TUOMO NIEMINEN

Adrenoceptor Antagonists and Haemodynamics

Contemporary Concepts

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ACADEMIC DISSERTATION

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LIST OF ORIGINAL COMMUNICATIONS

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I Nieminen T, Kööbi T and Turjanmaa V (2000): Can stroke volume and cardiac output be determined reliably in a tilt-table test using the pulse contour method? Clin Physiol 20:488-495.

II Nieminen T, Ylitalo R, Kööbi T, Ylitalo P, Kähönen M (2005): Effects of Adrenoceptor Blocking Drugs on Cardiovascular Responsiveness to Passive Orthostasis. A placebo-controlled double-blind study. Arzneimittelforschung/Drug Research 55:205-211.

III Nieminen T, Ylitalo R, Kööbi T, Ylitalo P, Kähönen M (2005): The vasodilatory effect of alfuzosin and tamsulosin in passive orthostasis. A randomised, double-blind, placebo-controlled study. Eur Urol 47:340-345.

IV Nieminen T, Uusitalo H, Turjanmaa V, Bjärnhall G, Hedenström H, Mäenpää J, Ropo A, Heikkilä P, Kähönen M (2005): Association between low plasma levels of ophthalmic timolol and haemodynamics in glaucoma patients. Eur J Clin Pharmacol 61:369-374.

V Nieminen T, Uusitalo H, Mäenpää J, Turjanmaa V, Rane A, Lundgren S, Ropo A, Rontu R, Lehtimäki T, Kähönen M. Polymorphisms of genes *CYP2D6*, *ADRB1* and *GNAS1* in pharmacokinetics and systemic effects of ophthalmic timolol. Submitted.

In addition, some unpublished data is presented.

ABBREVIATIONS

A	Adrenaline
ACh	Acetylcholine
ADRB1	Gene encoding the β_1-adrenergic receptor
ANS	Autonomic nervous system
AUC	Area under drug concentration in plasma versus time curve
AV	Atrioventricular
BP	Blood pressure
BPH	Benign prostate hyperplasia
CI	Cardiac index; cardiac output divided by body surface area
C_{max}	Maximal plasma concentration
CNS	Central nervous system
CO	Cardiac output
CYP	Cytochrome P450 enzymes
DAP	Diastolic arterial pressure
ECG	Electrocardiography
EM	Extensive metaboliser
FEV1	Forced expiratory volume in one second; a measure of pulmonary function
GDP	Guanosine diphosphate
GNAS1	Gene encoding the α-subunit of G-protein
GPCR	G-protein coupled receptors
GTP	Guanosine triphosphate
HR	Heart rate
ICG	Impedance cardiography
ICG_{WB}	Whole-body impedance cardiography
IM	Intermediate metaboliser
IOP	Intraocular pressure
ISA	Intrinsic sympathomimetic activity
LUTS	Lower urinary tract symptoms

MAP	Mean arterial pressure, DAP + ⅓ (SAP-DAP)
NA	Noradrenaline
PDE5	Phosphodiesterase type 5
PEF	Peak expiratory flow; a measure of pulmonary function
PM	Poor metaboliser
PR interval	Interval between the apex of P wave and the R peak; an ECG parameter
PWV	Pulse wave velocity
QT interval	Time between the beginning of Q wave to the end of T wave; an ECG parameter
QTc interval	QT interval corrected for HR
RMSSD	Square root of the mean squared differences of successive RRIs; a measure of HR variability
RRI	Interval between adjacent QRS complexes
SA	Sinoatrial
SAP	Systolic arterial pressure
SD	Standard deviation
SEM	Standard error of mean
SI	Stroke index; stroke volume divided by body surface area
SNP	Single nucleotide polymorphism
SV	Stroke volume
SVR	Systemic vascular resistance; total resistance in peripheral circulation
SVRI	Systemic vascular resistance index; the product of SVR and body surface area
$T_{½}$	Elimination half-life
T_{max}	Time to reach C_{max}

ABSTRACT

The effects of the sympathetic nervous system are mediated by adrenergic receptors or adrenoceptors. Drugs blocking these receptors, α- or β-antagonists, are widely used to diminish excessive sympathetic activity. Since heart and vasculature are major locations for adrenoceptors, α- and β-antagonists exert a variety of haemodynamic effects that have been studied quite extensively but not yet satisfactorily. This dissertation was aimed to complement the knowledge about the haemodynamic influence of adrenoceptor antagonists.

Several noninvasive methods have been introduced for assessment of haemodynamics. Whole-body impedance cardiography (ICG_{WB}) has been shown to agree well with invasive methods in many patient groups under different study settings. Finger-pressure pulse contour might be even easier to use and more readily available than ICG_{WB}, but the reliability of the pulse contour method has not been adequately documented. In the Study I, the paired stroke volume (SV) and cardiac output (CO) values by the pulse contour and ICG_{WB} were compared in 40 patients subjected to head-up tilt test. The pulse contour method tracked the changes in SV and CO relatively well, but it was not reliable in assessing absolute levels. ICG_{WB} was used in the later studies to assess the haemodynamic influence of the adrenoceptor antagonists.

The effects of propranolol, carvedilol, tamsulosin and alfuzosin were assessed in Studies II and III with 27 and 31 healthy volunteers, respectively, subjected to head-up tilt provocation. Tamsulosin and alfuzosin induced uniform effects: systemic vascular

resistance index (SVRI) decreased, while heart rate (HR) and cardiac index (CI) increased in head-up tilt, but not in supine position. The responses during carvedilol treatment did not differ from those with propranolol; HR and systolic arterial pressure (SAP) decreased.

The correlation between haemodynamic responses and the plasma concentration of aqueous and hydrogel formulations of ophthalmic timolol were measured in 25 glaucoma patients subjected to spirometry, head-up tilt and exercise test (Study IV). HR correlated to timolol level in supine, head-up tilt and exercise tests, gaining stronger association as the physical load increased. Arterial pulse wave velocity was inversely correlated with timolol level in supine position. In head-up tilt, CI diminished and SVRI increased as timolol concentration rose.

Timolol is metabolised by enzyme CYP2D6. The polymorphism in the gene *CYP2D6* had marked effects on the kinetics and systemic effects of aqueous ophthalmic timolol in 18 healthy volunteers (Study V): maximal plasma concentration and area under the curve were higher, elimination half-life was longer and elevation of HR from rest to maximal level tended to be higher for poor metaboliser (PM) than extensive metaboliser (EM) genotypes of *CYP2D6*. Single nucleotide polymorphisms in the genes *ADRB1* (β_1-adrenoceptor) and *GNAS1* (α-subunit of G-protein) did not, however, have consistent effects on the pharmacodynamics of ophthalmic timolol in the 18 healthy volunteers or the 19 glaucoma patients.

In conclusion, alfuzosin and tamsulosin induce clear vasodilatory effects, indicating that they are not purely uroselective α_1-antagonists. The α_1-blocking effect of the combined α_1- and β-antagonist carvedilol is not apparent in the tilt provocation, since the drug does not differ from the non-selective β-antagonist propranolol. Since PMs of *CYP2D6* have higher timolol concentrations than EMs, and since the plasma level of timolol correlates with several haemodynamic effects, the PMs may be more prone to systemic adverse events of timolol than EMs.

INTRODUCTION

Adrenergic receptors or adrenoceptors mediate the effects of adrenaline (A) and noradrenaline (NA) released by the adrenal medulla and the sympathetic nervous system. Rapid development of adrenoceptor antagonists started in the 1950's, as the existence of distinct α- and β-adrenoceptors was realised. Blocking of sympathetic stimulation is beneficial in a multitude of diseases and symptoms, and, consequently, the wide group of adrenoceptor antagonists is among the most common pharmaceuticals. Adrenoceptor antagonists cause many changes in the cardiovascular system and haemodynamics. The word cardiovascular is often understood narrowly, covering heart rate (HR) and blood pressures (BP), while the term haemodynamics has a broader meaning and describes the physical factors governing blood flow within the circulatory system, i.e. the factors controlling arterial pressures and systemic vascular resistance (SVR). In many cases, however, the words cardiovascular and haemodynamic are interchangeable.

A variety of technical approaches have been developed to assess the haemodynamic status in detail. Methods necessitating a pulmonary artery catheter are only feasible in critically ill patients, due to both technical and ethical factors. Therefore, there is a need for a method that enables semi-invasive and even noninvasive measurement of stroke volume (SV), cardiac output (CO), SVR, arterial pulse wave velocity (PWV) etc. For noninvasive continuous beat-to-beat measurements without a burden of operator-dependency, there are two techniques available: pulse contour method and impedance cardiography (ICG). A whole-body modification of ICG (ICG_{WB}) has

been comparable with gold-standard invasive methods in several studies. Compared with ICG, the use of the pulse contour method could possibly be even easier and more readily available, but its applicability e.g. in head-up tilt testing has not been proved.

The haemodynamic effects of propranolol, the first clinically used β-antagonist, have been examined extensively, and it is often used as a drug the other β-blocking drugs are compared with. Another non-selective β-antagonist, timolol, is currently mainly used as topical treatment of ocular hypertension and glaucoma. Despite the ophthalmic administration, timolol is absorbed to the systemic circulation with consequent β-blockade adverse effects in the circulatory and pulmonary systems. These effects have been characterised to some extent, but the association between them and the plasma concentration of timolol has not been established. Timolol is metabolised through cytochrome P450 enzyme CYP2D6, which is known to express marked genetic variations. However, the dependence of pharmacokinetics and pharmacodynamics of ophthalmic timolol on the *CYP2D6* genotypes is not clear. Carvedilol is a combined α_1- and β-antagonist widely used in the treatment of hypertension and congestive heart disease, but there are no published data on its effects on such advanced haemodynamic parameters as SV and SVR. The responses to orthostasis during carvedilol treatment are also unknown.

The α_1-blocking drugs alfuzosin and tamsulosin are solely used in the treatment of lower urinary tract symptoms (LUTS) due to benign prostatic hyperplasia (BPH). The haemodynamic effects of these α_1-antagonists have not been studied properly: only the responses of HR and BP are known.

The present study was designed and conducted to complement the knowledge on the haemodynamic effects of the adrenoceptor antagonists propranolol, carvedilol, timolol, alfuzosin and tamsulosin. Prior to estimating the drug effects, the reliability of the pulse contour method was evaluated.

Part I: REVIEW OF THE LITERATURE

1 CONTROL AND TESTING OF HAEMODYNAMICS

1.1 Autonomic nervous system

1.1.1 Overview

The autonomic nervous system (ANS) is a key regulator of physiological homeostasis; it controls HR, body temperature, BP, metabolism, circulation, respiration, and digestion. ANS is based on an architecture of reflex arch with efferent fibers transmitting impulses from the central nervous system (CNS) to regulate the functions of peripheral organs, and afferent fibers giving feedback information that modulates the efferent system. Anatomically, the efferent ANS is divided into three components: the sympathetic and parasympathetic divisions that both communicate with the third division, enteric nervous system (Furness and Costa 1980, Vizi and Labos 1991, Richerson 2003a). Most end-organs are innervated with the synergistic and often opposing effects of both sympathetic and parasympathetic systems.

Parasympathetic and sympathetic divisions have synapses in peripheral ganglia between the neurons originating in the CNS and the postganglionic efferents innervating the end-organs; the neurotransmission occurs with acetylcholine (ACh) via nicotinic ACh receptors. In the parasympathetic division, the postganglionic neurotransmitter ACh activates muscarinic ACh receptors. The postganglionic efferents of the sympathetic division release noradrenaline (NA) that acts through adrenergic receptors with the exception of sweat glands and vasodilatory neurons in the skeletal muscle, where the sympathetic system releases ACh. Beside the actual

neurotransmitters, both sympathetic and parasympathetic systems encompass a variety of cotransmitters and neuromodulators such as dopamine, adenosine triphosphate (ATP), angiotensin II, neuropeptide Y, enkephalin, somatostatin and vasoactive intestinal peptide (Lundberg 1996, Brodde and Michel 1999, Elenkov et al. 2000, Richerson 2003a).

The cholinergic nerve endings contain high concentrations of acytylcholinesterase, and, consequently, the transmitter ACh does not diffuse into the bloodstream. Thus, the effects of localised cholinergic discharge are discrete and last for a burst of a few milliseconds at a time (Levy 1997, Ganong 2003a). This enables swift and accurate regulation by the parasympathetic nervous system, reflected e.g. as beat-to-beat regulation of HR (Levy 1971, 1997). On the other hand, NA from the adrenergic nerve endings spreads into the neighbouring cells and circulation. The other two endogenous catecholamines, adrenaline (A) and dopamine, are also present in plasma. Consequently, the sympathetic division has more prolonged and diffuse effects than the parasympathetic system (Berne and Levy 1993b, Levy 1997).

ACh has four primary effects on the cardiovascular system: vasodilation in essentially all vascular beds (Feigl 1998), negative chronotrophy (DiFrancesco 1993, Brodde and Michel 1999), deceleration in conduction velocity or negative dromotrophy in the sinoatrial (SA) node, atrioventricular (AV) node and His-Purkinje system (Kent et al. 1974, Levy and Schwartz 1994), and negative inotrophy especially in atrial muscle (Higgins et al. 1973, Wickman and Clapham 1995, Mery et al. 1997). The target cell responses to the innervation by ANS are dependent on the densities of nine types of adrenoceptors (Fig. 1) and five types of muscarinic ACh receptors (Caulfield and Birdsall 1998, Darlison and Richter 1999, Guimaraes and Moura 2001).

1.1.2 Sympathetic nervous system

1.1.2.1 Background

The cell bodies of the sympathetic division are located in the intermediolateral column of thoracic and lumbar spinal cord between the levels T1 and L3. After leaving the CNS as part of thoracic and lumbar spinal nerves, the preganglionic efferent fibers synapse with the postganglionic neurons in sympathetic ganglia. Most of the synapses are in the paravertebral ganglia chains adjacent to the vertebral column all the way from the upper part of the neck to the coccyx. With few exceptions, the remaining sympathetic ganglia are in the adrenal medulla and the prevertebral plexus located in front of the aorta and along its major arterial branches (Elenkov et al. 2000, Richerson 2003a).

The end-organ effects of the sympathetic nervous system (Table 1) are mediated by adrenoceptors that constitute a family of structurally and functionally related proteins. They belong to G-protein-coupled receptors (GPCR) that are a wide group of receptors for hundreds of hormones and neurotransmitters, e.g. muscarinic acetylcholine receptors, serotonergic 5-HT receptors, rhodopsin and opiate receptors. All the GPCRs consist of a single polypeptide chain with up to 1100 amino acid residues that form seven transmembrane α_1-helices. One of the intracellular loops is larger than the others and interacts with the G-protein (Wess 1998, Hokfelt et al. 2000). The adrenoceptors are divided into three classes that are all further divided into three subclasses (Fig. 1).

Table 1. Responses of cardiovascular and pulmonary effector organs to sympathetic nerve impulses and circulating catecholamines. The direction and strength of the responses are indicated by the number and direction of arrows (Hoffman and Taylor 2001b, Richerson 2003b).

Effector organ	α_1	α_2	β_1	β_2
Heart				
S-A node			HR ↑↑	HR ↑
Atria			Contractility and conduction velocity ↑↑	Contractility and conduction velocity ↑
A-V node			Conduction velocity ↑↑	Conduction velocity ↑
His-Purkinje system			Conduction velocity ↑↑↑	Conduction velocity ↑
Ventricles			Contractility, conduction velocity, automaticity, rate of idioventricular pacemakers ↑↑↑	Contractility, conduction velocity, automaticity, rate of idioventricular pacemakers ↑↑↑
Arterioles				
Coronary	Diameter ↓	Diameter ↑		Diameter ↑↑
Skin and mucosa	Diameter ↓↓↓	Diameter ↑		
Skeletal muscle	Diameter ↓↓	Diameter ↑		Diameter ↑↑
Cerebral	Diameter ↓			
Pulmonary	Diameter ↓			Diameter ↑
Renal	Diameter ↓↓↓	Diameter ↑		Diameter ↑↑
Systemic veins	Diameter ↓↓	Diameter ↑		Diameter ↑↑
Lung				
Tracheal and bronchial muscle				Relaxation ↑
Bronchial glands	Secretion ↓			Secretion ↑
Kidney				
Renin	Secretion ↓		Secretion ↑↑	

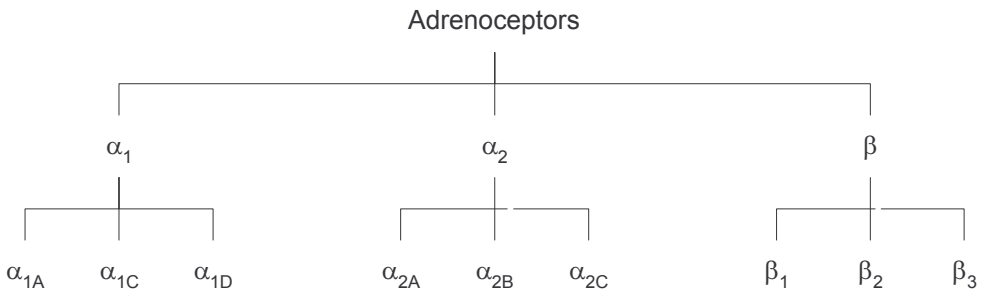

Fig 1. Adrenoceptor subtypes.

Adrenal medulla is analogous to a sympathetic ganglion: it is innervated by preganglionic sympathetic neurons that activate nicotinic ACh receptors on the postsynaptic target cells (chromaffin cells) (Richerson 2003a). When stimulated, the chromaffin cells release A into the bloodstream, and this is the sole source of A found in plasma. Thus, adrenal medulla serves as an endocrine component of sympathetic outflow, being crucial in the regulatory interplay between the ANS and the endocrine system (Miller and O'Callaghan 2002, Barrett 2003, Leal-Cerro et al. 2003).

1.1.2.2 G-proteins

The G-proteins are membrane proteins involved in transmembrane signalling. They consist of three subunits: α, β and γ. In the resting state, a G-protein exists as an αβγ-trimer, with guanosine diphosphate (GDP) attached to the α-subunit. As an agonist binds to a GPCR, a conformational change in the cytoplasmic domain of the receptor greatly increases the affinity of the GPCR to the G-protein αβγ-trimer. Association of GPCR with G-protein causes the bond of α-subunit to GDP to dissociate and to be replaced with guanosine triphosphate (GTP). This, in turn, causes βγ-dimer to separate from α-GTP. These two complexes are the active forms of a G-protein; they diffuse in the membrane and associate with various biochemical effectors, mainly enzymes and ion channels, which results in the activation or inactivation of various cascades. The cycle is completed as GTP is hydrolysed to GDP with the help of the GTPase of the α-subunit, whereupon the α-GDP complex dissociates from the effector and reunites with βγ-dimer (Conklin and Bourne 1993, Clapham and Neer 1997, Wess 1998).

Table 2. Adrenoceptors, different G-proteins and the related second messengers. L- and N-type channels are voltage-operated calcium channels. Adenylyl cyclase catalyses the formation of cyclic adenine monophosphate (cAMP). The directions of the effects are indicated by the arrows (modified from Hoffman and Taylor 2001b).

Adrenoceptor	G-protein	Biochemical effectors
β_1	G_s	↑ adenylyl cyclase, ↑ L-type Ca^{2+} channels
β_2	G_s	↑ adenylyl cyclase
β_3	G_s	↑ adenylyl cyclase
α_1	G_q	↑ phospholipase C and D
	G_q, G_i/G_o	↑ phospholipase A_2
	G_q	↑ Ca^{2+} channels ?
α_2	$G_{i\ 1, 2 \text{ or } 3}$	↓ adenylyl cyclase
	G_i	↑ K^+ channels
	G_o	↓ Ca^{2+} channels (L- and N-type)
	?	↑ phospholipase C and A_2

There are 20 known forms of the α-subunit (α_1-α_{20}), six of the β-subunit (β_1-β_6) and 12 of the γ-subunit (γ_1-γ_{12}). The specificity of intracellular GPCR effects is based on this variation within the G-protein (Wess 1998), mostly on α-subunit variation. G-proteins can be classified according to the involved combination of the three subunits into four main categories: G_s, G_i, G_q and G_o (Hoffman and Taylor 2001a, Roman 2003). Each type of G-proteins shows specificity to both receptors and the effectors with which they couple (Table 2).

Several polymorphisms have been discovered in the subunits of G-proteins. A single nucleotide polymorphism (SNP) of the β_3-subunit has been associated with low plasma renin activity, and this SNP may identify individuals with sodium-sensitive hypertension responsive to diuretics (Schunkert et al. 1998, Turner et al. 2001). In the gene *GNAS1* encoding α-subunit, there is a silent SNP at base locus 393, where thymine is replaced by a cytosine (T393C). This SNP is associated with the presence (+) or absence (-) of a restriction site for *Fok*I. Caucasian patients with *Fok*I+ allele respond better to the antihypertensiveness of β-antagonists than patients with *Fok*I- allele (Jia et al. 1999).

1.1.2.3 α-adrenoceptors

α_1-adrenoceptors

Although structurally closely related, the different adrenoceptors control synthesis and release of various second messengers (Table 2), and thus regulate distinct physiological cascades. Knowledge of the properties and distributions of the α- and β-adrenoceptor subtypes in each cell type and organ is necessary to understand the diverse effects of catecholamines and the drugs with sympathomimetic or sympatholytic effects.

Human α_1-adrenoceptors are currently divided into three subtypes (Fig. 1): α_{1A}, α_{1B} and α_{1D}, which are encoded in different genes in chromosomes 8, 5 and 20, respectively (Hieble et al. 1995, Brodde and Michel 1999). There are several splice variants of subtype α_{1A} differing in length and the sequence of their C-terminal domains (Hirasawa et al. 1995, Chang et al. 1998), but, importantly, no pharmacological or signalling differences have been observed (Chang et al. 1998).

The α_1-adrenoceptors mediate many actions of endogenous catecholamines, the main responses being contraction of arterial and venous smooth muscle (Table 1). Vascular smooth muscle tissues express mixtures of α_1-adrenoceptor subtypes (Rudner et al. 1999). The vasoconstrictory effect in humans may be primarily mediated by the subtype α_{1A}, but most probably several subtypes are involved (Van der Graaf et al. 1996, Zhong and Minneman 1999, Argyle and McGrath 2000). α_{1A} is the most abundant α_1-subtype in the heart, but the overall inotrophic and chronotrophic effects mediated by α_1-adrenoceptors are small compared with the effects mediated by β-adrenoceptors (Steinfath et al. 1992a, Steinfath et al. 1992b, Brodde and Michel 1999).

The α_1-adrenoceptors in the lower urinary tract contribute to the outflow resistance of urine (Walden et al. 1999, Roehrborn and Schwinn 2004). In stroma and capsule of the prostate, and in trigone of the bladder, α_{1A}-subtype overwhelmingly predominates with α_{1D}-adrenoceptors present to a lesser extent (Walden et al. 1999, Debruyne 2000). α_{1D}-subtype is the major adrenoceptor in the bladder detrusor muscle (Malloy et al. 1998, Hampel et al. 2002). The main locations for α_{1B}-adrenoceptors are CNS, spleen and lungs (Price et al. 1994, Langer 1999).

The only currently known non-synonymous polymorphism in α_1-adrenoceptors is the *Arg492Cys* in α_{1A}-adrenoceptor – a substitution of arginine by cysteine at amino acid position 492 – but it does not seem to present any noteworthy physiologic or pharmacologic variation (Liggett 2003, Sofowora et al. 2004).

α_2-adrenoceptors

Presynaptic α_2-adrenoceptors – mainly the sybtype α_{2A} – are probably present in all the arteries and veins, where they mediate a negative feedback to the release of NA (Starke 1987, Guimaraes et al. 1997, Langer 1997, Docherty 1998, Ho et al. 1998). In the endothelium of large arteries and microcirculation, activation of α_{2A}-adrenoceptors stimulates the release of nitric oxide (NO), and thus mediates endothelium-dependent relaxation (Angus et al. 1986, Richard et al. 1990, Bockman et al. 1993). These α_2-effects tend to attenuate vasoconstriction produced by activation of postsynaptic vascular α_1-adrenoceptors (Guimaraes and Moura 2001).

The postsynaptic α_2-adrenoceptors are found mainly in veins, but also in certain arteries with vasoconstrictory influence (Gavin et al. 1997, Guimaraes et al. 1997, He and Yang 1998, Guimaraes and Moura 2001, Dinenno et al. 2002a, Dinenno et al. 2002b). Also, stimulation of postjunctional α_2-adrenoceptors in the lower brainstem region is associated with reduced sympathetic outflow from the CNS, and this has been proposed to be the mechanism of the antihypertensive effect of clonidine (Hoffman 2001a). Pancreatic α_2-adrenoceptors inhibit insulin secretion (Yamazaki et al. 1982, Debuyser et al. 1991, Plant et al. 1991).

Each of the α_2-adrenoceptor subtypes show nonsynonymous polymorphism, some of them being of major clinical impact (Liggett 2003). E.g. deletion of amino residues 322-325 in α_{2C}-adrenoceptor causes a substantially uncoupled receptor and has been reported to have a fivefold increase in the risk of developing heart failure (Small et al. 2002). In addition, combination of this allele with the *Arg389* form of β_1-adrenoceptor brings about a tenfold risk for heart failure (Small et al. 2002).

1.1.2.4 β-adrenoceptors

β_1-adrenoceptors

Three β-adrenoceptor subtypes have been cloned so far: β_1, β_2 and β_3, coded in separate genes in chromosomes 10, 5 and 8, respectively (Bylund et al. 1994). β_1- and β_2-receptors mediate mainly cardiovascular and pulmonary responses to circulating A and to the NA released from sympathetic nerve terminals (Lands et al. 1967a, Lands et al. 1967b). In the heart, the ratio β_1/β_2 is about 60-70/30-40 in the atria and 70-80/20-30 in the ventricles (Brodde 1991), and both of these subtypes are involved in the positive chronotropic, inotropic, lusitropic (relaxant)

and dromotropic effects and the increased automaticity of pacemaker tissues (Newton et al. 1999, Pavoine and Defer 2005). Another important β_1-mediated effect is the increasing release of renin from the renal juxtaglomerular apparatus (Desaulles et al. 1978, Oates et al. 1978, Weber et al. 1983). NA and A are equally potent on the β_1-adrenoceptor (Hoffmann et al. 2004).

Two SNPs have been discovered in the β_1-adrenoceptor gene *ADRB1*: *Arg389Gly* that causes a substitution of arginine by glycine at amino acid position 389, and *Ser49Gly* that replaces serine with glycine at position 49 (Liggett 2003, Small et al. 2003). *In vitro*, the wild-type *Arg389* form (73 % frequency in Caucasians) of the *Arg389Gly* polymorphism produces increased high-affinity agonist binding and enhanced adenyl cyclase activities compared with the form *Gly389*, which has the frequency of the remaining 27 % (Mason et al. 1999, Liggett 2003, Small et al. 2003). The *ex vivo* experiments on human atrial preparations have yielded varying results: either the *Arg389Gly* polymorphism has not exerted any effects (Molenaar et al. 2002, Sarsero et al. 2003), or the *Arg389* variant has demonstrated greater inotropic and cyclic adenine monophosphate (cAMP) responses to NA than the *Gly389* variant (Sandilands et al. 2003). In clinical studies, no differences in HR, contractility or plasma renin activity responses to bicycle exercise between the *Arg389* and *Gly389* have been found (Buscher et al. 2001, Sofowora et al. 2003, Leineweber et al. 2004). *Arg389* homozygotes are at increased risk of developing hypertension (Bengtsson et al. 2001). On the other hand, the same patients have shown greater reduction of diastolic arterial pressure (DAP) during metoprolol or atenolol treatment compared with the variant allele in some (Johnson et al. 2003, Sofowora et al. 2003) though not all studies (O'Shaughnessy et al. 2000).

The *Gly49* allele (15 % frequency in Caucasians) of the *Ser49Gly* polymorphism is associated with greater agonist-promoted down-regulation and altered glycosylation of the β_1-receptor in hamster fibroblasts (Levin et al. 2002, Rathz et al. 2002). The SNP has not had any effects in the experiments with human atrial preparations (Sarsero et al. 2003). In clinical settings, the subjects with homozygosity for the *Gly49* allele had 5 bpm lower resting HR than the comparison group (Ranade et al. 2002), and there has been a trend toward a greater BP response to metoprolol in *Ser49* homozygotes compared with *Gly49* carriers (Johnson et al. 2003).

β_2-adrenoceptors

The role of β_2-adrenoceptors in the adrenergic cardiac effects (Table 1) is more prominent

than the sheer quantitative ratio β_1/β_2 would justify. This is due to the fact that β_2-adrenoceptors couple more effectively to adenylyl cyclase than β_1-adrenoceptors (Levy et al. 1993, Brodde and Michel 1999). These effects become even more pronounced in failing and aged hearts (Brodde and Michel 1999). In addition, β_2-adrenoceptors mediate bronchodilation, increased secretion from bronchial glands and vasodilation in most blood vessels. β_2-adrenoceptors exhibit about 35-fold higher affinity for A than for NA (Michel 1991, Hoffmann et al. 2004).

The three known SNPs within β_2-adrenoceptors are at amino acid positions 16, 27 and 164. These cause changes in agonist-promoted down-regulation, high affinity agonist binding and G_s coupling. *Ile164* allele has been reported to be associated with 4.8-fold risk of mortality in patients with heart failure (Liggett et al. 1998) and eightfold risk of having depressed exercise capacity (Wagoner et al. 2000, Wagoner et al. 2002). In asthma, a variant allele in the position 16 is markedly associated with tachyphylaxis to β-agonist (Israel et al. 2001). Combinations of certain polymorphisms relate to hyperreactivity of airways (Litonjua et al. 2004).

β_3-adrenoceptors

The first elucidated roles for the β_3-adrenoceptor were promotion of lipolysis in adipose tissue (Arch et al. 1984) and thermogenesis (Emorine et al. 1994, Enocksson et al. 1995). Later, slight β_3-mediated negative chronotropic (Gauthier et al. 1998, Moniotte et al. 2001) and inotropic (Gauthier et al. 1998) influence has been found. β_3-adrenoceptors are also present in blood vessels, where they mediate vasodilation in both peripheral vessels (Berlan et al. 1994, Shen et al. 1994) and coronary arteries (Dessy et al. 2004). The pharmacology of β_3-adrenoceptor is clearly distinct from that of β_1- and β_2-adrenoceptors: the first is not blocked by propranolol or other conventional β-antagonists, and it is about 10-30 fold more sensitive to NA than to A (Liggett 1992, Guimaraes and Moura 2001, Hoffmann et al. 2004). Polymorphism in the β_3-adrenoceptor may be related to obesity and type 2 diabetes in some populations, but the reports are not consistent (Liggett 2003, Small et al. 2003).

1.2 Haemodynamic parameters and regulation

1.2.1 Definitions

The cardiovascular and haemodynamic variables are intertwined with each other through many interdependencies and the

simultaneous operation of a multitude of regulation mechanisms (Fig. 2).

CO is defined as the product of HR and SV. SV is determined by myocardial contractility, preload and afterload. Myocardial contractility is a purely cardiac factor since it is a characteristic of cardiac tissues, even though modulated by various neural and humoral mechanisms. Given a constant contractility, the Frank-Starling's law states that the maximum tension cardiac muscle develops depends on the sarcomere length just before systole (Lakatta 1992). End-diastolic volume and pressure are surrogate parameters for sarcomere length, and all of these are measures of preload. The third crucial factor in sizing SV is the force that the contracting myocytes must overcome. This is often substituted for a more practical parameter, systolic arterial pressure (SAP), and both of these are measures of afterload (Boulpaep 2003a). Importantly, preload and afterload connect heart and vasculature into the same regulatory machinery, and they are called coupling factors since they are, in addition to being determinants of CO, also affected by CO and certain other cardiovascular characteristics (Berne and Levy 1993a).

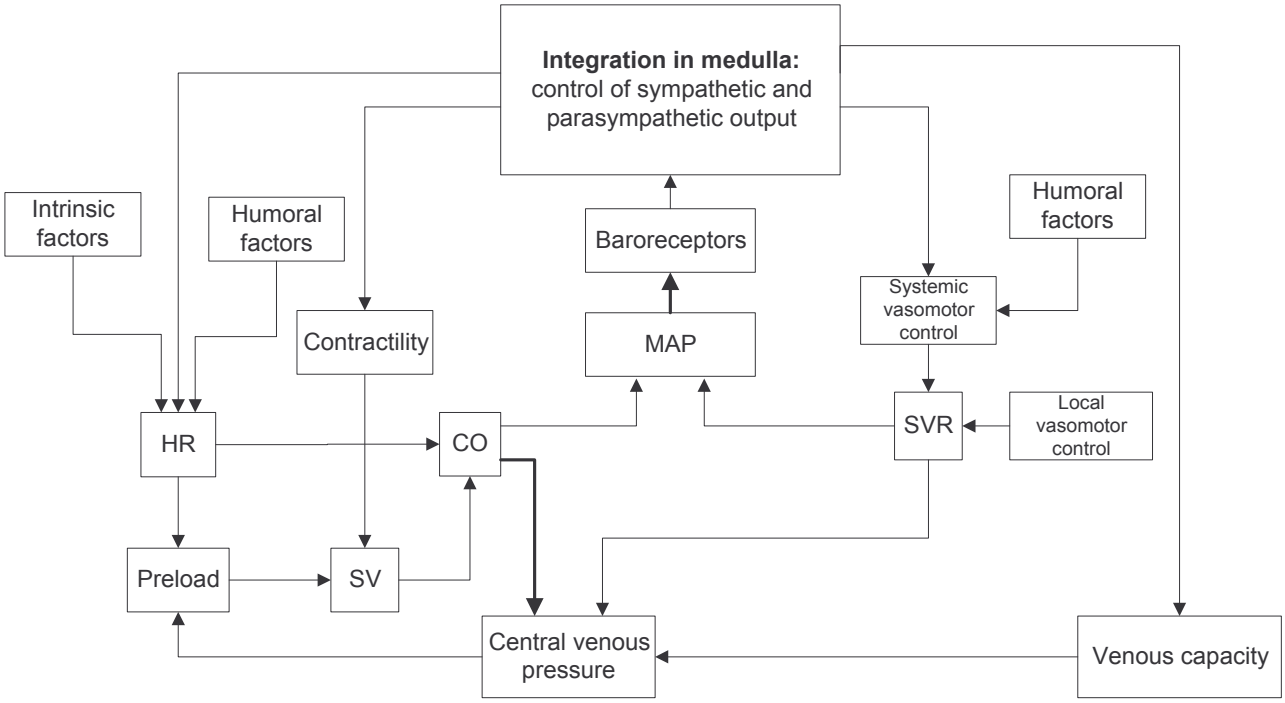

Fig. 2. The control of and interplay between haemodynamic parameters. Some interactions of a minor effect are ignored. The thick lines represent starting points of major feedback loops. Blood volume is assumed constant. CO, cardiac output; HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; SVR, systemic vascular resistance.

SVR, or total peripheral resistance, represents the sum of resistances in multiple parallel regional vascular beds. The site of the resistance is predominantly in the small arteries and arterioles, i.e. the resistance vessels. SVR is defined by the equation

$$\begin{aligned} SVR &= (MAP - P_{ra}) / CO \\ &\cong MAP / CO\left[mmHg \cdot \min / l\right] \\ &= MAP / CO \cdot 80\left[dyn \cdot s / cm^5\right] \end{aligned} \quad , \qquad (1)$$

where the mean right atrial pressure P_{ra} can be neglected since it is diminutive compared with the mean arterial pressure (MAP). The unit dyn is g/(s^2 · cm). The constant 80 in the last and most often used form of the equation is brought along by the change of the units of MAP and CO (Saksena 1983).

1.2.2 Regulatory mechanisms

Circulatory adjustments are necessary to secure adequate perfusion pressure and the subsequent blood supply to active tissues, as well as to increase or decrease heat loss, and – in times of challenges such as severe haemorrhage – to maintain the blood flow to the heart and brain. In healthy normotensive individuals, the kidney is central in setting long-term arterial pressure (Lohmeier 2001, Wyss and Carlson 2001, DiBona 2002), while ANS is thought to be primarily a short-term regulator maintaining the arterial pressure under acute challenges such as orthostasis, stress and physical exercise (Wyss and Carlson 2001). The pressure sensors that activate and deactivate ANS are stretch receptors called baroreceptors. The most important baroreceptors lie in the adventitia layer of aortic arch and carotid sinuses. If BP rises and the baroreceptors stretch, the firing by the baroreceptors to the medulla oblongata via the *nervi glossopharyngeus* (9^{th} cranial nerve) and *vagus* (10^{th}) increases. *Medulla oblongata*, especially *nucleus tractus solitarius*, is of primary importance in the cardiorespiratory reflex integration and regulation of sympathetic activity (Andresen and Kunze 1994, Colombari et al. 2001). Upon increased afferent activity, *medulla oblongata* excites vagal and inhibits sympathetic innervation to the pacemaker cells of the heart, ventricle, resistance arteries and capacity vessels, causing bradycardia, reducing inotrophy, and producing vasodilation and venodilation (Cohn 1992). These adjustments decrease BP back to the set point of the baroreceptor. In pathological states, the set point may be reset to a higher pressure level, and hypertension follows (Sleight 1991, Grassi et al. 1998, Stauss 2002).

Basicly, there are two parameters to adjust in order to maintain arterial pressure: CO and diameter of the resistance vessels (Fig. 2). CO can be varied by changing HR or SV. HR is mainly controlled by both sympathetic and parasympathetic divisions of ANS. When

these autonomic systems act concomitantly, the interactions are complex, and not simply additive algebraically (Levy 1997, Opie 1998). In resting state, there is a tonic discharge from both divisions, but the parasympathetic impulses prevail. Upon activation of sympathetic division, cholinergic stimulation decreases, and both of these changes as well as circulating catecholamines have a positive chronotrophic effect and increase HR.

Sympathetic stimuli strengthen the contractions – or increase contractility – of myocardial muscle, and a greater portion of the blood is expelled out of the ventricles and SV increases. This positive inotrophic effect is augmented by circulating catecholamines and certain pharmaceuticals, while parasympathetic stimuli have the opposite effect (Boulpaep 2003a).

The number and effect of cardiac α-adrenoceptors is low, and the cardiac sympathetic effects are mainly mediated by β_1- and β_2-adrenoceptors. However, the α-adrenergic stimulation has clear indirect effects, since α-adrenoceptor-mediated venoconstriction results in an increased venous return that facilitates cardiac filling and enhances preload, and arterial constriction results in an increased arterial resistance that augments arterial pressure and afterload.

Most vascular beds have an intrinsic property to adjust the caliber of the vessel as the perfusion pressure changes, probably at least partly due to a contractile response to stretching of smooth muscle in the walls of blood vessels. Other methods of local regulation comprise locally produced vasodilator metabolites and substances secreted by endothelium (Behrendt and Ganz 2002). The systemic regulation of the diameter of blood vessels consists of neural and humoral control. Arterioles and other resistance vessels are the most densely innervated ones, but all the blood vessels except capillaries and venules encompass fibers from the sympathetic nervous system. The parasympathetic system does not innervate blood vessels. The vasodilatory hormones include kinins, vasoactive intestinal peptide (VIP) and atrial natriuretic peptide (ANP), while NA, A, vasopressin and angiotensin II constrict blood vessels (Andresen and Kunze 1994, Marshall 1994, Boulpaep 2003c).

1.2.3 Arterial compliance and pulse wave velocity

Arterial compliance

$$C = dV / dP \cong \Delta V / \Delta p \qquad (2)$$

is a determinant of the storage capacity of the arteries during systole. During diastole, the

storaged blood flows from the arterial tree forward to periphery, securing continuous blood supply; this recoil is called Windkessel effect. Changes in compliance induced by drugs, physiologic responses, aging or disease may have a striking impact, since the resistance to the flow is inversely proportional to the 4^{th} power of the diameter of the tube, according to the Poiseuille's law (Boulpaep 2003b, LeWinter and Osol 2004).

The elasticity of proximal large arteries is a consequence of high elastin-to-collagen ratio in the arterial wall. The most prominent factor related to stiffer vessels is aging, which leads to progressive degeneration of arterial wall elastic fibers and increased collagen content (Avolio et al. 1998). Decreased arterial compliance i.e. increased stiffness is an important determinant of cardiovascular risk (Blacher et al. 1998, Blacher et al. 1999, Guerin et al. 2001, Laurent et al. 2001, McGrath et al. 2001, Barenbrock et al. 2002, Boutouyrie et al. 2002). The methods used for assessment of the arterial compliance are measurement of arterial PWV, volume-oscillometry method, or simultaneous measurement of arterial pulse pressure and the systolic change in arterial diameter using ultrasound (Stergiopulos et al. 1994, Asmar et al. 1995, Stergiopulos et al. 1995, Liang et al. 1998, Quick et al. 2000, Wiinberg 2000).

During each heart beat a pulse wave travels from the heart down the arterial wall in advance of blood flow (Lakatta 1989). The more rigid the wall of the artery, the faster the wave moves. Thus, PWV is inversely related to the arterial compliance. When the wave hits the major branching points, such as the renal and femoral arteries, these waves are reflected and they travel back to their point of origin (Pythoud et al. 1996). Normally, the reflected wave gets back to the starting point after the aortic valve is closed. This amplifies DAP and facilitates blood flow to the coronary arteries (O'Rourke 1991, London and Guerin 1999, Nichols and Edwards 2001). However, the increased PWV of the initial wave and the subsequent reflected wave may cause the wave to return to the aortic valve before this closes. This results in an increase in SAP rather than DAP (Nichols and Edwards 2001, Meeks 2002). It also decreases the contribution of the reflected wave to the filling of the coronary arteries. Subsequently, PWV is an independent predictor of cardiovascular mortality in high-risk individuals: the faster the PWV, the higher the risk (Blacher et al. 1999, London et al. 2001, Nichols and Edwards 2001, Shoji et al. 2001, Cruickshank et al. 2002, Safar et al. 2002b, Blacher et al. 2003). PWV can be estimated by a variety of methods, including Doppler ultrasound, analysis of the pulse contour, magnetic resonance tagging and impedance methods (Macgowan et al. 2002, Kööbi et al. 2003).

1.3 Tests on the control and function of haemodynamics

A variety of tests have been developed for clinical assessment of total autonomic activity on different organs: e.g. HR variability, heart rate turbulence (Guzik and Schmidt 2002), baroreceptor sensitivity (Parati et al. 2000), and passive orthostasis. In the following, HR variability and passive orthostasis are presented in more detail.

1.3.1 Heart rate variability

ANS is the most important controller of HR, and thus assessing and analysing the fluctuations in HR is a gateway to estimate autonomic function (Akselrod et al. 1981, Pagani et al. 1986, Bailey et al. 1996, Task Force 1996). All the analyses of HR variability are based on the R-R intervals (RRI) that are intervals between adjacent QRS complexes resulting from sinus node depolarisations. RRIs can be analysed in either time or frequency domain (Task Force 1996). Non-linear techniques, chaos theory and analysis of fractal behaviour have also been used, but the physiological interpretations of most of these methods are not yet established.

Time domain analysis comprises a variety of calculated indices. The simplest measure is the standard deviation of the RRIs (SDNN), but it is not well-defined on arbitrarily selected ECGs because of its dependence on the length of recording period. Another possibility is to analyse the differences between the adjacent RRIs. The mostly used of these parameters is square root of the mean squared differences of successive RRIs (RMSSD), and it is suitable especially for finding the short-term components of HR variability (Task Force 1996).

Frequency domain or spectral analysis of HR variability separates the different fluctuation frequencies within the data. The shift from time to frequency domain is most often performed with fast Fourier transform (FFT), but autoregressive model estimations are more suitable in certain cases. Spectral analysis of HR variability necessitates stationarity of the RRI data (Malliani et al. 1991, Öri et al. 1992). However, most physiologic phenomena are not stationary of nature, and the data is typically preprocessed with mathematical techniques linear detrending and filtering in order to increase stationarity (Penaz 1978).

Spectral methods produce a spectrum of HR variability, divided into four major frequency bands: high frequency (HF) 0.15-0.4 Hz, low frequency (LF) 0.04-0.15 Hz, very low frequency (VLF) 0.003-0.04 Hz and ultra low frequency (ULF) <0.003 Hz. These components represent fluctuations with a

periodicity of 2.5-7 s, 7-25 s, 25 s - 6 min and >6 min, respectively. Different indices can be calculated to describe the absolute and relative size of each of the components. Vagally controlled respiratory sinus arrhythmia (Katona and Jih 1975, Eckberg 1983, 2003) is a well-known cause for HR variability fluctuations. Since breathing frequency is roughly 0.25 Hz (equals to 15 times per minute), this dominates the HF component of the HR variability spectrum (Chess et al. 1975, Pagani et al. 1986, Bailey et al. 1996, Task Force 1996). The orally administered β-blocking drugs atenolol, bisoprolol and propranolol are known to increase the HF component due to decreased sympathetic and increased parasympathetic modulation (Pousset et al. 1996, Lin et al. 1999, Lampert et al. 2003).

The interpretation of the LF component is controversial. Vagal activity is known to be a major contributor (Chess et al. 1975, Akselrod et al. 1981, Akselrod et al. 1985), but the clinical and experimental studies with sympathomimetic and sympatholytic agents have yielded variable results (Chess et al. 1975, Akselrod et al. 1981, Akselrod et al. 1985, Pomeranz et al. 1985, Pagani et al. 1986, Breuer et al. 1993, van de Borne et al. 1997). Neither are the backgrounds of the VLF and ULF ranges adequately known. Vagal activity seems to largely modulate also these ranges (Akselrod et al. 1981, Pagani et al. 1986, Taylor et al. 1998), while renin-angiotensin system (Akselrod et al. 1981, Taylor et al. 1998) and thermoregulation (Lindqvist et al. 1989, Stauss 2003) have smaller roles.

A decrease in HR variability has clinical prognostic value, since it has been associated with increased mortality in patients with chronic heart failure (Galinier et al. 2000, Boveda et al. 2001, La Rovere et al. 2003) or coronary artery disease (Kleiger et al. 1987).

1.3.2 Passive orthostasis, head-up tilt

BP homeostasis is challenged by movement to an upright position: the body must accommodate to the effects of gravity that tend to shift large amounts of blood from central veins to abdomen and periphery. Factors such as the renin-angiotensin-aldosterone system play a role over a longer period, but the acute compensation is controlled by ANS.

Due to the increased hydrostatic pressure, venous return considerably decreases upon assuming the upright position. This leads to a decrease in cardiac filling and end-diastolic volume, and to a consequent drop of roughly 30-40 % in SV (Fig. 3). Since CO and MAP are directly proportional to SV, decline in BP immediately activates the compensatory mechanisms (Fig. 2) by the baroreceptors and so called cardiopulmonary receptors in atria, central veins and pulmonary circulation. The

resulting prompt decrease in the parasympathetic tonus and the increase in the sympathetic tonus accelerate HR by 20-25 % and increase inotrophy that compensates decreased cardiac filling. An important additional compensatory factor is the enhanced sympathetic activity of leg muscles which restricts the venous pooling of blood after standing up (Sundlöf and Wallin 1978, Mano and Iwase 2003). These changes combined, CO only decreases by approximately 15-20 %. As SVR increases at the same time, SAP remains unchanged, while DAP may even slightly increase (Tahvanainen et al. 2003).

Disorders of ANS resulting in sympathetic failure often cause orthostatic intolerance that varies from non-symptomatic decrease of BP to collapse and even transient asystole (Mano and Iwase 2003). Aging is associated with both reduced ability to withdraw the vagal activity and attenuated baroreceptor reflexes (Shimada et al. 1985, Smith et al. 1987, Shannon et al. 1991), which result in an increased risk of orthostatic intolerance. The mechanisms involved can often be diagnosed if the detailed haemodynamics is monitored during a head-up tilt (Dehn et al. 1984, Kenny et al. 1987, Maloney et al. 1988, Almquist et al. 1989, Milstein et al. 1989, Mathias et al. 1991, Kapoor 1999).

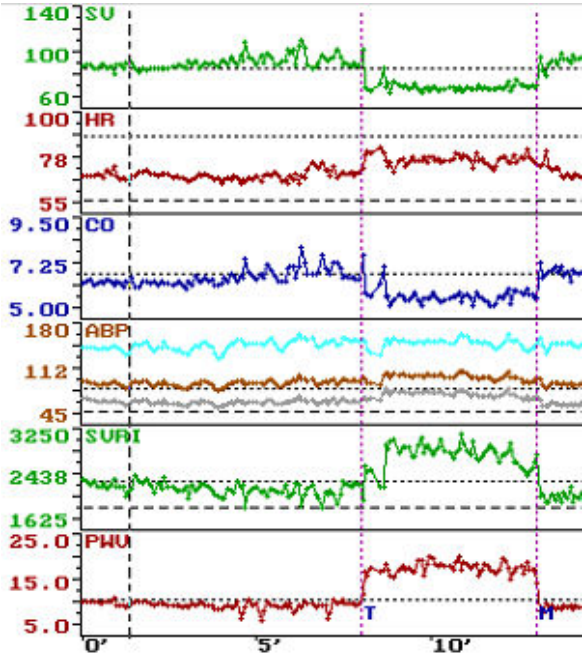

Fig. 3. Responses in stroke volume (SV), heart rate (HR), cardiac output (CO), arterial blood pressure (ABP), systemic vascular resistance index (SVRI) and pulse wave velocity (PWV) to a passive head-up tilt. The subject is turned from supine to 60˚ at the time point T and returned back to supine position at M. The ABP window includes systolic (uppermost line), mean (middle) and diastolic (lowermost) pressures.

1.3.3 Exercise test

Clinical exercise tests have been developed to examine the physical and especially cardiorespiratory capacity, and the level and mechanisms of performance restriction. The exercise test is most often performed by using an electronically braked bicycle or a treadmill ergometer. The standard exercise test focuses on the effects of stress on symptoms, 12-lead ECG, pulse oximetry and BP as functions of time and load. The purpose of this test is to identify ischemia, and generally it cannot

define the underlying pathophysiology in patients with exercise intolerance of nonischemic origin. When needed and appropriate, the additional measurements of pulmonary function and arterial blood gases provide important information on pulmonary gas exchange.

The main benefits of the bicycle ergometer are the possibility to quantify exactly the load and low level of ECG artefacts. The maximum oxygen consumption with a treadmill ergometer tends to be slightly higher than that with a bicycle ergometer because of the incorporation of arm movement and non-weight-bearing effect of sitting on the bicycle. Treadmill ergometer may be technically easier to perform.

1.3.4 Invasive measurement of cardiac output

By the means of physical assessment and clinical findings alone, the haemodynamic status is often poorly assessed (Connors et al. 1983, Eisenberg et al. 1984, Tuchschmidt and Sharma 1987, Connors et al. 1990, Steingrub et al. 1991). This is partly due to masking of haemodynamic changes by physiologic compensatory mechanisms: while a patient may have a significant decrease in CO, the initial compensatory vasoconstriction results in an increase in SVR that keeps BP unchanged. For more than half a century, numerous variations of indicator dilution techniques have been used to estimate a more exact haemodynamic status (Hamilton et al. 1932, Lassen et al. 1979). Even the classic direct and indirect Fick methods are specialised indicator methods with the blood-soluble gases oxygen (O_2) or carbondioxide (CO_2) as indicators (Lassen et al. 1979). Other commonly used indicators are e.g. cold or warm saline in thermodilution method (Lassen et al. 1979), indocyanine green (Iijima et al. 1997, Imai et al. 1997) and different radio isotopes (Yuille 1979). As a modification of the thermodilution method, a pulmonary artery catheter may incorporate a heating element that intermittently heats the blood up.

Essentially, the indicator dilution methods are based on the law of conservation of mass (Lassen et al. 1979). In thermodilution, for example, cold or warm saline is injected into or close to the right atrium, and the subsequent change in temperature is measured from the pulmonary artery. The cardiac output calculation is based on how the temperature returns to normal. Since the measurement is made over several heart beats, indicator dilution methods do not produce beat-to-beat data.

The greatest drawback of thermodilution and Fick methods is their invasiveness; the measurement is performed with a pulmonary artery catheter. Applying the catheter requires

special expertise, and it may involve complications (Mangar et al. 1993, Bernardin et al. 1994, Connors et al. 1996). From both technical and ethical points of view, the use of these CO measurement methods is limited to critically ill patients. Certain other dye-dilution methods are less invasive, since no connection to a central vein or artery is needed (Maarek et al. 2004).

1.3.5 Noninvasive measurement of cardiac output

1.3.5.1 Pulse contour method

The pulse contour method was initially developed as an invasive technique based on changes in intra-arterial pressure (McDonald 1974, Kööbi 1999). Later, a noninvasive finger pressure-derived pulse contour method has partly substituted its invasive counterpart, since the Finapres™ (Ohmeda, Louisville, CO, USA) device is able to mimic the intra-arterial BP patterns when placed on a finger (Imholz et al. 1988, Imholz et al. 1990, Hirschl et al. 1996).

Several models to assess SV from the pulse contour have been developed (Fogliardi et al. 1996, Cerutti et al. 2001). The computation is often based on three elements representing the major properties of aorta and arterial system: aortic characteristic impedance Z_0, which is a dynamic property of the aorta impeding pulsatile outflow from the ventricle, Windkessel compliance C_w, which is the ability of the aorta and arterial system to store SV elastically, and peripheral resistance R_p (Westerhof et al. 1971, Wesseling et al. 1993, Stergiopulos et al. 1999). Z_0 and C_w are nonlinearly dependent on the arterial pressure, and R_p is a time-varying variable. SVR is the sum of Z_0 and R_p. The basic functional principle of the three-element model is that SV is injected into the Windkessel compliance during systole, and it is dissipated into the periphery during diastole.

The finger pressure-derived pulse contour method has been compared with dye-dilution and ultrasound methods in a number of studies, with contradictory results (Stok et al. 1993, Hirschl et al. 1997, Girardis et al. 1998, Godje et al. 1998, Harms et al. 1999, Houtman et al. 1999, Rodig et al. 1999, Bein et al. 2004). The CO values in healthy participants during moderate exercise were 2.3 ± 3.9 l/min lower with the pulse contour method than with the CO_2 rebreathing method (Houtman et al. 1999). The reproducibility of the pulse contour method was clearly inferior to the CO_2 rebreathing method. Hirschl et al. (1997), too, reported that in critically ill patients the pulse contour method could not substitute the thermodilution – even if the pulse contour method was calibrated with thermodilution at the beginning of the measurements. However, the pulse contour method was able to show the

direction of changes in cardiac index: in 90 % of the readings, the values by both methods changed into the same direction. On the other hand, the study of Harms and colleagues (1999) on ten healthy young adults showed good agreement in SV measurement between the pulse contour method and thermodilution. The values were measured in various body positions, including head-up tilt. Some other recent studies with intra-arterial pulse contour measurement show more promising but still somewhat inconsistent results (Rodig et al. 1999, Cottis et al. 2003, Mahajan et al. 2003).

1.3.5.2 Ultrasonography

Several variations of ultrasonography have been shown to agree well with invasive methods in the measurement of SV and CO (Kööbi 1999, Hett and Jonas 2004). Transthoracic and transesophageal echocargiography is applicable for intermittent evaluation of a number of haemodynamic parameters, including SV, CO, global and regional wall motion, preload, afterload and valvular integrity (Ryan et al. 1992, Poelaert et al. 1999). Continuous haemodynamic assessment is possible with esophageal Doppler by using a probe inserted into the esophagus, but this is feasible only with critically ill or compromised patients due to the uncomfortableness of the probe (Perrino et al. 1991, Mythen and Webb 1995). Disadvantages of ultrasonography include operator-dependency and the need for recurrent repositioning of the probe (Cholley and Singer 2003, Turner 2003).

1.3.5.3 Impedance cardiography

Theory

Impedance cardiography (ICG) techniques to determine the CO have been available since the 1960s. In these methods, a constant alternating current is applied to a body in order to measure variations in electrical impedance. The electric current is applied to the body with current electrodes, and the voltage generated is measured by another pair of electrodes placed between the current electrodes. Then, impedance Z and its variation ΔZ are calculated according to Ohm's law. The electrical impedance consists of resistance and capacitance components. When the frequency of alternating current is 20-50 kHz, the capacitance component is negligible and the electrical impedance of the tissue is close to its resistivity, which is 150 Ω/cm for blood, 63 for plasma, 750 for cardiac muscle, 1275 for lungs and 2500 for fat (Baker 1989). According to an elementary principle in electricity, Kirchow law, electric current passes through the conductors with lowest impedance. Thus, under the conditions depicted above, the electric current is mainly

distributed via the blood vessels. In systole, the aortic volume increases by the amount of SV and there is a certain decline in the electrical resistivity or impedance ΔZ, the relation between these parameter being

$$\frac{SV}{V} = \frac{\Delta Z}{Z} \Leftrightarrow SV = \frac{\Delta Z}{Z} V , \qquad (3)$$

where V is the aortic volume just before the onset of the systole and Z is the diastolic impedance of the electric field involved. This is the base SV equation that has been refined for different electrode configurations, and correction factors for hematocrit, gender and body weight have also been introduced (Kööbi et al. 1997b, Moshkovitz et al. 2004).

Thoracic impedance cardiography

There are several different locations for the electrodes. In the thoracic impedance method (ICG_{TH}), one of the electrodes is placed at the root of the neck and another on the level of the xiphoid process. This electrode configuration mainly measures the electrical impedance changes in pulmonary artery and aorta (Moshkovitz et al. 2004). In all, at least four different SV equations for ICG_{TH} have been developed and used in clinical settings, the first of which was published by Kubicek and colleagues in 1966 (Moshkovitz et al. 2004). The thoracic impedance methods have been evaluated widely, but the results with the first generation devices were not consistent (Fuller 1992, Raaijmakers et al. 1999). The new generation devices have showed more promising agreement with thermodilution and direct Fick method (Ziegler et al. 1999, Charloux et al. 2000, Richard et al. 2001, Sageman et al. 2002, Van De Water et al. 2003).

Whole-body impedance cardiography

In the 1970s, the whole-body impedance cardiography (ICG_{WB}) was introduced (Tishchenko 1973). In this method, the current electrodes are placed on extremities, just proximal to wrists and ankles (Fig. 4). Voltage electrodes are placed proximally of the current electrodes. In addition to the original SV equation by Tishchenko (1973), Kööbi et colleagues (1997b), and Tsoglin and Frinerm (Miller et al. 1993, Tsoglin and Frinerman 1995) have developed equations for SV determination with ICG_{WB}.

Kööbi et al. (1997a, 1997b, 1999) have validated their algorithm in comparisons with thermodilution, and simultaneously with both thermodilution and Fick's direct oxygen method. The biases between the CO values were negligible and the limits of agreement comparable between the methods. ICG_{WB} estimated CO changes in head-up tilt adequately (Kööbi et al. 1997b). The repeatability of ICG_{WB} (0.57 l/min) was

clearly better than that of thermodilution (1.10 l/min).

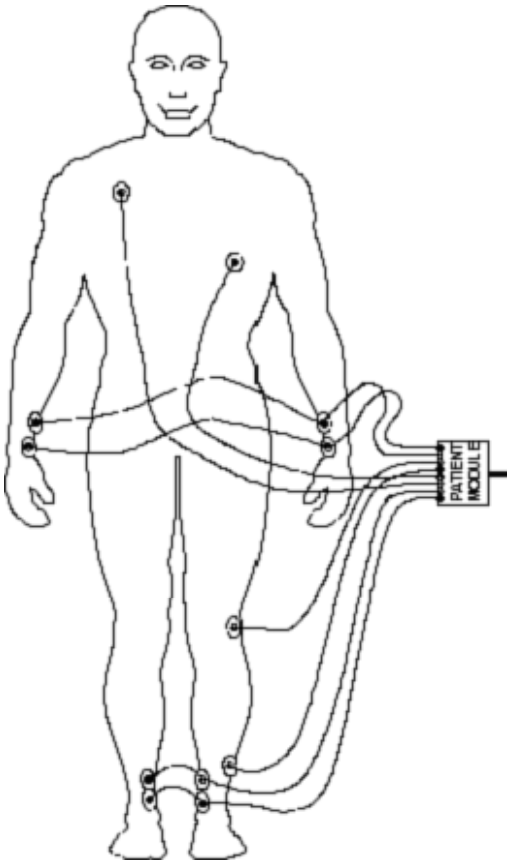

Fig. 4. The current electrodes in whole-body impedance cardiography are placed on wrists and ankles. Voltage electrodes are proximally of the current electrodes. *With the kind permission from JR Medical.*

The SV equation by Tsoglin and Frinerm (Miller et al. 1993, Tsoglin and Frinerman 1995) has been shown to agree well with thermodilution in patients with acute heart failure, congestive heart failure, and coronary artery disease, as well as during coronary artery bypass operation (Cotter et al. 2004, Moshkovitz et al. 2004). In some of the studies, ICG_{WB} has not agreed sufficiently well with the invasive methods (Imhoff et al. 2000). However, this is at least partly explained by the electrode configuration, which does not actually represent the entire body (Kööbi et al. 2001).

Limitations of bioimpedance

Regurgitation of blood due to significant aortic valve insufficiency or intracardiac and pericardiac shunts may cause overestimation of SV. Also, aortic dilatation, aneurysm and coarctation are conditions where impedance methods have not been validated. Neither ICG_{WB} nor ICG_{TH} allow measurement during motion or if the patient is restless (Moshkovitz et al. 2004).

Bioimpedance in the assessment of pulse wave velocity

Using electrodes placed on neck, manubrium sterni and left calf, bioimpedance can be used to assess arterial PWV by measuring the time delay between simultaneously recorded flow pulses and the distance between recording sites, i.e. between the root of aorta and popliteal artery. The method has been shown to agree well with PWV measured by the ultrasound Doppler method (Kööbi et al. 2003).

2 ADRENOCEPTOR ANTAGONISTS

2.1 α-adrenoceptor blocking drugs

2.1.1 α_1-antagonists alfuzosin and tamsulosin

Prazosin was the first drug in the family of agents with piperazinyl quinazoline nucleus. Its affinity to α_1-adrenoceptors is about 1000-fold greater than to α_2-adrenoreceptors. Originally, prazosin was used as a antihypertensive agent due to its remarkable vasodilatory effect (Graham 1983), but the main indication has shifted to alleviating lower urinary tract symptoms (LUTS) associated with benign prostatic obstruction (Hedlund et al. 1983, Bouffioux and Penders 1984). Terazosin and doxazosin, other close analogs of prazosin, have been used in LUTS patients with results comparable to those with prazosin (Kaplan et al. 1995, Kaplan et al. 1997, Tsujii 2000, Wilt et al. 2002a). These classic α_1-antagonists bind to α_1-receptors not only in lower urinary tract but also in blood vessels. This frequently brings along dizziness and orthostatic hypotension, especially when given to LUTS patients with pre-existing antihypertensive medication (Djavan and Marberger 1999, Tewari and Narayan 1999, Chrischilles et al. 2001).

Alfuzosin is structurally a quinozoline, but it differs from prazosin, terazosin and doxazosin by the absence of a piperidine moiety and the presence of a diaminopropyl spacer.

Tamsulosin, (R)-5-(2-[2-(2-Ethoxyphenoxy) ethylamino] propyl)-2-methoxybenzene-

sulphonamide hydrochloride, is the first clinically used α_1-antagonists without initial indication of hypertension (Abrams et al. 1995, Kawabe 1995).

Both alfuzosin and tamsulosin are used in the treatment of LUTS suggestive of benign prostatic obstruction. This is most typically caused by benign prostatic hyperplasia (BPH). Unlike the classic α_1-antagonists, alfuzosin and tamsulosin are not reported to cause tachyphylaxis, and the treatment can be started with the maintenance dose (de Mey 1998, Djavan and Marberger 1999, Tewari and Narayan 1999, Michel et al. 2001, Dunn et al. 2002, Michel and de la Rosette 2004).

Pharmacodynamics and haemodynamic effects

The beneficial urological effects of α_1-antagonists are due to urethral relaxation via the blockade of α_1-adrenoceptors in the urethra and prostate. Alfuzosin and tamsulosin relieve lower urinary tract symptoms as effectively as prazosin, doxazosin or terazosin, but have less circulatory adverse effects (Djavan and Marberger 1999, Michel et al. 2001, Wilt et al. 2002b, Wilt et al. 2003).

The concept of α_1-antagonist uroselectivity has been elaborated to describe the ratio of beneficial urinary effects compared with cardiovascular adverse effects (Andersson 1998). The physiological and pharmacological background of the uroselectivity has been widely investigated and discussed, but there are still many open questions; e.g. the exact roles of blood-brain barrier penetration, tissue-specific affinity states of the adrenoceptors and pharmacokinetics are still unsolved (Andersson et al. 1997, Debruyne 2000, Guimaraes and Moura 2001, Roehrborn 2001, Roehrborn and Schwinn 2004). Evidence for cell-type-specific affinity is the finding that the affinity of a certain prazosin derivative was higher to the α_1-adrenoceptors in native prostatic cells compared to cloned α_1-adrenoceptors (Mackenzie et al. 2000). Thus, a tissue-specific affinity state of the same receptor genotype could be a potential modulator of drug action (Mackenzie et al. 2000, Guimaraes and Moura 2001).

Tamsulosin is the first α_1-antagonist expressing receptor-subtype selectivity: it has moderately higher affinity to α_{1A} than to α_{1B}, and intermediate affinity to α_{1D} (Michel et al. 1996). This affinity profile is claimed to be a crucial factor explaining the prostate selectivity of tamsulosin (Roehrborn and Schwinn 2004), but some authors stress the importance of the pharmacokinetic pattern of the drug (Taguchi et al. 1998, Guimaraes and Moura 2001, Hein et al. 2001) and the tissue-specific affinity state of α_1-adrenoceptors (Mackenzie et al. 2000). A possible

pharmacokinetic explanation is the finding that tamsulosin shows the lowest level of inverse agonism among the commonly used α_1-antagonists (Hein et al. 2001).

In terms of pharmacological receptor selectivity, the classic α_1-antagonists and alfuzosin are nonsubtype-selective α_1-antagonists, i.e. they block all currently recognised three α_1-subtypes to about the same extent (Langer 1999, Guimaraes and Moura 2001, Roehrborn and Schwinn 2004). The relatively mild cardiovascular effects and functional uroselectivity by alfuzosin is explained entirely by its distribution in the body (Roehrborn 2001): in isolated human tissues, alfuzosin displays highest selectivity ratio for the prostate over the vascular tissue (ratio 544) compared to tamsulosin (90), doxazosin (51) and terazosin (19) (Eckert et al. 1999).

The decreases in SAP and DAP by alfuzosin have been similar or comparable to each other with all the three marketed formulations: in most studies, the BP changes in supine and standing positions have been non-significant, the maximal average reductions being 5 mmHg (Djavan and Marberger 1999, Michel et al. 2001, Roehrborn 2001, van Kerrebroec et al. 2002, Roehrborn and Schwinn 2004). The influence of alfuzosin on supine HR has been modest, even though tachycardia and palpitation have been reported as adverse drug reactions (Sanchez-Chapado et al. 2000, Michel et al. 2001, Roehrborn 2001, Roehrborn and Schwinn 2004).

The effect of tamsulosin 0.4 mg once daily on SAP and DAP has been either non-significant or clinically irrelevant, the order of magnitude being a drop of 0-2 mmHg (Chapple et al. 1996, de Mey 1998, Djavan and Marberger 1999, Michel et al. 2001, Roehrborn and Schwinn 2004). HR has been found either to remain on the pre-medication level or to show a small drop, but tachycardia and palpitation are possible adverse effects (Djavan and Marberger 1999, Lee 2000, Kloner et al. 2004, Roehrborn and Schwinn 2004).

Effects of tamsulosin and terazosin in diurnal and nocturnal orthostatic testing have been studied with 50 elderly normotensive male volunteers. In the tamsulosin group, there was only one subject who experienced symptomatic orthostatic reaction at least once out of the four performed orthostatic tests; seven subjects showed a reduction of at least 20 mmHg in SAP during orthostasis. The volunteers in the terazosin group experienced significantly more frequently both symptomatic and asymptomatic reactions (de Mey et al. 1998).

The knowledge on the effects of alfuzosin and tamsulosin on other cardiovascular parameters

except HR, SAP and DAP is scarce or lacking. As to alfuzosin, there are no reports on its effects on CO, SV or SVR, or corresponding indices. Responses on PWV or arterial compliance have not been reported, either, and the effects of alfuzosin on the head-up tilt responses are unknown.

The effects of tamsulosin on CO and SV have been measured in a study where the degree of α_1-blockade was assessed (Schafers et al. 1999). In that study, however, the subjects were pretreated with α-agonist phenylephrine infusion.

Pharmacokinetics

Oral bioavailability of alfuzosin is about 65 %. Maximal plasma concentration (C_{max}) is reached in 1-9 hours depending on the preparation used. In plasma, 90 % of alfuzosin is bound to proteins with elimination half-life ($T_{½}$) of 9 hours. In healthy elderly subjects, C_{max} and area under drug concentration in plasma versus time curve (AUC) do not differ significantly from those in younger adults. About 10 % of alfuzosin is excreted unchanged in urine, while the rest is metabolised in the liver by cytochrome P450 (CYP) enzyme 3A4, and the inactive metabolites are mostly excreted in feces.

Tamsulosin is totally absorbed from the small intestine. Due to the low level of first-pass metabolism, the bioavailability is close to 100 %. Tamsulosin in plasma is almost entirely bound to proteins. With the depot capsules in European markets, the C_{max} is reached in 6 hours, and the $T_{½}$ is 10-13 hours. Distribution volume is small, about 0.2 l/kg. About 9 % of tamsulosin is excreted unchanged in urine, while the majority is metabolised in the liver by CYP3A4 with the metabolites excreted in urine.

2.1.2 α_2-antagonists

Blockade of α_2-adrenoceptors with antagonists such as yohimbine increases sympathetic outflow from the CNS and inhibits the negative feedback on the release of NA from the nerve endings. This leads to activation of α_1-, β_1- and β_2-adrenoceptors with a consequent increase in HR and BP (Goldberg et al. 1983). However, α_2-adrenoceptor antagonists are not yet in frequent clinical use.

2.2 β-adrenoceptor blocking drugs

2.2.1 Non-selective (β_1 and β_2) β-antagonists

2.2.1.1 Propranolol

The β-adrenoceptor antagonists have drawn tremendous clinical attention because of their efficacy in the treatment of hypertension,

congestive heart failure, certain arrythmias and ischemic heart disease. All clinically used β-antagonists share the common feature of being competitive antagonists at β-adrenoceptors, but they can be distinguished e.g. by the following properties: relative affinity to β_1- and β_2-receptors, affinity to α_1-receptors, intrinsic sympathomimetic activity (ISA), local anaesthetic membrane-stabilising activity, lipid solubility, vasodilatory effects and pharmacokinetic properties (Haeusler 1990, Mickelson et al. 1990, Hoffman 2001a).

Propranolol, (R/S)-1-[(1-Methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol, was the first clinically used β-antagonist (Black and Stephenson 1962) and it remains the model β-antagonist with which all the other β-blocking drugs are compared. Propranolol is a racemic mixture of R- and S-enantiomers; essentially only S-enantiomer contrives β-blocking. The usage of propranolol for the treatment of angina pectoris or hypertension has substantially decreased, but it is used in the treatment of palpitations, several types of arrhythmia, benign positional tremor, hyperthyreosis and as a preventive against migraine.

Pharmacodynamics and haemodynamic effects

Propranolol has equal affinity to β_1- and β_2-receptors. Propranolol has no ISA effect. It has membrane-stabilising activity that does not bear clinical consequencies in oral dose regimes, but propranolol is not used as ophthalmic agent due to corneal anaesthesia (Lama 2002). Propranolol shows direct myocardial depressant effects.

Haemodynamic effects of propranolol are suppression of HR, CO, SV, BP and myocardial contractility especially in the state of elevated sympathetic tonus (Svendsen et al. 1979, Kendall and Beeley 1983, Dahlof 1990, Haeusler 1990, Asmar et al. 1991, Kähönen et al. 1998, Remme 1998, Hoffman 2001b, Kähönen et al. 2002). Followed by the reduction of HR, myocardial oxygen expenditure is reduced, too (Yamakawa et al. 1996). Short-term administration of propranolol increases SVR due to compensatory reflexes and blockade of vascular β_2-receptors (Kähönen et al. 2002). In the longer run, SVR returns to the initial level in normotensive individuals (Mimran and Ducailar 1988) and even decreases in hypertensive ones (Man in't Veld et al. 1988). Propranolol decreases PWV (Kähönen et al. 2002). All the β-adrenoceptor antagonists depress sinoatrial pacemaker, decrease spontaneous rate of depolarisation of ectopic pacemakers, slow conduction in the atria and in the AV node, and increase the refractory period of the AV node (Hoffman 2001b).

Pharmacokinetics

Propranolol is almost completely absorbed after oral administration, but the biologic availability is only around 25 % due to first-pass metabolism. There is no correlation between dose or plasma level and therapeutic effect (Shand et al. 1970, Zacest and Koch-Weser 1972). About 90 % of propranolol in plasma is bound to proteins. The volume of distribution is 4 l/kg, and C_{max} is achieved in 80-120 min; $T_{½}$ is 3-5 h (Power et al. 1995, Karol et al. 2000).

Propranolol is oxidatively metabolised through CYP1A6, CYP2D6 and CYP2C19, and oxidation is followed by conjugation reactions. Polymorphism in CYP2D6 does not seem to have clinical implications (Sowinski and Burlew 1997, Johnson et al. 2000, Karol et al. 2000, Huang et al. 2003).

2.2.1.2 Timolol

Timolol maleate, (S)-1-[(1,1-dimethylethyl) amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (Z)-2-butenedioate (1:1) (salt), is a non-selective β-antagonist originally only used in oral dose regimes for the treatment of cardiovascular diseases (Ulrych et al. 1972, Scriabine et al. 1973, Achong et al. 1975, 1976), but the current usage is mainly as ocular preparations. The efficacy of ophthalmic timolol in the reduction of intraocular pressure has been proven in a great number of studies (Heel et al. 1979, LeBlanc et al. 1985, Uusitalo and Palkama 1989), and timolol is currently used worldwide as a standard medication against glaucoma and ocular hypertension.

Pharmacodynamics and haemodynamic effects of ophthalmic timolol

Timolol does not have ISA, direct myocardiac depressant effect or membrane-stabilising activity. On weight bases, it is a 8-16 more potent β-antagonist than propranolol (Neufeld et al. 1983, Brooks and Gillies 1992).

Topically administered ophthalmic timolol reduces the intraocular pressure (IOP) by blocking the sympathetic nerve endings in the ciliary epithelium leading to a decrease in the production of aqueous humour (Neufeld et al. 1983).

In spite of the topical administration, ophthalmic timolol causes systemic adrenergic β-blocking due to absorption from the eye through the conjunctival epithelium, nasal mucosa, lacrimal channels and gastrointestinal tract into the systemic circulation (Doyle et al. 1984, Leier et al. 1986, Brazier and Smith 1988, Vuori et al. 1993, Dickstein and Aarsland 1996, Diggory et al. 1998, Korte et al. 2002, Nino et al. 2002). Gel formulations of

timolol have been developed with the goal to reduce systemic absorption and, correspondingly, systemic adverse effects, while maintaining the equivalent therapeutic activity compared with conventional ocular formulations, such as aqueous solution.

The absorbed fraction of ophthalmic timolol has been shown to induce various systemic adrenergic β-blocking effects in circulatory and pulmonary systems: Resting HR has decreased from negligible to 16 bpm with 0.25-0.5 % aqueous timolol (Doyle et al. 1984, Leier et al. 1986, Brazier and Smith 1988, Dickstein et al. 1988, Dickstein and Aarsland 1996, Umetsuki et al. 1997, Stewart et al. 1999, Stewart et al. 2002a, Stewart et al. 2002b) and from negligible to 8.5 bpm with 0.1-0.5 % gel formulations (Dickstein and Aarsland 1996, Stewart et al. 1999, Nino et al. 2002). The average reductions in the peak HR during exercise have been 7-24 bpm (aqueous) (Doyle et al. 1984, Leier et al. 1986, Brazier and Smith 1988, Dickstein et al. 1988, Dickstein and Aarsland 1996, Umetsuki et al. 1997, Stewart et al. 2002a, Stewart et al. 2002b) and 11-19 bpm (gel) (Dickstein and Aarsland 1996, Umetsuki et al. 1997, Stewart et al. 1999).

Neither aqueous nor gel formulations of timolol have exerted any noteworthy effects on SAP and DAP (Brazier and Smith 1988, Diggory et al. 1998, Korte et al. 2002, Nino et al. 2002, Stewart et al. 2002a, Stewart et al. 2002b), with the exception of a slight reduction in the nocturnal DAP in patients on 0.5 % aqueous eyedrops (Hayreh et al. 1999). As to ECG parameters, timolol has been shown to slightly shorten the corrected QT interval (QTc) and to slow atrio-ventricular conduction (PR interval) during tilt-test (Nino et al. 2002). Following instillation of 1 mg timolol maleate in each eye, HR and stroke index (SI) have decreased, and systemic vascular resistance index (SVRI) has increased significantly (Takahashi et al. 1989). However, the contemporary timolol drops only contain approximately 150 µg timolol in aqueous and 30 µg in gel formulations (manufacturers' documents), and effects of these small drops on SV, CO, SVR or the respective indices have not been reported. Also, the effect of timolol on arterial PWV is unknown. As a result of β_2-blockade, the average reductions in the pulmonary parameters peak expiratory flow (PEF) have varied from 0 to 7 %, and in forced expiratory flow in one second (FEV1) between 0 and 3 % of the original values (Dickstein et al. 1988, Diggory et al. 1998, Waldock et al. 2000, Korte et al. 2002). Even deaths have been reported due to bronchoconstriction (Fraunfelder and Barker 1984).

In the β-receptor blocking and binding activity studies of Kaila and colleagues (1991, 1993), the lowest timolol plasma level with changes in HR is around 0.20 ng/ml.

Pharmacokinetics

The bioavailability of ophthalmic timolol in healthy young volunteers has been 78 % in one study (Korte et al. 2002), but the corresponding value in elderly glaucoma patients is not known. As a comparison, the bioavailability after oral administration has only been 58-61 % (Ishizaki and Tawara 1978, El-Rashidy 1981, Wilson et al. 1982). About 60 % of timolol in plasma is protein-bound. $T_{½}$ is about 8 hours in eye tissues and 4-5 hours in plasma (Wilson et al. 1982, Vuori and Kaila 1995), and the volume of distribution is above 2 l/kg. Timolol is metabolised by CYP2D6 (see 2.4 for details) into inactive metabolites that are excreted via the kidney.

2.2.2 β_1-selective antagonists

Bisoprolol, metoprolol, atenolol and acebutolol are among the β-adrenoceptor antagonists with an essentially higher affinity to β_1- than to β_2-adrenoceptors. They are sometimes also called cardioselective, since β_1-adrenoceptor is the main β-receptor subtype in the heart. These drugs are mostly used in the treatment of hypertension and angina pectoris (Hoffman 2001b), but they were not studied in the present trials.

2.3 Combined α_1- and β-adrenoceptor blocking drugs

2.3.1 Carvedilol

Carvedilol, (R/S)-1-[9H-carbazol-(4)-yloxy]-3- [[2- (2-methoxyphenoxy]ethylamino]- 2-propranolol), is a nonsubtype-selective β-blocking drug that also antagonises α_1-receptors (Bristow 1997).

Carvedilol is used to treat angina pectoris, hypertension and chronic heart failure. Carvedilol possesses potentially relevant ancillary properties (e.g. antioxidant, antiproliferative and antiendothelin actions) that may confer an enhanced ability to protect the heart and vessels from the consequences of sympathetic hyperactivation (McTavish et al. 1993, Krum et al. 1995, Olsen et al. 1995, Bristow et al. 1996, Cohn et al. 1997, Dunn et al. 1997).

Pharmacodynamics and haemodynamic effects

Carvedilol does not have ISA or membrane-stabilising influence, and it does not cause the upregulation of the β_1-adrenoreceptors. Long-term oral administration of carvedilol (25-50 mg daily) has been shown to produce several

haemodynamic effects: a significant decrease in HR (Bristow et al. 1996, Cohn et al. 1997) and varied effects in lowering BP (Krum et al. 1995, Olsen et al. 1995), a significant reduction of SVR (Krum et al. 1995, Olsen et al. 1995), and a significant increase in SI that is probably compensatory due to the lowering of HR (Yin and Ting 1992, Slama et al. 1995). CO does not drop as clearly as with propranolol due to the vasodilatory effect of carvedilol (Hoffman 2001b). Also, carvedilol recovers PWV and arterial compliance in patients with hypertension (Van Bortel et al. 1995, Bristow 1997).

The α_1-adrenoreceptor-mediated vasodilator action of carvedilol counterbalances the negative inotropic effect from β-blockade, and thus can improve the acute tolerability of the drug by avoiding the drop of cardiac output (Morgan 1994).

Pharmacokinetics

Carvedilol is a racemic mixture of the enantiomers R(+)- and S(-)-carvedilol. Both forms block α_1-adrenoceptors, but only the S(-)-enantiomer blocks β-adrenoceptors (Morgan 1994). Carvedilol is extensively absorbed after oral administration (von Mollendorff et al. 1987), but the biologic availability is only approximately 25 % after first-pass metabolism (Morgan 1994). C_{max} is achieved within 1-2 h. Volume of distribution is about 1.5-2 l/kg, and 98-99 % of carvedilol is protein-bound in plasma (Feuerstein and Ruffolo 1995, Yue et al. 1995, Oldham and Clarke 1997, Tadolini and Franconi 1998).

Carvedilol is metabolised in the liver, predominantly by the cytochromes CYP2D6 and CYP2C9, into three active phenolic hydroxylated derivatives, which possess clear β-adrenoreceptor antagonist activity and a weak vasodilator activity. The derivatives are excreted via the bile into the feces as glucuronide and sulfate conjugates (Morgan 1994, Frishman 1998). Only 1-2 % of carvedilol is excreted unchanged through the kidneys (Kindermann et al. 2004).

After oral administration, the $T_{½}$ of carvedilol ranges 7-10 h. In poor CYP2D6 metabolisers, the plasma level rises 2-3 times higher than in extensive metabolisers. Carvedilol inhibits the catecholamine response of the human heart beyond the plasma elimination, probably because of its binding to an allosteric site of β-adrenoceptors.

2.3.2 Labetalol

Labetalol is another drug that competitively antagonises both α_1- and β-adrenoceptors. The clinically used formulations of labetalol hydrochloride contain four different stereoisomers, all with different relative

activities on the α_1-, β_1- and β_2-adrenoceptors (Gold et al. 1982). Labetalol is indicated for hypertension and hypertensive emergencies.

2.4 Drug elimination via cytochrome P450

Drug transformation reactions are classified as either phase I or phase II reactions. Diverse arrays of oxidative phase I reactions include e.g. hydroxylation, dealkylation and dehalogenation. In all these reactions, an oxygen atom is inserted into the substrate and the compound becomes more polar and soluble. Typically, a phase I reaction leads to the loss of pharmacological activity, even though there are examples of continuation or even enhancement of activity. If these metabolites are not promptly excreted into the urine, the metabolites are subject to a phase II reaction. These conjugation reactions result in the formation of a covalent bond between the parent compound or phase I metabolite and endogenously derived glucuronic acid, sulfate, glutathione, amino acids or acetate. This conjugation creates highly polarised molecules that are most often inactive and are excreted into the urine or feces (Wilkinson 2001, Hardikar and Suchy 2003).

The phase I reactions are mostly catalysed by cytochrome P450 (CYP) enzymes in liver and intestines. In humans, there are 57 known different active genes encoding CYP enzymes (Nelson et al. 2004). Even though many of these enzymes are capable of metabolising drugs, the great majority of CYP-mediated drug metabolism in catalysed by the CYP subfamilies CYP3A (45-60 % of the phase I reactions), CYP2D6 (20-25 %) and CYP2C (19-20 %).

The impact of genetic variation differs among the major CYP enzymes. The most important of the P450 enzymes, CYP3A, does not seem to be noteworthly affected by gene mutations. At the other extreme, CYP2D6, an important metaboliser especially in cardiovascular pharmacology, is highly polymorphic with about 100 phenotypes (Table 3) (Evans and Relling 2004, Siest et al. 2004). CYP2D6 metabolises carvedilol, labetalol, metoprolol, pindolol, propranolol, timolol, clonidine, debrisoquine, amiodarone, flecainide, lidocaine, nimodipine, indapamide etc. Individuals with certain combinations of the *CYP2D6* alleles can be classified into different categories according to the total metabolising activity of the combination. The most typical classification comprises four stages: If the level of CYP2D6 activity is very low or nil, the individuals are referred to as poor metabolisers, while individuals with a fully functional enzyme are called extensive metabolisers. Those with CYP2D6 activity between poor and extensive are intermediate, and the individuals with duplicated or

multiplicated functional *CYP2D6* genes are ultra-rapid metabolisers. Other classification schemes consist of 3-7 categories (Ingelman-Sundberg 2001, Phillips et al. 2001, Pirmohamed and Park 2001, Ma et al. 2002, Pirmohamed and Park 2003, Ingelman-Sundberg 2004b, a).

The polymorphism in CYP enzymes is of great importance in adverse effects, since poor metabolisers are at the risk of having higher drug plasma levels. On the contrary, the drug plasma levels in ultra-rapid metabolisers are often below therapeutic ranges (Ingelman-Sundberg 2004b). Due to this great variation in plasma levels, a new compound in drug development may even be discarded if a polymorphic CYP enzyme is largely involved in the metabolism (Pirmohamed and Park 2003).

Table 3. Most common CYP2D6 mutations: mutation frequencies in Caucasians and consequences in enzyme activity.

Allele code	Frequency in Caucasians (%)	Mutation description	Enzyme activity
*1	36.4	wild-type	normal
*2	32.4	1661G>C, 4180G>C	slightly decreased
*3	2.0	2549A>del, frameshift	none
*4	20.7	1846G>A, splicing defect	none
*5	2.0	gene deletion	none
*6	0.9	1707T>del, frameshift	none
*9	1.8	2613-2615delAGA	decreased
*10	1.5	100C>T	decreased
xN	2.0	Duplication or multiplication	increased

Part II: CLINICAL STUDIES

3 AIMS

The purpose of the study was to investigate the detailed haemodynamic effects of α_1- and β-adrenoceptor blocking drugs. More specifically, the substudies were designed and conducted for the following objectives:

1. To evaluate whether finger-pressure derived pulse contour method is appropriate in estimating haemodynamic responses to passive orthostasis.
2. To test the hypothesis that the α_1-adrenoceptor blocking drugs alfuzosin and tamsulosin do not produce marked haemodynamic effects in a head-up tilt.
3. To compare the haemodynamic influences of tamsulosin with the effects of the non-selective β-antagonist propranolol and the combined α_1- and β-antagonist carvedilol.
4. To test the hypothesis that there is a correlation between low plasma concentrations of the non-selective β-adrenergic antagonist timolol and cardiovascular parameters.
5. To evaluate whether variation in the genotypes of *ADRB1* and *GNAS1* modulate HR and BP responses to timolol, and to assess the contribution of genetic polymorphism of *CYP2D6* to the pharmacokinetics of ophthalmic timolol.

4 METHODS

4.1 Participants

4.1.1 Patients (I, IV, V)

In Trial I, the participant pool consisted of 230 physically active patients tested on a tilt table for collapse episodes or dizziness. Twenty-five of them had experienced presyncope/syncope during tilt-testing (tilt+), and 205 had not (tilt-). Twenty participants were chosen from each group; 21 males and 19 females, mean age 41 ± 19 yr. None of the patients had intracardiac shunts or severe valvular lesions.

Twenty-five glaucoma or ocular hypertension patients (11 females, 14 males, mean age 57 ± 10 yr) were enrolled in Study IV. The nineteen patients (9 females, 10 males, mean age 57 ± 10 yr) in Study V were a subgroup of the patients in Study IV. Patients with bronchial asthma, chronic obstructive pulmonary disease, inadequately compensated heart failure, bradycardia (HR<50), II or III degree AV-block, myocardial infarction within the last six months, or severe liver or renal disease were excluded. Furthermore, ocular contraindications were IOP>35 mmHg, recent intraocular or laser surgery, severely affected vision or visual fields, and abnormalities in ocular anatomy.

4.1.2 Healthy volunteers (II, III, V)

The inclusion and exclusion criteria for Studies II, III and V were equal: the volunteers were normotensive and physically active without history of cardiovascular or pulmonary disease. Pregnancy was an exclusion criterium.

In Study II, twenty-seven volunteers (15 females, 12 males, mean age 24 ± 2 yr) were randomised into four medication groups.

In Study III, thirty-one volunteers (17 females, 14 males, mean age 24 ± 2 yr) were randomised into three medication groups. 13 of these had already been included in Study II (see 4.2.2.1).

In Study V, eighteen volunteers (12 females, 6 males, mean age 23 ± 2 yr) were randomised into two treatment sequencies.

4.2 Study designs and measurement techniques

All the trial designs were approved by the Ethical Committee of the Hospital District of Pirkanmaa. In addition, the protocol for Study IV was approved by the Ethical Committee of the Hospital District of Uppsala, Sweden. A written informed consent was obtained from each subject prior to the study initiation, as necessitated in the Declaration of Helsinki.

4.2.1 Study I

4.2.1.1 Study design

The data acquisition was started after haemodynamics had stabilised in supine position on the tilt table with foot support in a silent laboratory. Thereafter, the table was kept horizontally for another 5 min before tilting the table up to 60° (2.3°/s) for 20 minutes (tilt-patients) before returning (2.3°/s) the table to the supine position. The tilt was cancelled earlier if presyncope or syncope developed (tilt+ patients).

CO and SV were measured beat-to-beat. The time periods of 30-60 s taken into analysis were just before lifting up the table, within five minutes after the lift-up, and one minute before set-down. Altogether, there were 120 measurement periods.

4.2.1.2 Pulse contour method

BP was measured continuously with Finapres (Ohmeda, Boulder, CO) and digitised by CircMon at a sampling rate of 200 Hz. The CircMon-generated BP file was down-sampled to 100 Hz using Matlab software (version 4.21c.1, MathWorks Inc., Natick, Massachusetts, USA). Beatfast software (TNO Biomedical Instrumentation Research Unit, Amsterdam, The Netherlands) was used to calculate beat-to-beat SV and derived haemodynamic parameters based on a non-linear, time-variable three-element Model flow model (Wesseling et al. 1993) and the individual characteristics of each patient: pulse contour, gender, age, height and weight. Average readings of SV and CO for the time

periods described above were calculated after discarding the values marked as poor by Beatfast.

4.2.1.3 Impedance cardiography

SV was measured by using ICG_{WB} (CircMon^{TM}, Model B202, JR Medical, Tallinn, Estonia). Disposable ECG electrodes (Blue sensor type R-00-S, Medicotest A/S, Ølstykke, Denmark) were used. A pair of electrically connected current electrodes was placed on extremities, just proximal to wrists and ankles. Voltage electrodes were placed proximal to the current electrodes with a 5 cm distance between the centers of the electrodes.

4.2.2 Studies II and III

4.2.2.1 Study design

The drugs in Study II (Fig. 5a) were non-selective β-antagonist propranolol (tabl. Propral 40 mg, Orion, Finland), non-selective α_1- and β-antagonist carvedilol (tabl. Cardiol 12.5 mg and 25 mg, Roche, Switzerland), α_1-antagonist tamsulosin and placebo (tabl./caps. lactos.). In the double-dummy design, the subjects took either placebo or the drug twice a day with 200 ml of water for three days:

1) placebo 8 am + 8 pm (n=7),
2) propranolol 40 mg 8 am + placebo 8 pm (n=7),
3) placebo 8 am + carvedilol 12.5 mg 8 pm for two days, and carvedilol 25 mg 8 am + placebo 8 pm since the third day (n=7),
4) tamsulosin 0.4 mg, 8 am + placebo 8 pm (n=6).

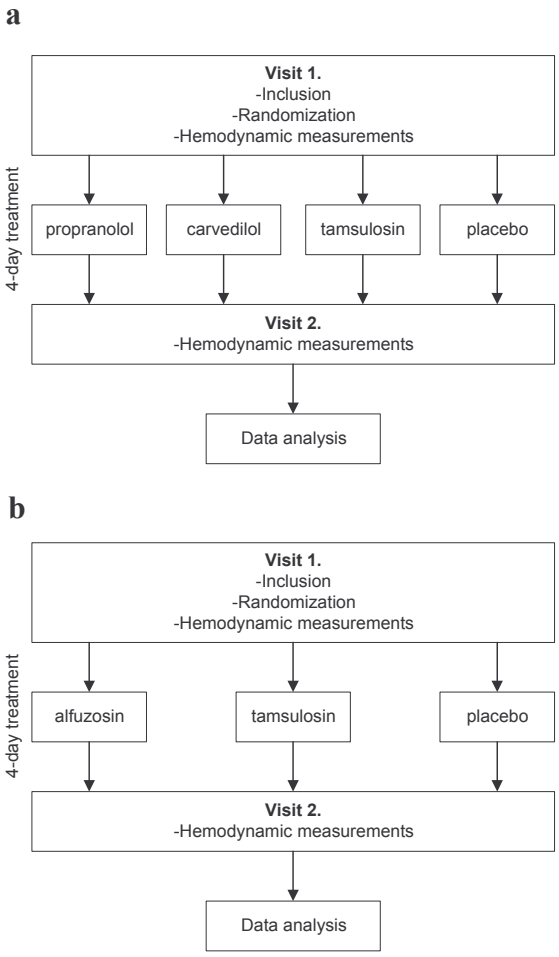

Fig. 5. Flows of the a) Study II and b) Study III.

Study II was a preliminary trial to estimate whether commonly used α_1-blocking drugs have measurable haemodynamic effects. The initial data gathering also included seven patients with α_1-antagonist alfuzosin (tabl.

Xatral 5 mg, Sanofi-Synthelabo, France), even though not presented with Study II results. After the interesting results with tamsulosin, the trial population was expanded to Study III (Fig. 5b), where the drugs were α_1-antagonist alfuzosin (tabl. Xatral 5 mg, Sanofi-Synthelabo, France), tamsulosin (caps. Omnic 0.4 mg, Yamanouchi Pharmaceutical, Japan), and placebo (tabl./caps. lactos.). The three-day regimen for the different treatment groups was as follows:

1) placebo 8 am + 8 pm (n=11),
2) tamsulosin 0.4 mg 8 am + placebo 8 pm (n=10),
3) alfuzosin 5 mg 8 am + 8 pm (n=10).

In the morning (8 am) of the fourth treatment day the subjects took their last drug dose 3.5-4 h before the beginning of the supine pre-tilt recording of cardiovascular variables. No other drugs or alcoholic beverages were allowed during the drug treatment, and the subjects fasted overnight until the study parameters were recorded.

The studies were carried out in a double-blind, randomised and placebo-controlled fashion. Before the drug treatment was started, the following continuous baseline recordings were taken after the subject had been in supine position for 20-30 minutes: beat-to-beat SAP, DAP, MAP, SV, CO, PWV, ECG, SVR and HR. The tilt-table was raised from a horizontal position to an upright 60° position for 8 minutes, after which it was let down again. As the subjects had rested on the horizontal tilt table for further 6 minutes, the post-tilt parameters were recorded. On the fourth treatment day, at 3.5-4 hours after the ingestion of the last drug dose, i.e. when the drugs are known to have effective concentrations in serum (manufacturer's documents, Morgan 1994, Power et al. 1995, Matsushima et al. 1998), the haemodynamic parameters were measured again prior to, during and after the tilt provocation as described above. During the tilt test, the subject was not allowed to speak, and he/she was allowed to breathe without controlled rate.

4.2.2.2 Measuring technique for haemodynamics

BP was recorded by finger blood-pressure measurement method (Finapres™ 2300, series FAX, Ohmeda, Louisville, CO, USA) and controlled by an experienced nurse with a brachial cuff according to the Riva-Rocci method.

SV was measured with ICG_{WB} (CircMon™, Model B202, JR Medical, Tallinn, Estonia). SVR was calculated from CO and MAP as SVR = MAP/COx80. SV, CO and SVR were transformed to the respective indices by

relating them to the body surface area (BSA): stroke index (SI=SV/BSA), cardiac index (CI=CO/BSA) and systemic vascular resistance index (SVRI=SVR x BSA). For PWV measurement, additional electrodes on neck, manubrium sterni and left calf were used.

4.2.3 Study IV

4.2.3.1 Study design

The study was carried out in two centres using a randomised, double-masked, two-period crossover design. Each subject was evaluated during five visits, four of which included head-up tilt test, ECG, spirometry, exercise test, eye examination including measurement of IOP, and blood sampling from the antecubital vein for the determination of haematocrit and plasma timolol concentration.

During the screening visit (1st visit, Fig. 6), the subjects were randomised to one of the two treatment sequences. Thereafter, the patients started a 23-day run-in glaucoma medication (one drop of dorzolamide three times daily) period followed by a five-day wash-out period without IOP lowering treatment before the visit for the baseline measurements (2nd visit). During the first four-week treatment period, half of the patients were treated with timolol 0.1 % hydrogel (Timosan, Santen Oy, Tampere, Finland) once daily, and with 0.9 % NaCl solution twice daily. The other half was treated with aqueous timolol 0.5 % (Oftan Timolol, Santen Oy, Tampere, Finland) twice daily and hydrogel vehicle once daily. Thereafter, the subjects were re-evaluated (3rd visit). A run-in medication period of 23 days followed, and it was succeeded by a wash-out period of five days. After the second baseline measurements (4th visit), the groups switched study medications for the second four-week treatment periods that were followed by one more set of physiologic measurements (5th visit).

4.2.3.2 Head-up tilt test protocol

The subjects were asked not to use caffeine-containing drinks (coffee, tea, cola drinks) and not to smoke in the morning before each study session, but they were advised to have a light breakfast.

The tilt test with continuous BP, ECG and ICG_{WB} monitoring was started 40 min after the drug delivery and after 10 min in supine position to stabilise haemodynamics. The first ten minutes the subject was still kept in supine position, followed by a transition to head-up tilt position (60º) on the tilt-table for five minutes. At 15 minutes from the start, the subject was again tilted down to supine position for another five minutes. Hence, the total duration of data acquisition was 20 minutes.

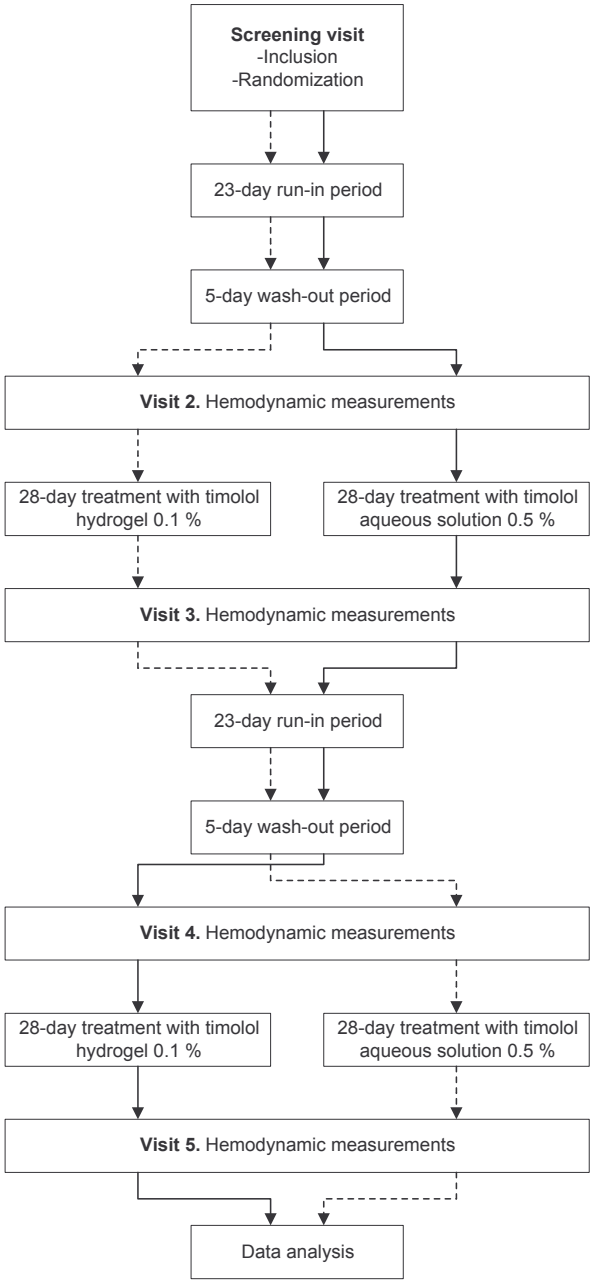

Fig. 6. Flow of Study IV with two preparations of ophthalmic timolol. The two types of lines represent the two patient groups with opposed treatment orders.

4.2.3.3 Spirometry, ECG and exercise test protocols

Forced expiratory volume in one second (FEV_1) was measured in a standard spirometry test about 60 minutes after the instillation of the drug.

Between the spirometry and exercise test the subject lied down in supine position for 10 minutes for registration of resting ECG. The maximal exercise test on a bicycle ergometer with electrical brakes was started approximately 90 minutes after the drug delivery. During the exercise test a 12-lead ECG (Mason-Likar) was measured every minute and BP every third minute (the third, the sixth and the ninth minute).

4.2.3.4 ECG analysis and cardiac autonomic measurements

The ECG signal was analysed with WinCPRS software (Absolute Aliens Oy, Turku, Finland) (Cooke et al. 1999). QRS detection algorithm modified from Engelse and Zeelenberg (1983) was used to define R peaks of QRS complexes with an accuracy of less than 2 ms and hence for obtaining instantaneous R-R intervals (RRI). The interval from the start of Q wave to the R peak was interactively defined, followed by software-based T wave apex and T wave end detection. Isoelectric line was defined by joining

the points 300 ms before the consecutive R peaks in the ECG signal. The end of T wave was defined as the crossing point of a tangent fitted to the descending part of each T wave and the isoelectric line. QT interval was measured as absolute time interval and as corrected QT interval (QTc) with the method of Hodges (1983), since in this study the HR changes between supine and head-up position were significant. In addition, P waves were detected as their apex to obtain PR interval, a measure of A-V conduction. For technical reasons, atrio-ventricular conduction time (PR interval) was defined as an interval between the apex of P wave and the R peak of the QRS complex, not as an interval from the start of P wave to the start of the Q wave of the QRS complex.

The mean ± SD of the RRI, HR, QTc and PR interval were calculated from a user-defined 240 s (4 minutes) period free from ectopic beats both in supine and in head-up tilted position. The square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals (RMSSD) was also calculated during the same periods. The first 30 s in head-up position was always excluded from the analysis because of nonstationarity and unstable haemodynamics after tilting up. In addition to the evaluation of the beat-to-beat values of RRI in time domain, spectral estimation of RRI variability in frequency domain was obtained from the four-minute stationary regions in the supine and in the head-up tilted position, using the published recommendations for HR variability (Task Force 1996). The spectral power of the R-R interval was calculated by fast Fourier transformation (Cooke et al. 1999). The time series were linearly interpolated, and resampled at 5 Hz. Then they were passed through a low-pass impulse response filter with a cut-off frequency of 0.50 Hz, linearly detrended, Hanning-filtered and fast Fourier-transformed to estimate power distribution (Press et al. 1989). Total power (variance) was divided by integration into three frequency bands: very low frequency band (VLF) at 0.004 Hz - 0.04 Hz, low frequency band (LF) at 0.04 Hz - 0.15 Hz and high frequency band (HF) at 0.15 Hz - 0.40 Hz. The integrated powers in the low frequency (LF) and high frequency (HF) bands were expressed in normalised units (nu). In addition, the ratio LF/HF was calculated.

4.2.3.5 Plasma timolol concentration

Venous blood samples of 5 ml were taken just before and 120 min after administration of the study drops. Timolol concentrations in plasma were analysed by using a sensitive radioreceptor assay (Kaila et al. 1993). The sensitivity of the assay is 0.02 ng/ml, with intra- and inter-assay variation of less than 10 %.

4.2.4 Study V

4.2.4.1 Study design

The glaucoma patients in this trial represented a subgroup of the patients of Study IV, and hence the study flow was equal for them. The healthy volunteers had a similar study course with three exceptions: They did not take any drugs during the run-in glaucoma medication phases, and they applied timolol for only two instead of four weeks in each treatment period. Furthermore, the day after each visit for haemodynamic measurements, the plasma concentration of timolol was determined at 5, 15, 30 and 45 minutes, and 1, 1.5, 2, 4, 8 and 12 hours after instillation of medication.

4.2.4.2 DNA extraction and genotyping of ADRB1 and GNAS1

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit and BioRobot M48 Workstation according to the manufacturer's instructions (Qiagen Inc., Hilden, Germany). DNA samples were genotyped by employing the 5' nuclease assay and fluorogenic allele-specific TaqMan MGB probes (Livak 1999) using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences of primers and probes used in the PCR were deduced from published sequences deposited in the GenBank and Celera databases and synthesised in conjugation with Applied Biosystems. PCR reaction containing genomic DNA, 1 × Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe was performed in 96-well plates using the standard protocol in a total volume of 25 µl. End-point fluorescence was measured and genotype calling carried out by the allelic discrimination analysis module after PCR resulting in a clear identification of three genotypes for both *ADRB1* and *GNAS1* polymorphisms.

4.2.4.3 CYP2D6 methodology

Presence of alleles *CYP2D6*3* and *CYP2D6*4* (Table 3) was assessed by use of a 5' nuclease assay. Each reaction used 5 ng of genomic DNA and was performed in duplicate using standard reaction conditions. The reagents used were Applied Biosystems (Foster City, CA, USA) Pre-Developed Assay Reagents (PDAR) PN:4312563 for the *CYP2D6*3*, and PN:4312504 for the *CYP2D6*4* (Livak 1999). Samples void of the **3* or the **4* variants were denoted as **1*.

4.3 Data handling and statistical analyses

Study I

Absolute values. Simultaneous SV and CO values estimated by pulse contour (PC) method and ICG_{WB} (SV_{PC}, CO_{PC} and SV_{ICG}, CO_{ICG}, respectively) were compared according to the recommendations by Bland and Altman (1986): The values of SV_{PC} were plotted against the corresponding values of SV_{ICG}. The differences between the paired SV values were plotted against the average of these paired SV values. Bias between the methods was defined as the mean difference between the corresponding SV values. The same analysis was also applied for CO. Limits of agreement were calculated as differences of ±2 SD of the measurement pairs. The percentages of patients in whom the discrepancy in SV values between the methods was within 10 % ($0.90 \leq SV_{PC}/SV_{ICG} \leq 1.10$), 20 %, 30 %, 40 % and 50 % were calculated. Student's paired *t*-test was used in the analysis of paired data. A p-value less than 0.05 was considered significant in all the studies I-V.

The bias between the methods and the standard deviation of bias were calculated separately for both the tilt- and the tilt+ group.

Relative values and changes in SV. Because the relative changes of SV values were of particular interest, the first SV value of each set of three measurements was considered as 100 %. Then the relative SV values in per-cents were calculated for the second and third measurements. The discrepancies in the second and third measurements were handled similarly to the absolute discrepancies: the percentages of patients in whom the discrepancy between the methods was within 10 %, 20 %, 30 %, 40 % and 50 % were calculated.

The changes in SV_{PC} were plotted against the changes in SV_{ICG}. The effect of head-up tilt was plotted for both methods.

Studies II and III

Means ± SEM are given. Changes with drugs at the baseline, i.e. before the tilt provocation, were compared with Student *t*-test for paired values supported with Bonferroni post hoc-test. Changes from the pre-treatment levels caused by the orthostasis within the study group were also tested with paired *t*-test using the Bonferroni test. Differences between the drug groups were evaluated with repeated ANOVA, supported by the Bonferroni test when carrying pair-wise comparisons between the drug groups (Figs. 9-10).

Study IV

Means ± SD are given. The changes in haemodynamic and pulmonary parameters were calculated as a difference between the measurements during timolol treatment and at the baseline. The data on both aqueous and gel

formulations of timolol were combined. Since timolol concentration values were not normally distributed, Spearman instead of Pearson correlation coefficients with significance levels were calculated for the relationship between the plasma concentration of timolol and the changes measured in circulatory/pulmonary parameters. The scatterplots of the timolol concentration and a circulatory/pulmonary parameter include the regression line with 95 % confidence interval limits.

Study V

CYP2D6-analyses. The *CYP2D6* allele **1* was deemed functional and alleles **3*, **4*, **5* nonfunctional. As a measure of total CYP2D6 activity, the participants were classified into three categories according to the number of functional *CYP2D6* alleles: the individuals with zero, one or two functional alleles are referred to as poor (PM), intermediate (IM) or extensive (EM) metabolisers, respectively (Kirchheiner et al. 2004). None of the participants carried duplicated or multiplicated functional *CYP2D6* alleles.

For the healthy volunteers, the pharmacokinetic parameters C_{max}, time to C_{max} (T_{max}), $T_{½}$ and AUC were calculated and compared between the three CYP2D6 activity groups using analysis of variance (ANOVA) separately for hydrogel and aqueous timolol preparations. Pairwise post-hoc comparisons were performed using least significant difference (LSD) correction for multiple tests. The effects of each *CYP2D6* group to the HR change from resting to maximal level during exercise were compared with ANOVA supported by LSD correction.

For the glaucoma patients, the plasma concentration at 120 minutes after the drug instillation was compared between the IM and EM groups, using Student T-test for independent samples.

ADRB1- and GNAS1-analyses. The Gly carriers of *Ser49Gly* polymorphism of *ADRB1* were combined into one group, and Ser homozygotes to another. Also for the *Arg389Gly* polymorphism, glycine carriers were combined for analysis. As to *GNAS1*, the cytocine carriers of the *T393C* polymorphism were considered as one group, and thymine homozygotes as the other.

The effect of *ADRB1* and *GNAS1* polymorphisms for the SAP, DAP and HR responses were analysed with linear regression using age, gender and plasma concentration of timolol as covariates. The significant differences were followed up with pairwise post-hoc comparison with LSD correction. The concentration threshold for the systemic β_1-mediated effects is approximately 0.20 ng/ml

(Kaila et al. 1991). Since the aim of the study was to estimate the effects of *ADRB1* and *GNAS1* polymorphisms during timolol treatment, the concentration values below 0.20 ng/ml were excluded from the *ADRB1* and *GNAS1* analyses.

5 RESULTS

5.1 Comparison of noninvasive methods in the assessment of SV and CO (I)

The average SV- and CO-values differed between the methods ($p<0.001$): the SV values by ICG_{WB} ranged from 23 to 117 ml, but by the pulse contour method from 5 to 91 ml. The bias in SV between the methods was 19 ± 14 ml (Fig. 7), the limits of agreement being -9 and 48 ml; the agreement plot is shown in Fig. 8. The percentages in different discrepancy ranges are given in Table 4.

The CO values by ICG_{WB} ranged from 2.20 to 8.75 l/min, and by the pulse contour values from 0.51 to 6.13 l/min. The bias between the methods was 1.55 ± 1.14 l/min, the limits of agreement being -0.72 and 3.83 l/min.

Table 4. Cumulative percentages of discrepancies between stroke volume values evaluated by pulse contour (PC) method and impedance cardiography (ICG). Discrepancy before scaling refers to the ratios between paired absolute SV_{PC} and SV_{ICG} values. The percentages with scaling are the discrepancies of the second and third paired measurements, when the first ones are scaled to 100 %. *Published with the kind permission from Blackwell Publishing.*

Discrepancy		
Range (%)	Before scaling (%)	With scaling (%)
10	14	36
20	28	66
30	43	83
40	68	95
50	82	98

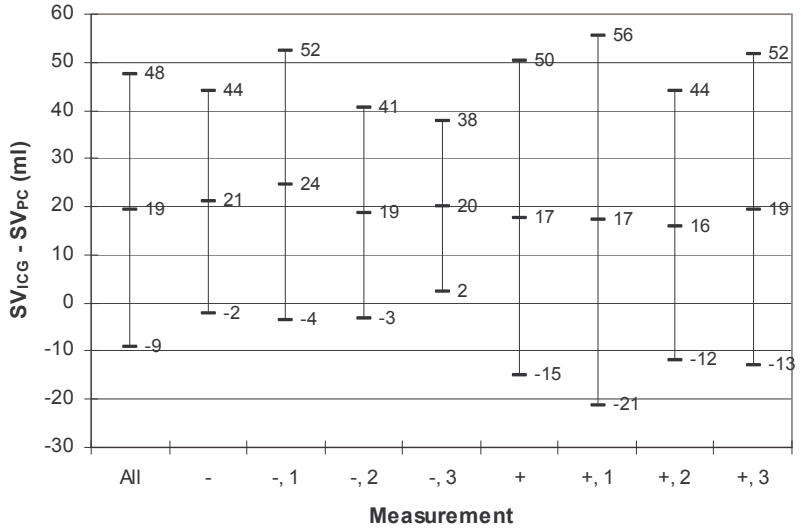

Fig. 7. Bias and limits of agreement (2SD) in stroke volume (SV) between whole-body impedance cardiography (SV_{ICG}) and pulse contour method (SV_{PC}) for all measurements (All) and separately for tilt-negative (-) and tilt-positive (+) patients in the supine position (1), five minutes after the beginning of the tilt (2) and before the end of the tilt (3). The corresponding data values are shown by each point. *Published with the kind permission from Blackwell Publishing.*

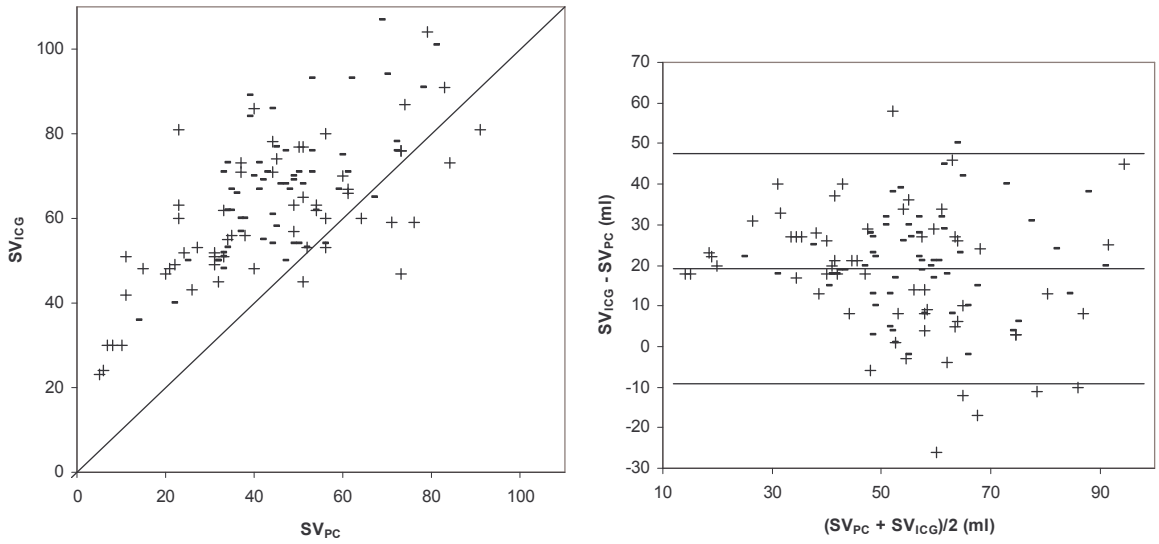

Fig. 8. Left: Stroke volume (SV) values measured by whole-body impedance cardiography (SV_{ICG}) plotted against the pulse contour method (SV_{PC}). The line of equality is shown. **Right:** Differences of measured SV values (SV_{ICG} - SV_{PC}) plotted against the average of these two values, (SV_{ICG} + SV_{PC})/2. The average and limits of agreement (±2 SD) are also shown. "-" and "+" refer to ”tilt-negative” and ”tilt-positive” patients, respectively. *Published with the kind permission from Blackwell Publishing.*

5.2 Effects of oral adrenergic antagonists on haemodynamics (II, III)

Supine haemodynamics

None of the volunteers reported any adverse drug reactions during the treatment periods.

In the recumbent position before the passive head-up tilt, both propranolol and carvedilol decreased SAP significantly more than placebo ($p<0.01$), and HR more than placebo and tamsulosin ($p<0.05$) (Table 5). Propranolol also decreased PWV more than placebo ($p<0.05$). Tamsulosin and alfuzosin did not change significantly any of the variables compared with placebo.

Cardiovascular responsiveness to passive orthostasis

The most pronounced changes in the cardiovascular responsiveness to orthostasis occurred in SVRI responses (Figs. 9 and 10), which were remarkably reduced in head-up tilt position in the subjects with tamsulosin and alfuzosin compared with the pre-treatment responses ($p<0.05$). The response curve with placebo differed from that with tamsulosin ($p<0.05$) and tended to differ from that with alfuzosin ($p<0.10$). In the propranolol group, SVRI tended to increase from the value without the drug ($p=0.10$). In the tamsulosin group, the SVRI response curve to the orthostasis differed significantly from that in the propranolol and carvedilol groups ($p<0.05$).

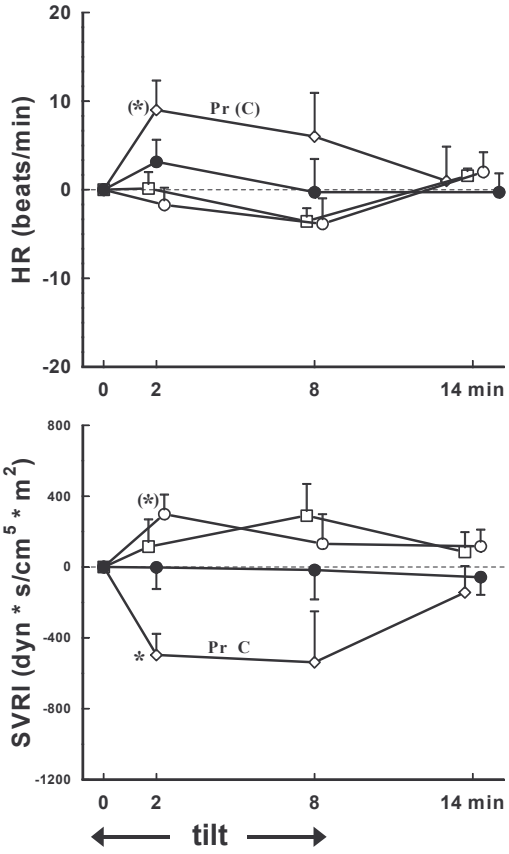

Fig. 9. Drug-induced changes (means ± SEM, n=6-7) from pre-treatment levels (dotted 0-line) in the responses of heart rate (HR) and systemic vascular resistance index (SVRI) to passive orthostasis during the 8-min tilt-provocation test. Symbols: closed circles = placebo, open circles = propranolol, squares = carvedilol, diamonds = tamsulosin. ^{Pr} $p\leq0.05$ compared with propranolol curve, ^{C} $p\leq0.05$ compared with carvedilol curve, $^{(C)}$ $p\leq0.1$ compared with carvedilol curve, * $p\leq0.05$ compared with the value before the drug, (*) $p\leq0.1$ compared with the value before the drug (0-value). *Published with the kind permission from Editio Cantor Verlag.*

During the passive orthostasis, SAP was initially (2 min) decreased more with tamsulosin than before the drug ($p<0.05$). The time-course curves of SAP and DAP did not, however, differ significantly between propranolol, carvedilol, tamsulosin and placebo.

Table 5. Summary of the haemodynamic effects of the tested drugs. The arrows show the direction of effects ($p\leq0.05$), in parentheses if $0.05 < p \leq 0.10$. A "−" denotes lack of effect, and an empty space that the parameter was not assessed.

	Propranolol	Timolol	Carvedilol	Alfuzosin	Tamsulosin
Supine position					
HR	↓	↓	↓	−	−
SAP	↓	(↑)	↓	−	−
DAP	−	−	−	−	−
SI	−	−	−	−	−
CI	−	−	−	−	−
SVRI	−	−	−	−	−
PWV	↓	↓	−	−	−
Head-up tilt					
HR	−	↓	−	↑	↑
SAP	−	−	−	−	↓
DAP	(↑)	−	−	−	−
SI	−	−	−	−	−
CI	−	↓	−	↑	↑
SVRI	(↑)	↑	−	↓	↓
PWV	−	−	−	−	−
Exercise					
HR		↓			
SAP		−			
DAP		−			
ECG and HR variability					
QTc interval		−			
PR internal		−			
RMSSD		−			
Spectral indices		−			
Pulmonary function					
FEV1		−			

HR, heart rate; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; SI, stroke index; CI, cardiac index; SVRI, systemic vascular resistance index; PWV, pulse wave velocity; QTc, HR-corrected interval between the beginning of Q wave to the end of T wave; PR, interval between the apex of P wave and the R peak; RMSSD, a measure of HR variability, see 1.3.1 for details; FEV1, forced expiratory volume in one second.

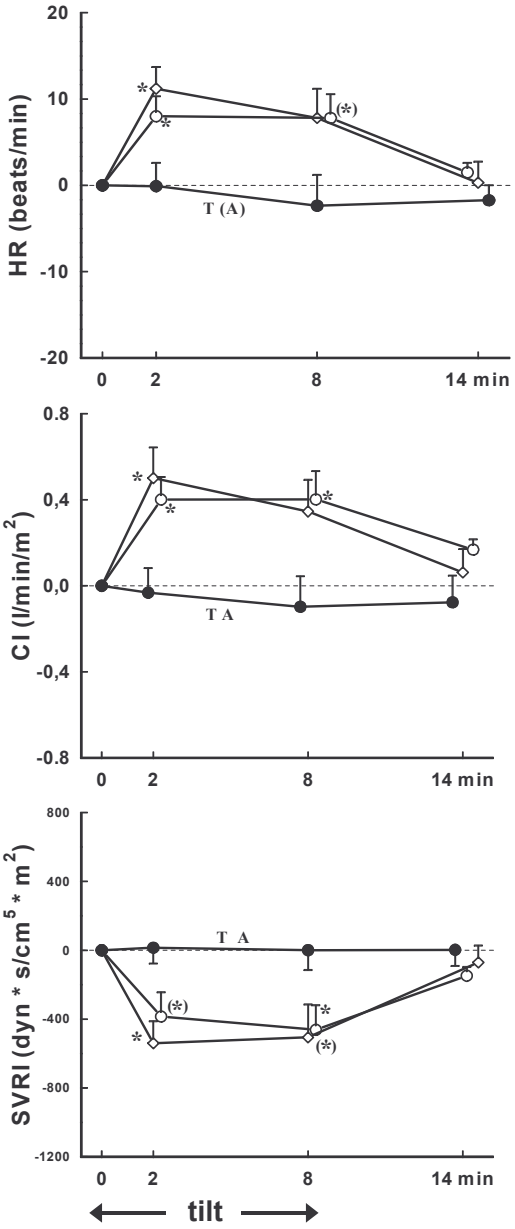

Fig. 10. Drug-induced changes (means ± SEM, n=10-11) from pre-treatment levels (dotted 0-line) in the responses of heart rate (HR), cardiac index (CI) and systemic vascular resistance index (SVRI) to passive orthostasis during the 8-min tilt-provocation test at 4 h after giving the drug to healthy volunteers. Symbols: closed circles = placebo, open circles = alfuzosin, diamonds = tamsulosin. ^{A} $p<0.05$ compared with alfuzosin curve, $^{(A)}$ $p<0.1$ compared with alfuzosin curve, ^{T} $p<0.05$ compared with tamsulosin curve, * $p<0.05$ and (*) $p<0.1$ compared with the value before the drug (0-value). *Published with the kind permission from European Association of Urology.*

The HR response curve with tamsulosin differed from that with propranolol ($p=0.05$) and tended to differ from that with carvedilol ($p=0.10$) (Fig. 9). HR response was elevated in both the tamsulosin and alfuzosin groups compared with the pre-treatment rates ($p<0.05$). The placebo HR time-course curve differed from the curve in the tamsulosin group ($p<0.05$), and tended to differ from that in the alfuzosin group as well ($p<0.10$) (Fig. 10).

SI response to the tilt provocation was not significantly influenced by any of the treatments. During the tilt-up position CI decreased significantly from the pre-tilt values in the subjects with placebo, while this decrease was markedly antagonised by both α_1-antagonists. The CI response curve in the tamsulosin group did not, however, differ significantly from those in the propranolol and carvedilol groups. The drugs did not significantly affect PWV.

5.3 Effects of low plasma levels of timolol on the haemodynamics (IV)

Supine position and head-up tilt test

In supine position before the head-up tilt, the resting HR ($R=-0.52$, $p<0.001$) and PWV

(R=-0.34, p=0.04, Fig. 11) declined with rising timolol concentration.

After tilting the bed up, ophthalmic timolol effectively suppressed the rise in HR (R=-0.36, p=0.03). SI did not change with timolol concentration (R=-0.10, p=0.56), while CI diminished as timolol concentration rose (R=-0.39, p=0.02). The change in SVRI and timolol concentration had a relatively strong positive relation (R=0.38, p=0.02). The changes in SAP, DAP and PWV were not associated with timolol level in the upright position.

Exercise test

In supine position before the exercise on bicycle ergometer, the resting HR was inversely dependent on the timolol concentration (Fig. 12a) with a Spearman correlation coefficient R=-0.33 (p=0.04). The relationship grew stronger in the course of the test: at 60W (Fig. 12b) and 120W (Fig. 12c), the correlation was R=-0.32 (p=0.04) and R=-0.50 (p<0.01), respectively. The correlation between the plasma concentration of timolol and heart rate reached R=-0.67 (p<0.0001) at the maximum load (Fig. 12d) of 183 ± 49 W. No significant correlations were obtained in SAP and DAP.

ECG parameters, HR variability and pulmonary function

These parameters were measured over two 4 min periods during the tilt test: before the head-up tilt and during the upright position. The corrected QT interval (QTc) and PR interval were not dependent on the timolol concentration. Neither were HR variability parameters RMSSD and RRI spectral indices correlated with timolol concentration (unpublished data).

The pulmonary parameter measured, FEV1, did not correlate with timolol (R=0.20, p=0.20) (unpublished data).

5.4 Pharmacogenetics of ophthalmic timolol (V)

Pharmacokinetic parameters in healthy volunteers

Ten of the 18 participants were classified as extensive metabolisers (EM) of *CYP2D6*, six as intermediate metabolisers (IM) and two as poor metabolisers (PM).

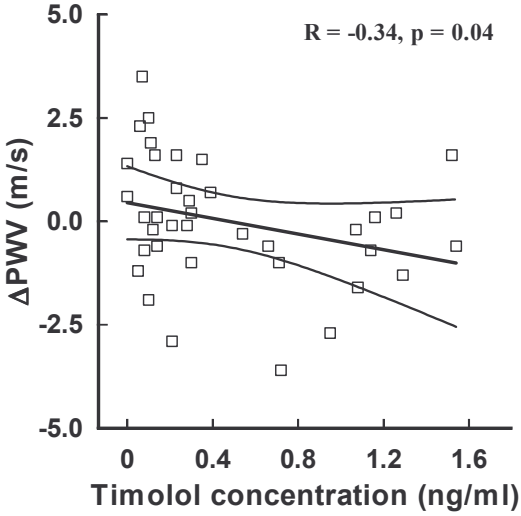

Fig. 11. The effect (Δ) of timolol level on pulse wave velocity (PWV) in supine position before the head-up tilt. The rectangles are the difference between the value with timolol medication and the value at the baseline without medication. Linear regression line with 95 % confidence interval limits and correlation coefficient are shown. *Published with the kind permission from Springer Science and Business Media.*

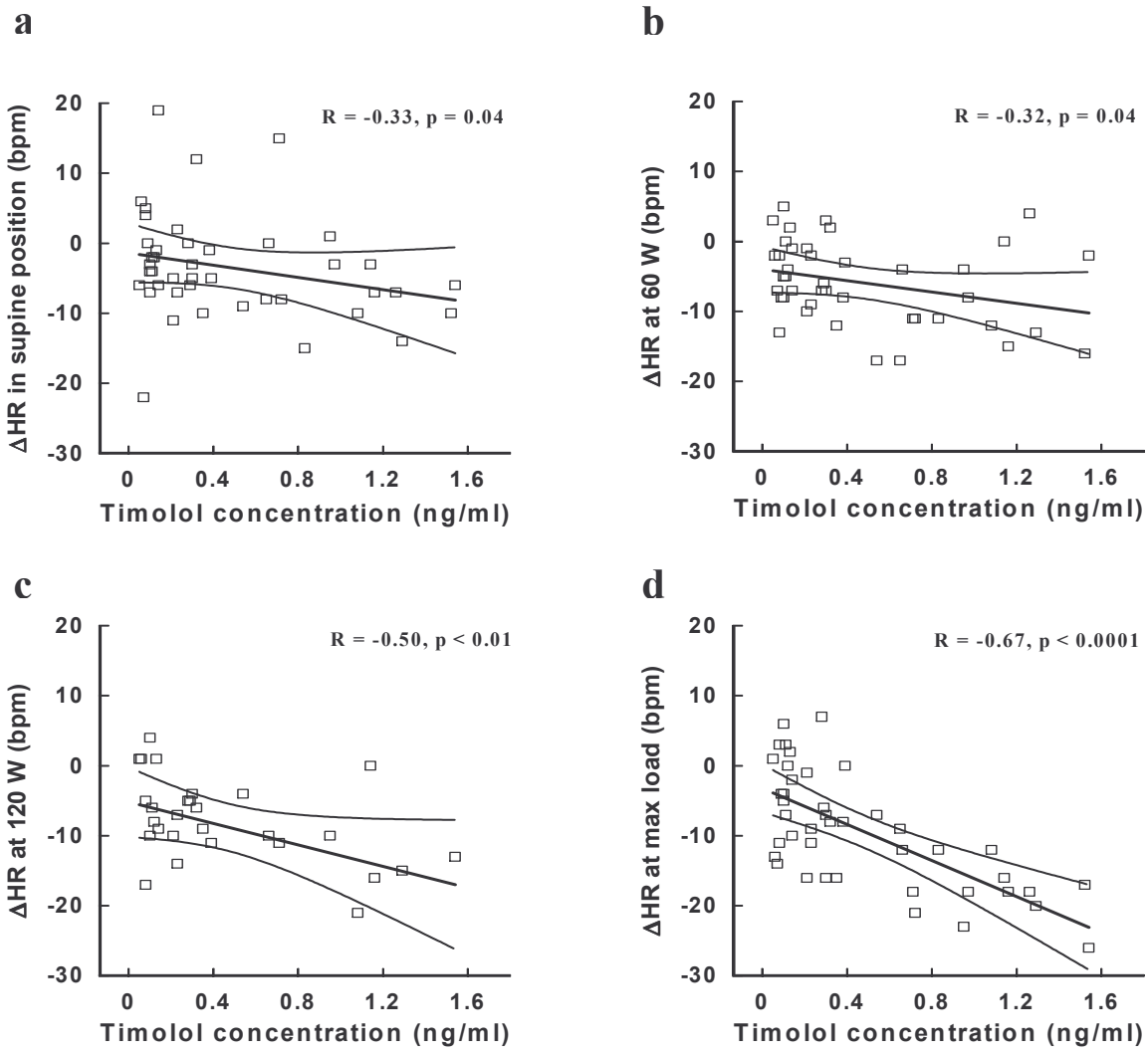

Fig. 12. Suppression of the heart rate (HR) as a function of timolol concentration at four measurement points during the exercise test: the change (Δ) a) in the supine position before the exercise, b) at 60 Watts, c) at 120 Watts, and d) at the maximum load. The rectangles are the difference between the value with timolol medication and the value at the baseline without medication. Linear regression line with 95 % confidence interval limits and correlation coefficient are shown. *Published with the kind permission from Springer Science and Business Media.*

During the treatment with aqueous formulation of timolol, $T_{½}$ ($p<0.01$) and AUC ($p<0.01$) differed between the CYP2D6 activity groups, and C_{max} tended to differ ($p=0.06$) (Table 6). During the hydrogel treatment, the groups did not present any statistically significant differences. The corresponding plasma concentration curves are in Fig. 13.

In the pairwise post-hoc comparison during the treatment with aqueous 0.5 % timolol, IM and EM groups did not differ from each other at any of the calculated four variables. The PM group presented higher C_{max} than IM ($p=0.05$) and EM ($p=0.02$) groups. T_{max} did not differ between the groups, while $T_{½}$ was longer ($p<0.01$) and AUC higher ($p=0.02$ and $p<0.01$) in PMs than in IMs and EMs, correspondingly.

The effect of CYP2D6 genotype on the decrease of maximal HR

In healthy volunteers with aqueous timolol, the elevation of HR from rest to maximal level during exercise tended to differ between PMs and IMs ($p=0.079$, 95 % confidence interval -33.7 to 2.1 bpm), and PMs and EMs ($p=0.076$, 95 % confidence interval -32.2 to 1.8 bpm). The CYP2D6 activity groups did not differ from each other during treatment with timolol hydrogel.

The plasma concentration of timolol in glaucoma patients

16 of the 19 patients were EMs and three IMs. No significant differences between the groups were detected in the plasma concentrations at 2 h.

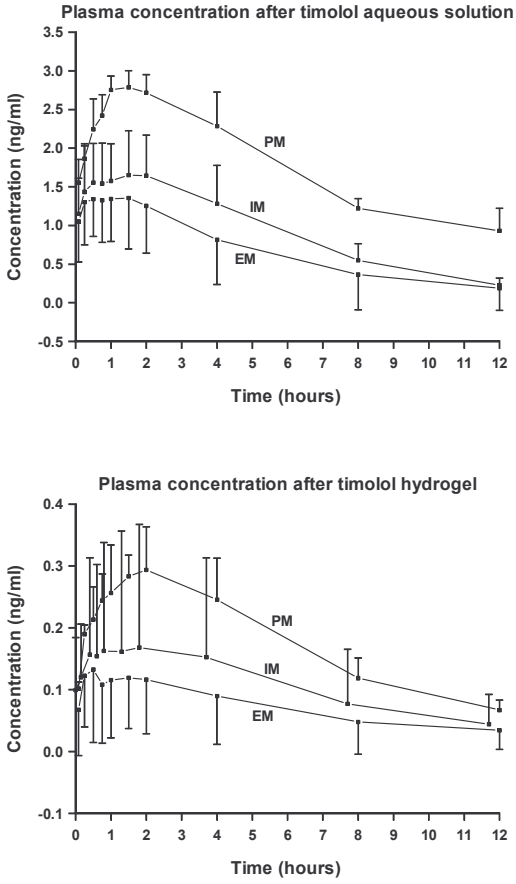

Fig. 13. Upper panel: Concentration curves for the three CYP2D6 activity groups after instillation of aqueous formulation of ophthalmic timolol. The data points present mean ± SD. Poor (PM), intermediate (IM) and extensive (EM) metabolisers had zero, one and two functional CYP2D6 alleles, respectively. Lower panel: The curves with hydrogel formulation of timolol. Note the different concentration scales in the panels.

The effect of ADRB1 and GNAS1 polymorphisms on the BP and HR responses

In the supine position before the head-up tilt, none of these polymorphisms had significant effects on HR, SAP or DAP. When tilting the bed up to 60º, the subjects with two *Ser49* alleles had higher SAP (p=0.03) and DAP (p<0.01) than the Gly carriers. During the maximal exercise test, the subjects with CC alleles of *GNAS1* had lower DAP (p=0.04) than the T carriers.

Table 6. Pharmacokinetic parameters maximal plasma concentration (C_{max}), time to C_{max} (T_{max}), elimination half-life ($T_{½}$) and area-under-curve (AUC) are shown for both aqueous and hydrogel treatments and each CYP2D6 activity group. PM=poor metabolisers (no functional *CYP2D6* alleles), IM=intermediate metabolisers (one functional allele) and EM=extensive metabolisers (two functional alleles). Mean, standard deviation (SD), minimum and maximum values are given.

Treatment	Parameter	*CYP2D6*	Mean	SD	Min	Max
Aqueous 0.5 %						
	C_{max}	PM	2.79	0.22	2.63	2.94
	(ng/ml)	IM	1.73	0.59	0.82	2.43
		EM	1.57	0.65	0.79	2.58
	T_{max}	PM	1.42	0.35	1.17	1.67
	(h)	IM	1.63	0.71	0.42	2.17
		EM	1.09	0.76	0.25	2.17
	$T_{½}$	PM	6.12	0.89	5.49	6.75
	(h)	IM	3.30	0.48	2.54	3.74
		EM	3.07	1.16	1.97	6.08
	AUC	PM	21.40	2.62	19.54	23.25
	(ng·h/ml)	IM	11.32	3.72	5.11	15.81
		EM	8.13	5.46	3.39	22.19
Hydrogel 0.1 %						
	C_{max}	PM	0.30	0.06	0.26	0.34
	(ng/ml)	IM	0.19	0.19	0.05	0.54
		EM	0.16	0.11	0.05	0.42
	T_{max}	PM	1.92	0.35	1.67	2.17
	(h)	IM	1.60	1.46	0.25	4.17
		EM	1.08	1.16	0.25	4.17
	$T_{½}$	PM	4.59	0.40	4.31	4.87
	(h)	IM	3.83	1.07	3.17	5.07
		EM	4.37	1.67	2.51	6.75
	AUC	PM	2.13	0.49	1.78	2.48
	(ng·h/ml)	IM	1.26	1.50	0.12	4.02
		EM	0.78	0.77	0.02	2.27

6 DISCUSSION

6.1 Pulse contour method in the assessment of haemodynamics

Noninvasive measurement of SV or CO is indispensable in most haemodynamic studies, since technical and ethical grounds restrict the usage of invasive methods to critically ill patients. There are two main alternatives of noninvasive techniques that enable continuous assessment of beat-to-beat SV and CO: pulse contour method and impedance cardiography. The reliability of whole body impedance cardiography (ICG_{WB}) has been proven in a number of studies with different study designs and equipment (Kööbi et al. 1997a, Kööbi et al. 1997b, Kööbi et al. 1999, Cotter et al. 2004, Moshkovitz et al. 2004). The pulse contour method would potentially be even easier to use and more readily available, but its reliability has not been adequately proved e.g. during head-up tilt test. Therefore, the present studies were started with an evaluation of whether the pulse contour method could substitute ICG_{WB} in further haemodynamic trials.

The CO values estimated by the pulse contour method were relatively low for physically active subjects (average 3.29 l/min), while the values by impedance cardiography were more physiological (average 4.85 l/min). These results are in agreement with those of Houtman et. al. (1999), who showed that CO values obtained by the pulse contour method were lower than the values by the rebreathing CO_2 method. It seems that the pulse contour method underestimates CO significantly.

The accuracy of the pulse contour method has been poorest in patients with unstable haemodynamics (Dos Santos et al. 1994,

Irlbeck et al. 1995). Therefore, it was expected that the measurements would differ most in the phase of (pre)syncope, but the bias between the methods turned out to be almost equal in the different phases of the test. However, the diversity was broader in the tilt+ patients (SD of bias of 1.35 l/min in CO measurements) than in the tilt- patients (SD 0.88 l/min) ($p<0.05$).

Knowledge of the absolute CO level in tilt-table tests is important, because the low basal CO may be the factor causing orthostatic hypotension, especially in the elderly. Correction for bias in the pulse contour method will not eliminate the disagreement, because the limits of agreement are relatively large, particularly in syncopal patients. Furthermore, calculating SVR on the basis of erroneous CO is problematic and may even lead to management errors.

Beside the absolute values, the ability of the pulse contour method to reflect SV changes in each patient was scrutinised. When the bias between the methods was eliminated by scaling the first measurement to 100 %, the difference between the methods in the second and third measurements did not exceed 20 % in 66 % of the cases, while the corresponding result without the scaling was only 28 %. It indicates that the methods agree better in tracking changes in SV and CO.

One source of inaccuracy in the pulse contour method is the estimation of systemic haemodynamic parameters on the basis of local characteristics: the aortic pulse contour is approximated by the contour in a finger. However, BP is regulated by many systemic and local mechanisms as well as by arterial wall properties (Boulpaep 2003c, Ganong 2003b), and the compliance of arteries varies from one artery to another. This may be of less significance in healthy young people, but in elderly atherosclerotic patients the individual differences can be significant. The actual pressure contour, especially under unstable haemodynamic conditions, may vary considerably in different parts of the body.

Another possible source of inaccuracy in the pulse contour method is inherently linked to the approximation of the aortic cross-sectional area by the patient's age, sex, height and weight. In the case of a dilated aorta, the resulting CO values will be smaller than the true output and, vice versa, overestimating the aortic area increases the CO. An error of 1 mm in diameter approximation leads to an error of 5-10 % in the CO value, since the cross-sectional area and CO are proportional to the square of the diameter.

6.2 Detailed influence of oral adrenergic antagonists on haemodynamics

Haemodynamic effects of the non-selective β-antagonist propranolol and the combined α_1- and β-antagonist carvedilol have been extensively studied, except for detailed knowledge of the haemodynamic changes in passive orthostasis. The cardiovascular changes due to the α_1-antagonists alfuzosin and tamsulosin are known only for BP and HR – the knowledge on the effects on cardiac indices and PWV is poor or nonexistent. In the present trials, the noninvasive ICG_{WB} enabled the assessment of a variety of haemodynamic parameters difficult to estimate otherwise.

Effects on supine haemodynamics

In the propranolol and carvedilol groups, the baseline SAP and HR decreased markedly compared with placebo or tamsulosin, as reported previously in many papers (Svendsen et al. 1979, Morgan 1994, Kähönen et al. 1998, 2002). The baseline CI and SI did not change significantly, which is in harmony with the studies showing that propranolol does not have essential negative inotrophy at rest (Broeder et al. 1993, Kähönen et al. 2002).

Arterial PWV is often used as a surrogate to measure arterial stiffness, and it has recently been proposed to be an independent predictor of cardiovascular mortality in high-risk individuals: the faster the PWV, the higher the risk (Safar et al. 2002a, Blacher et al. 2003). Oral β-antagonists with different profiles have been shown to either decrease (bisoprolol, carvedilol, metoprolol and propranolol) or increase (celiprolol) PWV (Asmar et al. 1991, Yin and Ting 1992, Merli et al. 1993, Slama et al. 1995, Van Bortel et al. 1995, Kähönen et al. 1998, 2002). In the present data, the decrease of PWV with propranolol is in concert, and the unchanged PWV values with carvedilol in discord, with the previous studies. The latter may be due to the relatively low size of the carvedilol group.

Three different formulations of alfuzosin have been marketed: original standard formulation 2.5 mg three times daily, sustained release formulation 5 mg twice daily, and prolonged release formulation 10 mg once daily. All these formulations have only minor BP depressor effects: in most studies, the BP changes in supine and standing positions have been non-significant, the maximal average reductions being 5 mmHg (Michel et al. 2001). Tamsulosin has been used in doses ranging from 0.2 mg to 0.8 mg once daily. Tamsulosin 0.4 mg once daily is the most common dosing, since larger doses bring along adverse drug reactions such as dizziness or rhinitis in up to 75 % of the patients (Wilt et al. 2003). The effect of tamsulosin 0.4 mg on SAP and DAP

has been clinically irrelevant, 0-2 mmHg (Chapple et al. 1996, de Mey 1998, Michel et al. 2001). Also in the present studies, the decrease of supine SAP and DAP remained practically unchanged with alfuzosin 5 mg twice daily and tamsulosin 0.4 mg once a day.

In recumbent position, the sympathetic activity of α_1-adrenoceptors is low (Chapple et al. 1996, Lepor et al. 1997). Hence, even a total blockade of α_1-receptors would only induce minor cardiovascular effects. In contrast, standing up markedly activates the sympathetic system, and, consequently, vascular α_1-blockade by classic antagonists such as prazosin, doxazosin and terazosin often causes symptomatic orthostatic reactions. Even fainting has been reported with these α_1-antagonists (Mets 1995, Chrischilles et al. 2001, Roehrborn and Schwinn 2004).

Effects on the cardiovascular responsiveness to passive orthostasis

The cardiovascular responses to alfuzosin and tamsulosin were relatively uniform, and the activation of vascular α_1-adrenoceptors upon orthostasis was clearly interfered with by both of them. The volunteers in both groups experienced a significant and marked drop in systemic vascular resistance compared with the pre-drug change. This suggests that the drugs are not purely uroselective α_1-antagonists. The drop in the SVR lowered SAP 8 mmHg and 3 mmHg in tamsulosin and alfuzosin groups, respectively, compared with the change prior to medication. The marked acute decrease in BP in the tamsulosin group could have clinical relevance in the elderly men with benign prostatic hyperplasia: as the patients suddenly arise during the night due to nocturia, the drop in BP could induce dizziness and possibly even fainting. This has actually been documented as an infrequent adverse effect of both alfuzosin and tamsulosin in ambulatory patients (Roehrborn and Schwinn 2004).

The relationship between the usage of α_1-antagonists (prazosin, doxazosin, indoramin, terazosin, alfuzosin and tamsulosin) and hip/femur fractures has been recently assessed in a large population-based case-control study (Souverein et al. 2003). The researchers found an association between hip fractures and α_1-antagonists, when the medication was taken for a cardiovascular disease. However, the treatment with α_1-antagonists for prostatic hyperplasia was not related to an increased risk of fractures. This implies that the drop in SAP found in this study with tamsulosin is not clinically significant as for the risk of falling, or that the haemodynamic response to head-up tilt in healthy volunteers differs from that of elderly men. Extrapolating the observed vasodilatory effects to the elderly is not

straightforward because of contradicting mechanisms: Aging is associated with a reduction in the adrenergic influence on physiological processes, and this would attenuate the responses observed (Guimaraes and Moura 2001). However, the autonomic compensatory mechanisms in the elderly are not as efficient as in the young (de Mey 1998), which could cause even more pronounced haemodynamic responses with the presence of α_1-blockade in aged patients.

The decrease in BP activates a sympathetic baroreflex that results in a β_1- and β_2-adrenoceptor-mediated compensatory increase in HR. This relative tachycardia was further enhanced by both alfuzosin and tamsulosin, probably because of inhibited vasoconstriction, and because α_1-blockade does not result in notable negative chronotropic effects on the heart (Brodde and Michel 1999). A passive head-up tilt impedes venous return, which leads to a decrease in preload and SV, as was also found in the present studies. Alfuzosin and tamsulosin did not significantly change the SI response compared with the pre-treatment and placebo values, but the drugs significantly antagonised the decrease in CI in the head-up tilt as a result of the increase in HR. Thus, the clear drop in SVRI was counteracted by the relative increase in CI, which explains why changes in BP are small in spite of the marked inhibition of vasoconstriction.

Tamsulosin differs from both propranolol and carvedilol as regards HR and SVRI responses to passive orthostasis. The difference between propranolol and carvedilol was not statistically significant in terms of any of the variables measured. Thus, the α_1-blocking effect of carvedilol is not apparent in the tilt provocation, and the drug follows smoothly the haemodynamic profile of a non-selective β-antagonist.

6.3 Association between low plasma levels of timolol and haemodynamics

None of the earlier reports show specific correlations between the low plasma concentration of timolol and circulatory/pulmonary influence, even though such a correlation has been shown to exist for oral timolol (Wilson et al. 1982). On the other hand, oral and/or intravenous metoprolol and propranolol do not present correlation between the cardiovascular effects and plasma concentration (Wilson et al. 1982, Zineh et al. 2004). These conflicting results with different β-adrenoceptor antagonists raise the interesting question whether the low plasma levels caused by topical timolol carry an association with cardiovascular actions.

In Study IV, glaucoma or ocular hypertension patients were subjected to passive head-up tilt, ECG, bicycle ergometer exercise test and spirometry for assessment of detailed haemodynamic changes. All the patients were treated with both aqueous and hydrogel formulations of timolol using a randomised, double-masked crossover multicenter design. Since the study was aimed to estimate the quantitative relation between timolol concentration and the cardiovascular effects, the data on the two treatments were combined, and, thereafter, the correlations between the plasma level of timolol and the circulatory/pulmonary effects were calculated. The timolol plasma concentrations varied between non-detectable (<0.04 ng/ml) and 1.54 ng/ml, which is in agreement with earlier studies: the average plasma concentration of timolol after using aqueous solution has varied between 0.46 and 1.38 ng/ml (Urtti 1994, Vuori and Kaila 1995, Dickstein and Aarsland 1996, Shedden et al. 2001, Korte et al. 2002, Nino et al. 2002), while after the application of the gel formulation it has been 0.14-0.71 ng/ml (Dickstein and Aarsland 1996, Shedden et al. 2001, Nino et al. 2002).

The plasma concentration of timolol was determined 120 min after the drug instillation, while the orthostasis was started only 40 min and the exercise test 90 min after the drug. These time differences may be limitations to the study, but, on the other hand, the plasma levels of timolol remain relatively stable over the time period from 40 to 120 minutes (Fig. 13).

The degree of adrenergic β-blockade is often demonstrated by the extent of reduction in maximum HR, because estimation of β-blockade is difficult at lower HR levels due to low levels of β-sympathetic activity (Johnsson and Regardh 1976). The present data is strongly supportive to this concept: the higher the HR, the greater the correlation between the plasma level of the β-blocking agent timolol and HR (Fig. 12a-d).

Although the rise in HR is clearly suppressed proportionately to timolol concentration, SAP and DAP were not significantly dependent on timolol level, which is in agreement with earlier results (Korte et al. 2002, Nino et al. 2002, Stewart et al. 2002a, Stewart et al. 2002b). This is probably due to sufficiently strong baroreceptor-activated compensatory vasoconstriction. Evidently, higher plasma levels after *per os* administration of timolol overweigh the compensatory mechanisms and lower BP, since the oral dose regimes with plasma levels of around 100 ng/ml (Fourtillan et al. 1981, Wilson et al. 1982) have successfully been used as antihypertensive treatment (Brogden et al. 1975, Lohmoller and Frohlich 1975). Following the drop in HR, CI decreased, while the drug did not exert a

noteworthy influence on the SI at these plasma levels.

The vasoconstrictory effect of timolol was reflected in the positive correlation between the drug concentration and SVRI in the tilt-test. This was probably mostly a compensatory reaction due to the attenuation of HR, but, theoretically, the blockade of vasodilatory β_2-adrenoceptors might also play a part in it. Another β_2-blockade effect, bronchoconstriction, was not found in this study, which is in concert with some, but not all, of the previous studies (Umetsuki et al. 1997, Diggory et al. 1998, Waldock et al. 2000, Korte et al. 2002). In the present study and all these previous ones, patients with known asthma were excluded from timolol exposure, but differing prevalence of subclinical reversible obstructive airways disease among the study populations may explain the different levels of pulmonary symptoms (Diggory et al. 1998, Waldock et al. 2000). Timolol 0.5 % aqueous solution was the formulation used in all the studies with pulmonary reactions, but the actual plasma levels were not quantified.

The present data reveals the interesting novel finding that there is an inverse correlation between PWV and the plasma level of timolol even at these concentrations well below the levels after *per os* administration. The clinical consequences of the finding remain to be explored.

HR variability mirrors the autonomic activity transmitted to the sinoatrial node (Pagani et al. 1986). Orally administered β-blocking drugs are known to increase HR variability due to decreased sympathetic and increased parasympathetic modulation (Pousset et al. 1996, Lampert et al. 2003). In the present study, the changes in HR variability did not correlate with timolol concentration in spite of the marked effects of timolol on HR. This is in agreement with an earlier report, where gel and aqueous formulations of timolol did not change significantly any of HRV parameters (Nino et al. 2002). The ECG parameters PR interval and QTc time did not associate with timolol level. Again, these findings are probably due to the relatively low plasma concentrations of timolol.

6.4 Pharmacogenetics of ophthalmic timolol

After oral ingestion of timolol, the pharmacokinetic parameters C_{max} and AUC have been significantly higher and the $T_{½}$ longer in *CYP2D6* PMs than in EMs (McGourty et al. 1985, Lennard et al. 1989). Peak HR during exercise has been significantly lower in PMs than in EMs, with no significant difference on resting HR or IOP

(Lewis et al. 1985, al-Sereiti et al. 1990). However, two previous studies with ophthalmic timolol have yielded conflicting results. In the first of them (Huupponen et al. 1991), ocular drug administration paradoxically caused higher peak plasma concentrations in EMs than PMs, and the authors concluded that the variation in the systemic absorption of ocular timolol contributes to timolol plasma levels more than the CYP2D6 phenotype. In another study, exercise HR following timolol eye drops was reduced significantly more in the PMs than EMs, and plasma timolol concentration was higher in PMs (Edeki et al. 1995). However, timolol was applied directly to the nasal mucosa in this latter study, not to the *cul de sac* as applied when used as ocular treatment. As the authors noted, this change in the instillation routine most probably increased the bioavailability of timolol, leading to elevated plasma concentrations of timolol with less interpersonal variation within each phenotype. This methodological limitation was avoided in the present study.

The genotypic definitions of PM, IM and EM vary to some extent (Kirchheiner et al. 2004, Zineh et al. 2004). For simplicity and due to the relatively low number of study subjects, we classified the individuals with 0, 1 or 2 functional *CYP2D6* alleles as PMs, IMs and EMs. Our results show that CYP2D6 activity grouping has a clear association to the pharmacokinetic parameters C_{max}, $T_{½}$ and AUC, but only when timolol is given as aqueous solution. The plasma concentrations of timolol remain essentially lower after hydrogel than after aqueous preparation. On the other hand, the lower the timolol levels, the lower the CYP2D6 activity may be sufficient to metabolise the drug. This may explain why the pharmacokinetics of timolol hydrogel is not dependent on the *CYP2D6* group.

Previous studies with oral metoprolol, another β-antagonist metabolised through CYP2D6, have been somewhat inconsistent in the pharmacokinetic differences between individuals with 1 or 2 functional *CYP2D6* alleles: plasma concentrations and AUC have been either equal between the groups (Rau et al. 2002) or higher in the individuals with only one functional allele (Koytchev et al. 1998). In the present data, the differences in pharmacokinetic parameters did not reach statistical significance between IMs and EMs among healthy volunteers, and neither did the plasma concentrations of timolol at 2 h after drug delivery among glaucoma patients. Had the study population been bigger, the significance might have been reached.

One of the EMs among the healthy volunteers showed a C_{max} of 2.58 ng/ml and AUC of 22.2 ng·h/ml, which ranked to third and second highest in the whole study population, respectively. In a recent study, where the

relationship between metoprolol pharmacokinetics and CYP2D6 activity were examined (Zineh et al. 2004), such a deviation was explained by the use of certain CYP2D6 inhibitors. However, none of the present volunteers used concomitant drugs, and the deviations from general pattern may be at least partly due to untypical bioavailability of ophthalmic timolol in these individuals. Also, nonfunctional *CYP2D6* alleles of frequencies below 2 % in Caucasians were not detected, and, consequently, possible carriers of those alleles were misclassified to a higher CYP2D6 activity group.

Interestingly, the rise of HR from the basal to maximal level tended to depend on the *CYP2D6* group, when the healthy volunteers were treated with aqueous timolol solution; the increase in HR among PMs was 16 and 15 bpm lower than among IMs and EMs, correspondingly. The results with hydrogel had the same trend of *CYP2D6* dependence without reaching statistical significances, probably partly due to the relatively low sample size. These findings have relevance in clinical practice because of the great number of people on treatment with ophthalmic timolol and the relatively high prevalence of PM genotype: e.g. 5-10 % among Caucasians, 1-2 % among the Asians, and 0-19 % among the African Americans (Siest et al. 2004). Several authors have concluded that there are no safety reasons for clinical *CYP2D6* genotyping in conjunction with β-antagonist therapy (Zineh et al. 2004). However, the cardiovascular effects of timolol should be avoided when timolol is used as ophthalmic treatment. Since these effects are dependent on the *CYP2D6* genotype, knowing the actual genotype might be valuable particularly in patients who experience adverse effects.

The functional importance of the polymorphism in the β_1-adrenoceptor gene *ADRB1* has been assessed in several studies with partly inconsistent results. Patients homozygous for the *Arg389* allele have shown greater reduction of diastolic arterial pressure (DAP) during metoprolol and atenolol treatment compared with the variant alleles in some (Johnson et al. 2003, Sofowora et al. 2003) but not all (O'Shaughnessy et al. 2000) of the previous studies. In our data, the *Arg389Gly* polymorphism did not differentiate the BP and HR responses in any of the measurements during timolol treatment.

As to the *Ser49Gly* polymorphism, the *Ser49* homozygotes have shown a trend toward a stronger response to metoprolol in 24-hour and daytime DAP compared with *Gly49* carriers (Johnson et al. 2003). Contrary to this, the present *Gly49* carriers faced a stronger attenuation of SAP and DAP upon standing up than *Ser49* homozygotes. In addition to the different physiological designs (ambulatory vs. orthostatic BP), other possible explanations for

the different BP responses are β-subtype selectivity and the plasma concentration of the antagonists: Metoprolol is a β_1-selective antagonist, while timolol antagonises both β_1- and β_2-adrenoceptors – both of which are present in the human heart. Also, the different plasma concentrations may induce varying β_1-AR responses, and the timolol concentrations after ophthalmic administration are essentially lower than in oral dose regimens. Furthermore, the rather low number of individuals in each genotype group may contribute to these inconclusive findings. These arguments may also explain why the cytosine homozygotes of *T393C* of gene *GNAS1* had lower DAP during maximal exercise than the T carriers, even though most of the thymine carriers had been classified as good responders to β-blockade in an earlier study (Jia et al. 1999). The competing results in different studies imply that the effects of the studied SNPs of *ADRB1* and *GNAS1* may not be great enough to explain systematically the interpersonal differences in the responses to β-antagonist therapy.

6.5 Clinical implications and future prospects

Recent technological advances in the noninvasive measurement of haemodynamics provided for the present assessment of more accurate and extensive cardiovascular status than earlier. Most of the new findings complement the existing knowledge, such as the correlation between HR and concentration of timolol even at low plasma levels. This is of particular clinical value when a CYP2D6 poor metaboliser attends a clinical exercise test. If the ophthalmic timolol treatment is not perceived or taken into account, the HR remains lower than expected, which may lead to undiagnostic ECG changes, wrong conclusions and even mismanagement.

Previous understanding is challenged e.g. by the unexpectedly clear vasodilatory effects of alfuzosin and tamsulosin. Some of the BPH patients on α_1-antagonist treatment also use phosphodiesterase type 5 (PDE5)-inhibitors for erection disorders. Since the PDE5-inhibitors sildenafil, vardenafil and tadalafil are also known to relax vascular smooth muscle (Pomara et al. 2004), concomitant use of drugs of these two classes may provoke accentuated vasodilation. BP has not markedly decreased in the previous few trials with co-administration of these drugs (Auerbach et al. 2004, Kloner et al. 2004), but the advanced haemodynamic parameters have not been assessed. A new study is warranted to clarify the detailed haemodynamic effects of alfuzosin and tamsulosin in the elderly LUTS patients, also with concomitant usage of other vasodilatory drugs, such as PDE5-inhibitors.

7 CONCLUSIONS

The pulse contour method significantly underestimates the absolute values of SV and CO in supine position and head-up tilt compared with the whole-body impedance cardiography. In tracking changes the methods agree relatively well. Therefore, the finger pressure-derived pulse contour method seems only suitable for tracking the direction of SV and CO changes, and thus impedance cardiography was used in the present drug tests.

Both alfuzosin and tamsulosin have clear haemodynamic effects in the passive head-up tilt provocation, indicating that they are not purely uroselective α_1-antagonists. This is most strikingly evident in the decrease in SVR and increase in CO. The effects measured for alfuzosin and tamsulosin did not significantly differ from each other. The uroselectivity of these α_1-antagonists may be overestimated, if only BP and HR are measured.

Tamsulosin clearly differs in the haemodynamic responses compared with propranolol and carvedilol. The latter two produced practically equal responses.

The pharmacokinetics of ophthalmic timolol is clearly dependent on the *CYP2D6* genotype. Since there is a correlation between the plasma level of timolol and several haemodynamic effects, especially HR, the CYP2D6 poor metabolisers may be more prone to systemic adverse events of timolol than extensive metabolisers. Although HR at rest and in exercise is conversely related to the plasma level of timolol, BP is kept unchanged due to the compensatory increase in SVR.

The polymorphisms in *ADRB1* and *GNAS1* modulate SAP and DAP responses to ophthalmic timolol, but the directions of effects are not consistent with earlier results.

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ORIGINAL COMMUNICATIONS