly lower in patients (median, 0.08 pg/mL) compared with control subjects (median, 0.33 pg/mL) ($P < 0.01$, Mann-Whitney U test), whereas increased basal levels of IL-6 were observed in patients (median, 1.47 pg/mL) compared with control subjects (median, 0.99 pg/mL) ($P < 0.05$, Mann-Whitney U test). This difference in basal IL-6 levels was found to be caused by increased IL-6 levels in a subgroup of patients who were in the later stage of their ECT course. Statistically, the basal levels of IL-6 correlated positively to the number of ECT treatments ($r = 0.80$; $P = 0.01$, Spearman test). The levels of IL-1ra showed no significant differences between patients and control subjects. The basal levels of cytokines were not associated with MADRS scores before or after ECT series.

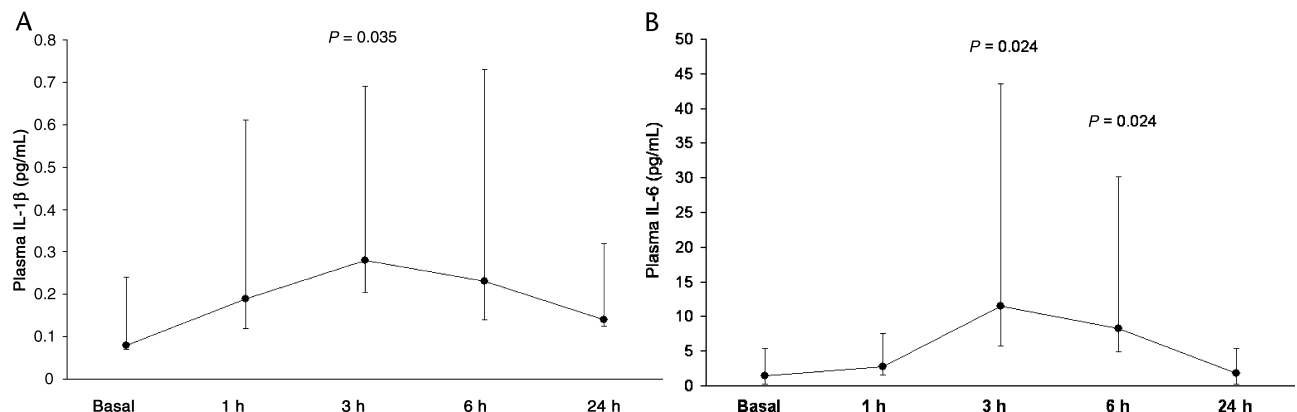


FIGURE 1. A, Plasma levels of IL-1 β (A) and IL-6 (B) in different time points. IL-1 β levels were significantly increased at the 3-hour time point compared with basal levels, and B, IL-6 levels were significantly increased at the 3- to 6-hour time points. Dots represent median cytokine levels, and error bars represent upper and lower quartiles. Statistical differences between different time points after ECT compared with basal levels have been tested using Wilcoxon signed ranks test and Bonferroni correction.

DISCUSSION

The present study provides the first human evidence about the ECT-associated production of proinflammatory cytokines IL-1 β and IL-6. The increase in plasma levels of both cytokines was rapid, and the effect was short-lasting. The findings with IL-6 are in line with those observed postictally in patients with spontaneous epileptic seizures.^{12,13} However, increased plasma levels of IL-1 β have not been found in epileptic patients; instead, IL-1ra has been found to be elevated.¹³

Cerebral release of inflammatory cytokines may be regarded as response to seizures. This phenomenon has been observed in experimental epilepsy models,^{9,11} after electroconvulsive shock in mice¹⁵ as well as in epileptic human patients.^{12,13} Interestingly, we found that the stimulus dose (charge) used in seizure induction correlated to the IL-6 response. The biological meaning of stimulus dose was recently elucidated by Swartz,¹⁶ stating that stimulus dose in ECT is correlated to the number of neurons that are depolarized, that is, volume of seizure focus. This indicates that IL-6 release may be related to the volume of seizure focus. This is in accordance with previous experimental studies in which IL-6 expression in neurons has been reported after electroconvulsive shock in mice, and the mechanism was found to be depolarization of neuronal cell membranes.¹⁵ It should be noted that IL-6 release was not correlated to the duration of seizures, which showed small range of variation (Table 1).

Until now, the few studies on immunological effects of ECT suggest mainly immunosuppressive effects for this treatment. A study in patients with major depression showed an acute decrease in the absolute number of total blood lymphocytes and T8⁺ and Leu11⁺ cells 1 hour after a single ECT.⁶ Another study found that the clinical improvement of severely depressed patients during repeated ECT was accompanied by a gradual and significant decline in tumor necrosis factor α level (which was increased before the ECT), reaching levels comparable with those in healthy controls at the end of the study.⁸ Thus, ECT may have variable effects on different cytokines, such as tumor necrosis factor α and IL-6/IL-1 β , and the short-term and long-term effects may be different. Kronfol et al⁷ observed an acute and short-lasting increase of natural killer cell activity after ECT. This finding may relate to ECT-associated production of IL-6 and IL-1 β observed in the present study. In general, it seems likely that ECT-induced cytokine release from central nervous system is responsible for changes in immune system cell activity observed after ECT.

In keeping with previous studies,^{17–19} basal plasma levels of IL-6 were higher in patients with depression than in control subjects. In contrast to earlier observations,^{17,20} levels of IL-1 β were lower than in controls. Our findings must be interpreted cautiously because the patients were studied at different stages of their ECT series and that basal levels of cytokines serve mainly as a control level for comparison of acute changes in cytokine levels induced by ECT, which is the primary scope of the present study. Thus, changed basal plasma levels of IL-6 and IL-1 β in our patients indicate only that ECT may have long-term effects of basal IL-1 β and IL-6 levels. However, the finding that basal IL-6 levels correlated positively to the number of ECT treatment sessions indicates that IL-6 levels may be increased during the ECT course.

Our study has also some limitations. The number of patients is quite low, and cytokine measurements were done at different time points in ECT schedule. The effect of ECT on cytokine release may be different in the beginning of the treatment and in the later phase of the treatment. Whether cytokine release is associated with the treatment response is also difficult to test with relatively small patient population. These questions clearly need further investigation. We also found that cytokine levels after ECT show relatively high variation. This was partially explained by stimulus strength in the case of IL-6; however, it seems clear that cytokine responses are also regulated by genetic factors.

Clinical correlates for the proinflammatory response to ECT remain open. The induction effect of ECT on the production of IL-1 β and IL-6 was short-lasting in our patients. A transient effect may differ from that of chronic exposure to proinflammatory cytokines in depression. IL-1 β and IL-6 are able to stimulate the production of neurotrophic factors, such as nerve growth factor.^{21–23} Therefore, transient increase in IL-1 β and IL-6 may initiate cascade of growth factors, resulting in plasticity in neuronal circuits associated with depression, potentially explaining the therapeutic effects of ECT.

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