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α_2-ADRENOCEPTOR-MEDIATED VASCULAR RESPONSES INDUCED BY DEXMEDETOMIDINE

by

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Jussi P. Posti: *α_2-Adrenoceptor-mediated vascular responses induced by dexmedetomidine*

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ABSTRACT

α_2-Adrenoceptors are cell-surface G protein coupled receptors that mediate many of the effects of the catecholamines noradrenaline and adrenaline. The three human α_2-adrenoceptor subtypes are widely expressed in different tissues and organs, and they mediate many different physiological and pharmacological effects in the central and peripheral nervous system and as postsynaptic receptors in target organs. Previous studies have demonstrated that α_2-adrenoceptors mediate both vascular constriction and dilatation in humans. Large inter-individual variation has been observed in the vascular responses to α_2-adrenoceptor activation in clinical studies. All three receptor subtypes are potential drug targets. It was therefore considered important to further elucidate the details of adrenergic vascular regulation and its genetic variation, since such knowledge may help to improve the development of future cardiovascular drugs and intensive care therapies.

Dexmedetomidine is the most selective and potent α_2-adrenoceptor agonist currently available for clinical use. When given systemically, dexmedetomidine induces nearly complete sympatholysis already at low concentrations, and postsynaptic effects, such vasoconstriction, can be observed with increasing concentrations. Thus, local infusions of small doses of dexmedetomidine into dorsal hand veins and the application of pharmacological sympathectomy with brachial plexus block provide a means to assess drug-induced peripheral vascular responses without interference from systemic pharmacological effects and autonomic nervous system regulation.

Dexmedetomidine was observed to have biphasic effects on haemodynamics, with an initial decrease in blood pressure at low concentrations followed by substantial increases in blood pressure and coronary vascular resistance at high concentrations. Plasma concentrations of dexmedetomidine that significantly exceeded the recommended therapeutic level did not reduce myocardial blood flow below the level that is observed with the usual therapeutic concentrations and did not induce any evident myocardial ischaemia in healthy subjects. Further, it was demonstrated that dexmedetomidine also had significant vasodilatory effects through activation of endothelial nitric oxide synthesis, and thus when the endothelial component of the blood vessel response to dexmedetomidine was inhibited, peripheral vasoconstriction was augmented. Hand vein constriction responses to α_2-adrenoceptor activation by dexmedetomidine were only weakly associated with the constriction responses to α_1-adrenoceptor activation, pointing to independent cellular regulation by these two adrenoceptor classes. Substantial inter-individual variation was noted in the venous constriction elicited by activation of α_2-adrenoceptors by dexmedetomidine. In two study populations from two different continents, a single nucleotide polymorphism in the *PRKCB* gene was found to be associated with the dorsal hand vein constriction response to dexmedetomidine, suggesting that protein kinase C beta may have an important role in the vascular α_2-adrenoceptor signalling pathways activated by dexmedetomidine.

Key words: dexmedetomidine; receptor, adrenergic, adrenoceptor, alpha 2; alpha 1; myocardial perfusion; blood pressure; endothelium; nitric oxide; constriction; dilatation; hand vein; GWAS; candidate genes

Jussi P. Posti: *Deksmedetomidiinin α_2-adrenoseptorivälitteiset verisuonivaikutukset*

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TIIVISTELMÄ

α_2-Adrenoseptorit ovat solukalvolla sijaitsevia G-proteiinikytkentäisiä tunnistajamolekyylejä, jotka välittävät katekoliamiinien, noradrenaliinin ja adrenaliinin, vaikutuksia elimistössä. Ihmisen kudoksissa tavataan kolmea erilaista α_2-adrenoseptorien alatyyppiä. Ne välittävät monia erilaisia fysiologisia ja farmakologisia vaikutuksia keskus- ja ääreishermostossa ja hermopäätteiden kohde-elimissä. α_2-Adrenoseptorien aktivaatio aiheuttaa sekä verisuonten laajenemista että niiden supistumista. Kliinisissä lääketutkimuksissa on havaittu, että α_2-adrenoseptorien aktivaation välittämissä verisuoniston vasteissa on huomattavia yksilöiden välisiä eroja. Koska kaikki α_2-adrenoseptorialatyypit ovat mahdollisia lääkeaineiden vaikutuskohteita, on tärkeää tutkia sydän- ja verenkiertoelimistön toiminnan adrenergista säätelyä ja sen geneettistä vaihtelua. Näiden ilmiöiden ymmärtäminen voi tulevaisuudessa edistää sydän- ja verisuonisairauksien lääkehoidon ja kriittisesti sairaiden potilaiden tehohoidon kehittämistä.

Deksmedetomidiini on selektiivisin kliinisessä käytössä oleva α_2-adrenoseptoreja aktivoiva lääkeaine. Deksmedetomidiinin annostelu verenkiertoon aiheuttaa lähes täydellisen sympaattisen hermoston lamauksen jo pieninä pitoisuuksina; postsynaptiset vaikutukset, kuten verisuonten supistuminen, tulevat esiin suuremmilla pitoisuuksilla. Deksmedetomidiinin aiheuttamia ääreisverenkierron verisuonivasteita voidaan silti tutkia kohdennetusti, ilman autonomisen hermoston ja muiden elinten reseptorien aktivaatiota, kun käytetään apuna hartiapunoksen puudutusta ja annostellaan hyvin pieniä lääkemääriä paikallisesti kämmenselän laskimoon.

Tässä tutkimussarjassa deksmedetomidiinin annostelu verenkiertoon aiheutti selkeästi kaksivaiheisen verenkiertoelimistön vasteen; koehenkilöillä havaittiin aluksi verenpaineen lasku alle lähtötason, minkä jälkeen verenpaine nousi ja sepelvaltimoiden virtausvastus lisääntyi. Kliinisesti käytettyjä hoitopitoisuuksia huomattavasti suuremmat lääkepitoisuudet eivät kuitenkaan heikentäneet terveiden koehenkilöiden sydänlihaksen verenkiertoa sen enempää kuin tavanomaiset hoitopitoisuudet, eikä sydänlihaksen hapenpuutetta ollut todettavissa. Deksmedetomidiinin ääreisverenkiertoon kohdistuvia vaikutuksia tutkittaessa havaittiin, että deksmedetomidiini myös laajentaa verisuonia suonten sisäkalvon typpioksidisynteesin aktivaation kautta. Typpioksidin synteesin esto voimisti supistusvasteita. Deksmedetomidiinin kyky supistaa α_2-adrenoseptorien välityksellä kämmenselän laskimoita ei yksilötasolla ollut kytköksissä samojen suonten α_1-adrenoseptorivälitteiseen supistukseen, minkä perusteella voidaan päätellä, että ihmisessä nämä kaksi adrenergistä järjestelmää toimivat erilaisten solunsisäisten kytkentöjen kautta. α_2-Adrenoseptorivälitteisessä supistuksessa havaittiin hyvin suuri yksilöllinen vaihtelu. Kahdessa eri maanosia edustavassa tutkittavien henkilöiden ryhmässä todettiin *PRKCB*-geenin vaihtelun liittyvän tähän vaste-eroon. Tämän perusteella voidaan olettaa, että proteiinikinaasi C beeta voi olla tärkeässä asemassa välittämässä α_2-adrenoseptorivälitteistä kämmenselän laskimoiden supistusta.

Avainsanat: deksmedetomidiini; reseptori, alfa2, adrenerginen, adrenoseptorit; alfa1; sydänlihaksen verenkierto; verenpaine; endoteeli; typpioksidi; supistuminen; laajentuminen; kämmenselän laskimo; GWAS, kandidaattigeeni

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ABBREVIATIONS

ADCY	Adenylyl cyclase
ADRA2(A-C)	$\alpha_{2A\text{-}C}$-AR genes
AUC	Area under time-response curve
BMI	Body mass index
BP	Blood pressure
CBF	Cerebral blood flow
CNS	Central nervous system
CMR	Cerebral metabolic rate
CO	Cardiac output
CVS	Cardiovascular system
CVR	Coronary vascular resistance
ECG	Electrocardiogram
ECL	Extracellular loop
ED_{50}	Effective dose 50 %; dose causing 50 % of the maximal drug effect
DHV	Dorsal hand vein
G protein	Guanine nucleotide binding regulatory protein
GPCR	G protein coupled receptor
GRK	G protein coupled receptor kinase
GWAS	Genome-wide association study
HR	Heart rate
ICL	Intracellular loop
ICU	Intensive care unit
KO	Knock-out

LC	Locus coeruleus
L-NMMA	N^G-monomethyl-L-arginine
LTF	Light transmission through finger
LVDT	Linear variable differential transformer
MBF	Myocardial blood flow
mRNA	Messenger ribonucleic acid
NA	Noradrenaline
NO	Nitric oxide
NOS	Nitric oxide synthase
PET	Positron emission tomography
PKC	Protein kinase C
PNS	Peripheral nervous system
PTX	Pertussis toxin
RGS	Regulator of G protein signalling protein
RPP	Rate-pressure product
SNP	Single nucleotide polymorphism
SV	Stroke volume
TM (1-7)	Transmembrane domain (1-7)
TTE	Transthoracic echocardiography

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original publications that are referred to in the text by the Roman numerals I-IV.

I Snapir A, Posti J, Kentala E, Koskenvuo J, Sundell J, Tuunanen H, Hakala K, Scheinin H, Knuuti J, Scheinin M (2006). Effects of low and high plasma concentrations of dexmedetomidine on myocardial perfusion and cardiac function in healthy male subjects. *Anesthesiology.*105: 902-10.

II Snapir A, Talke P, Posti J, Huiku M, Kentala E, Scheinin M (2009). Effects of nitric oxide synthase inhibition on dexmedetomidine-induced vasoconstriction in healthy human volunteers. *Br J Anaesth.* 102: 38-46.

III Posti JP, Valve L, Ruohonen S, Akkila J, Scheinin M, Snapir A (2011). Dorsal hand vein responses to the α_1-adrenoceptor agonist phenylephrine do not predict responses to the α_2-adrenoceptor agonist dexmedetomidine. *Eur J Pharmacol*. 25: 70-4.

IV Posti JP*, Salo P*, Ruohonen S, Valve L, Muszkat M, Sofowora GG, Kurnik D, Stein CM, Perola M, Scheinin M, Snapir A (2012). A polymorphism in the protein kinase C gene *PRKCB* is associated with α_2-adrenoceptor-mediated vasoconstriction. *Submitted for publication.*

*equal contribution

1. INTRODUCTION

α_2-Adrenoceptors (α_2-ARs) are cell membrane proteins belonging to the superfamily of G protein coupled receptors (GPCRs). They mediate cellular signaling and respond to noradrenaline (NA) released from nerve cells and adrenaline secreted by the adrenal medulla. Three different subtypes of α_2-ARs, namely α_{2A}, α_{2B} and α_{2C}, have important physiological regulatory functions in different tissues and organs of the body. α_2-ARs in the central and peripheral nervous system modulate the release of NA and adrenaline, and postsynaptic receptors mediate many effects in target tissues. In the cardiovascular system (CVS), the postsynaptic effects of α_2-AR activation include contraction of vascular smooth muscle cells and an opposing effect, production of the vasodilating agent nitric oxide (NO) in endothelial cells, leading to complex net effects on vascular tone and blood flow. This complexity makes them attractive but challenging targets for therapeutic drugs, *i.e.* both antagonists and agonists have been considered as potential therapeutic agents. The classic cardiovascular response to systemic infusion of therapeutic doses of an α_2-AR agonist is biphasic, with an initial short-term increase in blood pressure (BP) followed by a long-lasting decrease in BP and heart rate (HR). The initial response is presumably a result of activation of vascular postsynaptic α_2-ARs, this being later masked by central sympatholysis. The currently available α_2-AR agonist drugs have potent hypotensive, sympatholythic and sedative effects, and they are used in clinical practice for sedation of patients in intensive care units, as adjuncts for general anaesthesia, for BP reduction, and for attenuation of sympathetic overstimulation during opioid and alcohol withdrawal. α_2-AR agonists may also possess organoprotective properties. The lack of subtype-selectivity of current α_2-AR-targeting drugs has been a major limitation in their application. Dexmedetomidine is a highly selective α_2-AR agonist. Dexmedetomidine (Precedex®) was approved by the US Food and Drug Administration in 1999 for sedation of patients undergoing mechanical ventilation in intensive care settings. In 2011, Orion Pharma (Espoo, Finland), the company who originally developed the drug, received European marketing authorisation from the European Medicines Agency for dexmedetomidine (Dexdor®). The current series of studies employed dexmedetomidine as a pharmacological tool to investigate the effects of α_2-AR activation on the CVS.

Despite regulatory approval and a growing interest in the clinical uses of dexmedetomidine in the intensive care setting and as an anaesthetic adjunct, its effects on myocardial perfusion and cardiac function have not been fully explored in humans. The α_2-AR-mediated endothelium-dependent vasodilatation opposing α_2-AR-mediated vasoconstriction has been well documented, but the relative importance of stimulation of NO synthesis for the net effect of dexmedetomidine on vascular tone has not been determined. Several previous studies have reported very substantial inter-individual variability in the dosage of dexmedetomidine required to cause constriction of dorsal hand veins (DHV). A familial, probably genetically determined, factor has been postulated to explain a large share of this variability. This individual variability in the vascular responses to α_2-AR activation may influence vascular responsiveness to sympathetic stimulation in health and disease and may have an impact on the outcome of treatment

with α_2-AR agonists – either on the favourable therapeutic effects or on the adverse effects. However, it is not known whether the receptors themselves respond differently in different individuals, or whether the individual responsivity of DHVs is determined by factors inherent to the vessel itself, downstream of the receptors that initiated the signaling cascade. This thesis comprises a series of studies that were designed to investigate the roles of α_2-ARs in dexmedetomidine-induced effects on the CVS and to explore clinical and genetic determinants of the responses to dexmedetomidine. A secondary aim of the studies was to develop and validate new approaches for the *in vivo* evaluation of drugs active at α_2-ARs. Three of the studies were clinical trials with healthy subjects and one was a combined whole-genome / candidate gene investigation that made use of the results of one of the clinical trials (Study III) and an external study population that was investigated elsewhere in a similar clinical pharmacological setting.

2. REVIEW OF THE LITERATURE

2.1. α_2-Adrenoceptors

2.1.1 The α-adrenoceptors

Adrenoceptors (ARs) are part of the large guanine nucleotide binding regulatory protein (G protein) coupled family of cell surface receptors. G protein coupled receptors (GPCRs) represent the largest family of membrane proteins in the human genome (Zhang *et al.*, 2006). They transduce signals from extracellular exogenous and endogenous ligands and regulate many intracellular effector systems, and are responsible for much of the transmembrane signaling in response to hormones, neurotransmitters and therapeutic drugs (Wettschureck and Offermanns, 2005). The ARs are comprised of three major categories, α_1, α_2 and β, each represented by three subtypes in mammals. The ARs have been thoroughly investigated because they are mediators of many physiologically important effects, such as regulation of cardiovascular, neuronal, endocrine, vegetative, and metabolic functions.

In 1948, Raymond Ahlquist proposed that the ARs consist of two receptor types, α and β, based on the rank orders of potency of five different catecholamine analogues. He postulated that excitatory effects were mediated by α-ARs, whereas β-ARs mediated the inhibitory actions of catecholamines (Ahlquist, 1948). In 1974, it was suggested that postsynaptic α-ARs mediating responses in effector organs should be called α_1-ARs and presynaptic α-ARs regulating the release of NA from nerve endings should be called α_2-ARs (Langer, 1974, Starke *et al.*, 1974). Soon, this classification of α-ARs that was based on anatomical locations was shown to be inappropriate because α_2-ARs were also demonstrated to mediate inhibition of glycolysis and lipolysis (Schimmel, 1976), to have postsynaptic actions regardless of their anatomic location (Berthelsen and Pettinger, 1977), and to mediate excitatory responses in vascular smooth muscle (Drew and Whiting, 1979). Further advances in the understanding of α-ARs came from radioligand binding assays, which, beginning in the early 1980's, began to demonstrate that there were subtypes of both α_1-ARs and α_2-ARs (Bylund and U'Prichard, 1983). However, the relationships between ligand binding sites and functional receptors were not always easy to discern. For instance, there has been a dispute regarding some species differences in α_1-AR antagonist affinity. This led to the assumption that a fourth α_1-AR existed, which was termed the α_{1L}-AR. Only recently has it been demonstrated with the use of genetically modified knock-out (KO) mice that the prostatic α_{1L}-AR phenotype requires the expression of the α_{1A}-AR gene and thus the α_{1L}-AR is not currently recognized as a true receptor subtype (Ventura, 2012). Similarly, it was first thought that also a fourth mammalian α_2-AR subtype existed, since the rodent α_{2A}-AR differed from its human counterpart in terms of its affinity for some agonists and antagonists, and the receptor was for some time called the α_{2D}-AR (Lanier *et al.*, 1991). It was subsequently shown that the different ligand binding properties could be largely explained by a single amino acid substitution in TM5 and two amino acids in extracellular loop 2 (ECL2) (Link *et al.*, 1992, Laurila *et al.*, 2007) (Figure 1).

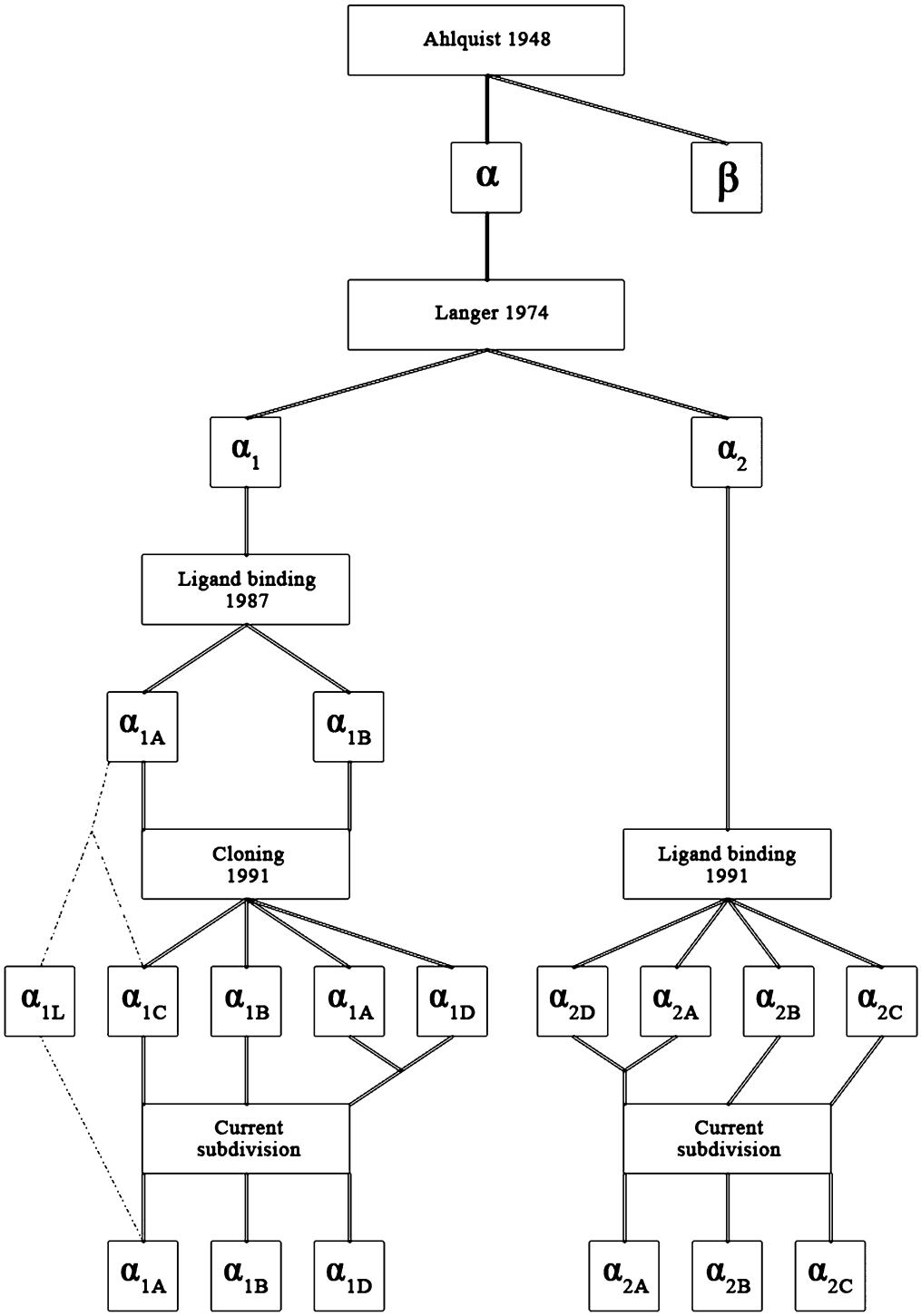

Figure 1. Classification of α-ARs. Advances in molecular biology have enabled the thorough characterization of the six α-ARs that have been cloned, expressed and sequenced. On the basis of structural similarity, localization and pharmacology, these six receptors are grouped into two subfamilies, α_1- and α_2-ARs. Modified from Docherty 1998 and Quaglia *et al*. 2011.

The ARs share considerable amino acid sequence homology. The membrane-spanning domains show the highest degrees of similarity, followed by the first two cytoplasmic loops. The third cytoplasmic loop and the amino and carboxyl termini are the most divergent structures within the AR family (Raymond *et al.*, 1990, Strasser *et al.*, 1992). All human AR genes have been found to be polymorphic, which opens up the possibility that genetic differences in AR structure and function may be relevant *e.g.* for a person's cardiovascular risk profile (Flordellis *et al.*, 2004) and also for genetically targeted drug development.

2.1.2 Subtypes and structure

α_2-ARs are cell surface proteins that mediate many physiological effects in several tissues and organs of the body. The functions mediated by α_2-ARs vary by receptor subtype, tissue and cell type, and the pre- or postsynaptic location of the receptors. The subtypes differ in their pharmacological properties, sensitivity to phosphorylation, desensitization, and internalization. The present classification of α_2-ARs into three subtypes, α_{2A}-, α_{2B}- and α_{2C}-ARs, was first based on the relative affinities of antagonists (Bylund, 1985, Petrash and Bylund, 1986, Bylund, 1988) and was then confirmed by molecular cloning (Kobilka *et al.*, 1987, Regan *et al.*, 1988, Lomasney *et al.*, 1990). After the discovery of three distinct human α_2-AR genes, the receptors were initially labelled α_2C10, α_2C2 and α_2C4 based on the localization of their genes on human chromosomes 10, 2 and 4. The cloned receptors were found to correspond to the pharmacologically identified α_2-AR subtypes α_{2A}, α_{2B} and α_{2C} (Kobilka *et al.*, 1987, Regan *et al.*, 1988, Lomasney *et al.*, 1990, Bylund, 1992). Two additional α_2-AR subtype genes (encoding receptor paralogs) were isolated in the zebrafish genome, which contains five distinct α_2-AR genes, whereas mammals have only three subtypes (receptor orthologs) (Ruuskanen *et al.*, 2005) (Figure 1).

α_2-ARs are monomeric polypeptides that in humans consist of 450 (α_{2A}-AR and α_{2B}-AR) or 461 (α_{2C}-AR) amino acids. α_2-ARs share the common molecular properties of all rhodopsin-like GPCRs. According to insights derived from computational modeling, α_2-ARs consist of seven α-helical transmembrane domains (TM1-TM7) that are connected by three intra- (ICL) and three extracellular (ECL) hydrophilic loops (Trabanino *et al.*, 2004, Freddolino *et al.*, 2004). The extracellularly located N-terminus is glycosylated in subtypes A and C *via* two asparagine residues (Lomasney *et al.*, 1991). Rhodopsin has two palmitoylated cysteines in its C-terminus. In the human α_{2A}-AR and α_{2B}-AR, a single cysteine-linked palmitate is present in the intracellular C-terminus, but no cysteine is found in this region of the human α_{2C}-AR. The TM domains form a binding pocket for ligands inside the core of the receptor protein (Figure 2). Like many other GPCRs coupled to G_i- and G_o-type G proteins, the α_2-ARs have long third intracellular loops (ICL) consisting of approximately 150 amino acids. The amino acid similarity between the human α_2-AR subtypes is about 50 % in the entire proteins and about 75 % in the TM domains (Herz *et al.*, 1997). The seven TMs are well conserved between the receptor subtypes, whereas the amino terminus, the carboxyl terminus, and the third ICL are the most divergent domains (Lomasney *et al.*, 1990). All aspects of the interplay of GPCRs and other components of cell membranes are not completely understood, partly because high-resolution structures of GPCRs have not been available. Crystal structures of GPCRs first became available for bovine rhodopsin (Palczewski *et al.*, 2000) and the β_2-AR (Rasmussen *et al.*, 2007). In rhodopsin, the ligand (retinal) binding

site is covered by ECL2 that connects TM4 and TM5 (Palczewski *et al.*, 2000), and it has been suggested that other rhodopsin-like GPCRs have a similar structure (Shi and Javitch, 2004). The binding pocket of β_2-AR was identified in electron-density maps to be close to the extracellular side of the TMs, in agreement with the retinal binding site of rhodopsin (Rasmussen *et al.*, 2007). No crystal structures of α_2-ARs have been published, but combined mutagenesis and computer modelling studies suggest that their structures closely resemble that of the β_2-AR, with the ligand binding site covered by an ECL2 constrained by a disulphide bridge (Laurila *et al.*, 2011).

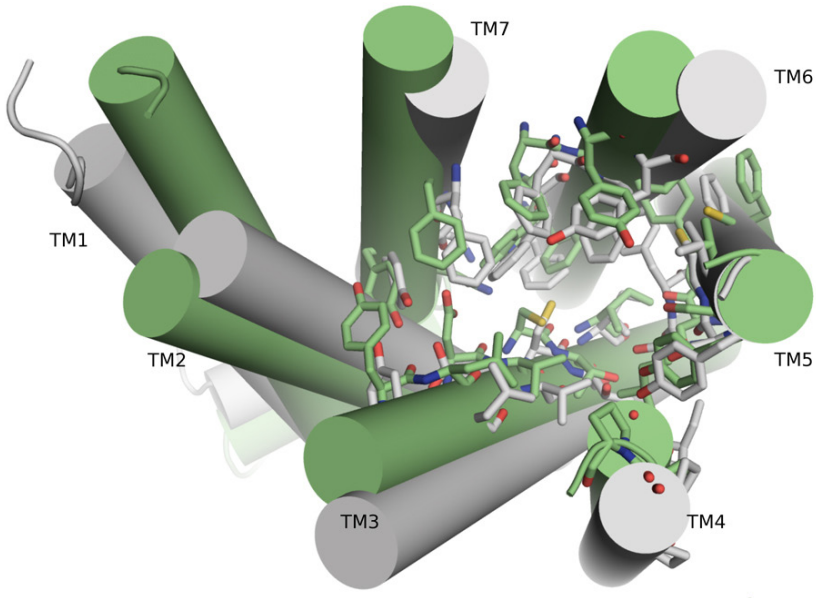

Figure 2. Molecular model and the binding cavity of α_{2A}-AR viewed from the extracellular side of the cell membrane, based on the bovine rhodopsin (green) and human β_2-AR structures (grey). The surface of the binding cavity is formed by amino acids of the TM helices; for clarity, not all amino acids that face the binding cavity are shown. Courtesy of Dr. Henri Xhaard, PhD.

2.1.3 Signaling pathways and regulation

According to currently held views, the structures of rhodopsin-like GPCRs exist in an equilibrium between an inactive and an active state, the former being stabilized by inverse agonists, and the latter induced and/or stabilized by agonists and by constitutively activating mutations (Gether *et al.*, 1997, Nyronen *et al.*, 2001, Xhaard *et al.*, 2005, Rosenbaum *et al.*, 2009). It has been suggested that a common activation mechanism of rhodopsin-like GPCRs exists allowing extracellular ligands to transduce receptor activation to intracellular G proteins (Oliveira *et al.*, 2003, Schwartz *et al.*, 2006).

Receptor-activated heterotrimeric G proteins are bound to the intracellular surface of the cell membrane and they mediate signals from activated GPCRs to intracellular effector systems (Hamm, 2001). G proteins consist of an α-subunit, which binds and hydrolyses GTP, and a non-dissociable βγ-subunit complex. In the absence of an agonist, GPCRs are in a low-affinity state in a complex with a heterotrimeric G protein in its GDP-bound

conformation. The activated receptor interacts with the G protein, evoking a change in its conformation that results in the dissociation of GDP from the α-subunit and its replacement with GTP. This is followed by dissociation of the GTP-bound α-subunit from the activated receptor as well as from the βγ-subunit complex. Then the activated α-subunit and the βγ-complex can both interact with effectors. The α-subunit has an integral GTPase activity, which hydrolyses GTP to GDP and terminates the signaling. This is followed by reassociation of the GDP-bound α-subunit and the βγ-complex, which completes the G protein activity cycle (Clapham and Neer, 1997, Hamm, 1998, Hamm, 2001, Cabrera-Vera *et al.*, 2003) (Figure 3).

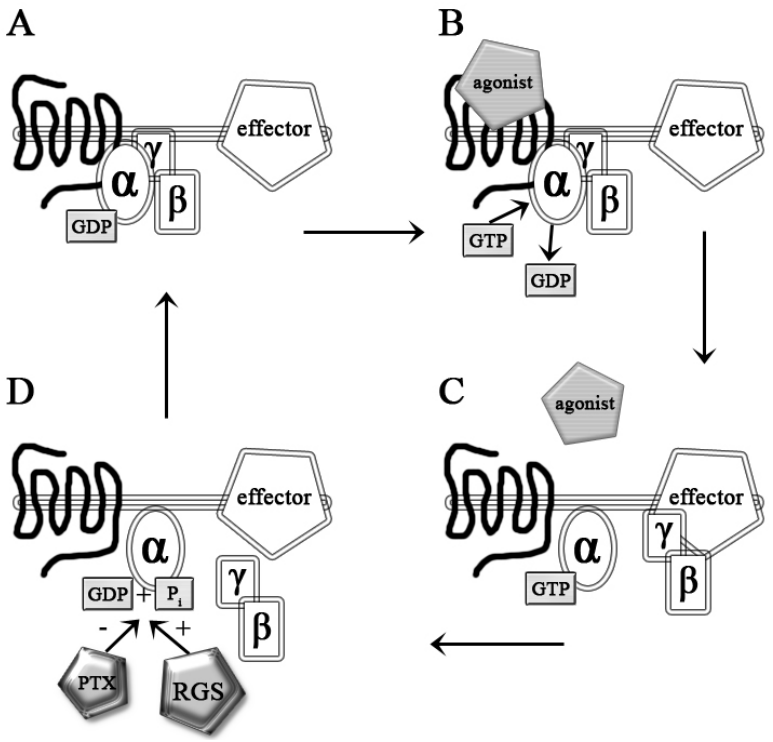

Figure 3. The GPCR cycle. A, In the basal state, the receptor is in a low-affinity mode with G-protein Gα subunits in the GDP-bound conformation. B, When a ligand (agonist) activates the GPCR, it induces a conformational change in the receptor that promotes the exchange of GDP for GTP on the Gα subunit. In the traditional view of heterotrimeric G protein activation, this exchange triggers the dissociation of the Gα subunit, bound to GTP, from the Gβγ dimer and the receptor. C, Both Gα-GTP and Gβγ (shown in the figure) are capable of activating different second messenger pathways and effector proteins, while the receptor is able to activate the next G protein. D, Gα has an integral GTPase activity that hydrolyses GTP to GDP and terminates the signaling. This is followed by reassociation of Gα-GDP and the Gβγ complex that terminates the G protein activity cycle. Pertussis toxin (PTX) can ADP-ribosylate most of the members of the Gαi/Gαo family; ADP-ribosylated G proteins are unable to interact with the receptor, thus PTX treatment results in the uncoupling of the receptor and the G protein. RGS proteins attenuate GPCR signalling by binding to Gα and accelerating the GTPase leading to accelerated termination of the G protein signalling. $G\alpha_i$ and $G\alpha_q$ subtypes are sensitive to GTPase acceleration activity. Modified from Wetschureck & Offermanns, 2005.

G proteins are divided into four functional subcategories: G_s, which activate adenylyl cyclases (ADCYs), $G_{i/o}$, which inhibit ADCYs, inhibit voltage-dependent Ca^{2+} channels, and activate inwardly rectifying, hyperpolarizing K^+ channels, $G_{q/11}$, which activate phospholipase C, and $G_{12/13}$, which can activate many signalling cascades including RhoA and tyrosine kinases. A total of 17 genes for α-subunits, 5 genes for β-subunits, and 12 genes for γ-subunits are known in humans (Offermanns, 2003, Wettschureck and Offermanns, 2005). The functions of the members of the G_i/G_o family have often been studied using a toxin from the bacterium *Bordetella pertussis* (pertussis toxin; PTX) that is able to ADP-ribosylate the α-subunits of the G_i/G_o family, thus interfering with intracellular signaling (Offermanns, 2003) (Figure 3).

The best-known signalling pathway of all α_2-AR subtypes is inhibition of ADCYs through activation of G_i that results in inhibition of cAMP formation (Cotecchia *et al.*, 1990, Eason *et al.*, 1992, Chabre *et al.*, 1994, Pohjanoksa *et al.*, 1997). However, this pathway alone cannot account for all of the actions mediated by α_2-ARs in different cell types, tissues and organs. The effects of G protein-mediated α_2-AR coupling to intracellular effector mechanisms may differ depending on the receptor subtype and the cell type. For instance, α_{2A}-ARs seem to inhibit L-type Ca^{2+} channels in neurons of the locus coeruleus (LC), whereas in the vasculature, α_{2B}-ARs may activate a similar effector mechanism (Kamibayashi and Maze, 2000, Owesson *et al.*, 2003). Indeed, even the same receptor subtype has been reported to couple to different effector systems in different environments. A recent *ex vivo* study showed that in the endothelium of the rat mesenteric artery, α_{2A}-ARs and to a lesser extent also α_{2B}-ARs, mediate vasodilatation by activating endothelial nitric oxide synthase (NOS) (Wong *et al.*, 2010), while, based on studies on α_2-AR-KO mice, it seems that α_{2B}-ARs mediate vasoconstriction evoked by vascular smooth muscle contraction (Link *et al.*, 1996, MacMillan *et al.*, 1996). Additionally, coupling *via* G_q- and G_s-mediated pathways and different downstream effector circuitries has been reported for all α_2-AR subtypes, at least in transfected cells (Table 1). The vast range of different possible effector cascades from modulation of ADCYs and phospholipase C to regulation of cell growth and vasoconstriction *via* stimulation of mitogen-activated protein kinases imply that the precise functions of different α_2-AR subtypes in different environments are far from definitively clarified, and the relevance of distinct intracellular signalling pathways for the physiological functions of α_2-ARs is only partially understood.

Activation of a GPCR does not solely transduce signaling to an isolated effector system. GPCR activation by an agonist triggers concurrent regulatory processes that can lead to receptor desensitization and internalization. The regulation of receptor activity complements the biological meaningfulness of signal transduction. GPCR desensitization attenuates receptor-mediated signalling as a function of time and therefore this confers protection against overstimulation of the signalling system. It also enables filtering towards multi-source receptor input and thus integrates the information flow that is essential for efficient cellular functioning.

The GPCR desensitization paradigm is classically defined as a consequence of synergism of different mechanisms that result in uncoupling of receptors from their cognate G proteins by phosphorylation and β-arrestin binding (Ferguson, 2001).

Table 1. Suggested α_2-AR signalling pathways

Receptor	G protein	Mediator / Second messenger	Effect	Cell / tissue type	Receptor origin	Studies
α_{2A}-AR[1, 2, 3, 4] α_{2B}-AR[2] α_{2C}-AR[1, 2, 4]	G_i	ADCY ↓	cAMP ↓	CHLF[1] CHO[2, 4] HEK 293[3]	wtH-α_2-ARs [1, 2, 4] wtP-α_{2A}-AR[3]	Cotecchia *et al.*, 1990[1] Eason *et al.*, 1992[2] Chabre *et al.*, 1994[3] Pohjanoksa *et al.*, 1997[4]
α_{2A}-AR α_{2C}-AR	G_i	PLC ↑[I] IP_3 ↑[II]	$[Ca^{2+}]_{rel}$↑	CHLF COS-7	wtH-α_2-AR	Cotecchia *et al.*, 1990
α_{2A}-AR[3, 5, 7] α_2-AR[6]	G_q[3, 7] G_q+ R183C[5] PTXi[6]	PLC ↑[I] IP_3 ↑[II]	$[Ca^{2+}]_{rel}$↑	HEK 293[3, 5] COS-7[5] DCT[6] PMC[7]	wtP-α_{2A}-AR[3, 5] endog[6, 7]	Chabre *et al.*, 1994[3] Conklin *et al.*, 1992[5] Gesek, 1996[6] ZhuGe *et al.*, 1997[7]
α_{2A}-AR[2, 3] α_{2C}-AR[2] α_{2B}-AR[2, 4, 8]	G_s	ADCY ↑	cAMP ↑	CHO[2, 4] HEK 293[3] Sf-9[8]	wtH-α_2-ARs[2] wtP-α_{2A}-AR[3] wtH-α_{2B}-AR[8]	Eason *et al.*, 1992[2] Chabre *et al.*, 1994[3] Jansson *et al.*, 1995[8] Pohjanoksa *et al.*, 1997[4]
α_2-AR	G_i	βγ-dimers[I] GIRK ↑[II]	$[K^+]_{inf}$↑	AtT20[12] MBLA[13]	wtH-α_2-ARs[12] endog[13]	Surprenant *et al.*, 1992[12] DeBock *et al.*, 2003[13]
α_2-AR	G_i		$[Ca^{2+}]_{rel}$↑ $[Ca^{2+}]_{inf}$↑	HSRA[14] HORA[15]	endog[14] endog[15]	Parkinson and Hughes, 1995[14] Hughes *et al.*, 1996[15]
α_2-AR	$G_?$	RhoK ↑[17] PI3K ↑[16, 17] PKC ↑[6, 16, 17]	$[Ca^{2+}]_{rel}$ ↑[6] $[Ca^{2+}]_{inf}$ ↑[16] $[Ca^{2+}]_{sens}$ ↑[17]	DCT[6] CMV[16] RA[17]	endog	Gesek *et al.*, 1996[6] Yamboliev *et al.* 2005[16] Kim *et al.* 2011[17]
α_{2A}-AR	$G_{i/o}$	ADCY [I] L-type VDCC ↑[II]	$[Ca^{2+}]_{inf}$ ↑	PMC	endog	ZhuGe *et al.*, 1997
α_{2A}-AR	G_i	βγ-dimers[I] PLC ↑[II] IP_3 ↑[III]	$[Ca^{2+}]_{rel}$↑	COS-7 CHO HEL	wtH-α_{2A}-AR, (CHO, COS-7) endog (HEL)	Dorn *et al.*, 1997
α_2-AR	$G_{i/o}$	βγ-dimers[I] P/Q-type VDCC[II] ↓	$[Ca^{2+}]_{inf}$↓	MVD[18] RCRN[19]	endog[16] endog[17]	Waterman, 1997[18] Li and Bayliss, 1998[19]

α_{2A}-AR	G_i	βγ-dimers[I] PLCβ ↑[II] $[Ca^{2+}]_{rel}$ ↑[III] CaM ↑[IV] Pyk2 ↑[V] Src ↑[VI] Shc ↑[VII] mSOS / Grb2 [VIII] Ras ↑[IX] Raf ↑[X]	MAPK-ERK ↑	HEK-293	wtH-α_{2A}-AR	Della Rocca *et al.*, 1997
α_2-AR	G_i G_o	βγ-dimers[I] N-type VDCC ↓[II]	$[Ca^{2+}]_{inf}$↓	RSCGN	endog	Delmas *et al.*, 1999
α_{2B}-AR	$G_?$	PLA_2 ↑[I] AA_{rel} ↑[II] LOX / COX ↑[III] MMP ↑[IV] $HB\text{-}EGF_{rel}$ [V] EGFR ↑[VI]	MAPK-ERK ↑	LLC-PK_1	wtR-α_{2B}-ARs	Cussac *et al.*, 2002a
α_{2B}-AR	$G_{i/o}$	[PLA_2 ↑, AA_{rel} ↑]α Shc ↑[I] mSOS / Grb2[II]	Ras / MAPK-ERK ↑	RPTC LLC-PK_1	endog (RPTC) wtR-α_{2B}-ARs (LLC-PK_1)	Cussac *et al.*, 2002b
α_2-AR	$G_?$	Src ↑[I] PI3K ↑[II] $[Ca^{2+}]_{inf}$ ↑[III] EGFR ↑[IV]	MAPK-ERK ↑	PPLV	endog	Roberts, 2001 Roberts, 2003
α_2-AR	$G_?$	Rho/RhoKP ↑[I] MP ↓[II]	MLC ↑	PPLV	endog	Roberts, 2004
α_{2A}-AR α_{2B}-AR α_{2C}-AR	$[G_{i/o}]\beta$	PLC ↑[I] AA_{rel} ↑[II] P450-EOX ↑[III] Src ↑[IV]γ MMP ↑[V] $HB\text{-}EGF_{rel}$ [VI] EGFR ↑[VII]	MAPK-ERK ↑ Akt ↑	PC12	wtH-α_2-ARs	Karkoulias *et al.*, 2006

α_2-AR	$G_{i/o}$	βγ-dimers^{I} [ADCY ↑]δ PLC ↑^{II} PKC ↑^{III} ADCY ↑^{IV} [PKA ↑^{V}]ε	[ADCY ↑]ζ [NA_{rel}]ε	RTA	endog	Fresco *et al.*, 2007
α_{2A}-AR	G_i	eNOS ↑	NO_{rel}	RMA[18] RMA, RA[19]	endog[20] endog[21]	Figueroa *et al.*, 2001[20] Wong *et al.*, 2010[21]

Mediator / Second messenger and Effect columns: Superscript Roman numerals denote positions in the second messenger cascades: ↑, stimulation; ↓, inhibition; ADCY, adenylyl cyclase; cAMP, cyclic AMP formation; PLC, phospholipase C; IP_3, inositol triphosphate; $[Ca^{2+}]_{rel}$, release of intracellular calcium; G_q + R183C, G_q with mutated α subunit (arginine 183 replaced with cysteine); PTXi, pertussis toxin-insensitive mechanism; βγ-dimers, G protein-derived beta-gamma dimers; GIRK, G protein-dependent inward-rectifying K^+ channels; $[K^+]_{inf}$, influx of extracellular potassium; VDCC, voltage-dependent calcium channels; $[Ca^{2+}]_{inf}$, influx of extracellular calcium; RhoK, Rho kinase; PI3K, phosphatidylinositol 3-kinase; PKC, Protein kinase C; $[Ca^{2+}]_{sens}$, calcium sensitization; CaM, calmodulin; Pyk2, focal adhesion kinase family protein-tyrosine kinase; Src, Src tyrosine kinase; Shc, Shc phosphorylation of adaptor protein; mSOS/Grb2, formation and recruitment of the Grb2-Sos(-Shc) complex to the cell membrane; Ras, activation of Ras GTPase through guanine nucleotide exchange; Raf, phosphporylation cascades initiated by proto-oncogene c-RAF; MAPK-ERK, activation of MAPK-ERK by phosphorylation; PLA2, phospholipase A_2; AA_{rel}, release of arachidonic acid; LOX / COX, generation of AA metabolites by cyclooxygenase and/or lipoxygenase; MMP, matrix metalloproteinases; $HB\text{-}EGF_{rel}$, release of heparin-binding EGF-like growth factor; EGFR, transactivation of epidermal growth factor receptor; MP, myosin phosphatase; MLC, myosin light chain phosphorylation; P450-EOX, P450-dependent epoxygenase; Akt, Akt serine/threonine protein kinase; PKA, protein kinase A; NA_{rel}, noradrenaline release; eNOS, endothelial nitric oxide synthase; NO_{rel}, release of nitric oxide;

Greek characters in Mediator / Second messenger and Effect column: α, the relationship between MAPK and PLA2 was not directly investigated in the study; β , the role of pertussis toxin sensitive G proteins is only mentioned in the introduction; γ, α_{2A}- and α_{2C}-ARs may directly activate MAPK-ERK and Akt by phosphorylation induced by Src activation; δ, ADCY may be directly activated by βγ-dimers; ε, ADCY was suggested to be activated by both α_2-AR and adenosine A_{2A} that leads to NA release induced by PKA activation; ζ, pathway that is solely activated by α_2-AR ends with ADCY activation

Cell / tissue type and Receptor origin columns: Superscript Arabic numerals refer to the studies; CHLF, transfected Chinese hamster lung fibroblasts; CHO, transfected Chinese hamster ovary cells; HEK 293, transfected human embryonic kidney 293 cells; wtH-α-ARs, human wild-type α-ARs; wtP-α_{2A}-AR, wild-type porcine α_{2A}-ARs; COS-7, transfected kidney cells of the African green monkey; DCT, kidney distal convoluted tubule cells; PMC, porcine myometrial cells; endog, receptors are expressed endogenously; Sf-9, transfected *Spodoptera frugiperda* insect cells; AtT20, transfected AtT20 mouse pituitary tumour cells; MBLA, mouse basolateral amygdala neurons; HSRA, human subcutaneous resistance arteries; HORA, human omental resistance arteries; CMV, canine mesenteric vein smooth muscle cells; RA, rat aorta smooth muscle cells, HEL, human erythroleukemia cells; MVD, mouse vasa deferentia; RCRN, rat caudal raphe neurons; RSCGC, rat superior cervical ganglion neurones transfected with various G_α subunits; LLC-PK_1, transfected porcine kidney renal epithelial cells; wtR-α_{2B}-ARs, rat wild-type α_{2B}-ARs; RPTC, rat proximal tubule cells; PPLV, porcine palmar lateral vein; PC12, transfected rat adrenal medulla pheochromocytoma cells; RTA, rat tail artery; RMA, rat mesenteric artery; RA, rat aorta

GPCR phosphorylation is typically directed towards serine and threonine residues, but sometimes also towards tyrosine residues located in the third ICL and the C-terminus. Phosphorylation-mediated GPCR desensitization occurs as a result of activation of specific GPCR kinases (GRKs) and at least two second messenger-dependent kinases, cAMP-dependent protein kinase (protein kinase A) and protein kinase C (PKC). GRKs recognize agonist-activated GPCRs and phosphorylate them in a manner that increases their affinity to bind β-arrestins (Lohse *et al.*, 1990, Kurose and Lefkowitz, 1994, Pei *et al.*, 1994, Ferguson, 2001). Phosphorylation mediated by protein kinase A and PKC may be triggered regardless of the activation state of a GPCR, and it does not involve β-arrestin coupling (Lohse *et al.*, 1990, Hausdorff *et al.*, 1989, Pitcher *et al.*, 1992). It has been shown that β-arrestin-coupled GPCR desensitization is not always dependent on phosphorylation (Jala *et al.*, 2005), and GRK2 and some other GPCR-interacting proteins are able to uncouple receptors from their G proteins without phosphorylation (Ferguson, 2007).

GPCR signalling can be terminated at the Gα-subunit level by regulator of G protein signalling (RGS) proteins that are negative modulators of G protein signalling. The RGS proteins are a family of more than 30 intracellular proteins and they exhibit control over G protein signal strength and may thus affect agonist efficacy and potency, resembling the actions of phosphorylation and β-arrestin binding. RGS proteins attenuate GPCR signalling by binding to the Gα-GTP-complex as well as by accelerating the GTPase activity (Ross and Wilkie, 2000, Hollinger and Hepler, 2002). This leads to accelerated termination of G protein signalling and thus reassociation of the heterotrimeric G protein. G_i and G_q subtypes are sensitive to GTPase acceleration activity because of their naturally relatively slow rate of hydrolysis of GTP.

GPCR internalization provides control over the cell membrane receptor density and signal duration and strength. GPCR coupling with β-arrestin induces conformational changes in β-arrestin, leading to exposure of its C-terminus that interacts with the β2-adaptin subunit of the clathrin adaptor AP-2 complex that targets the GPCR-arrestin complex to clathrin-coated pits. Subsequently, the receptor-arrestin complex is internalized through clathrin-coated vesicles, followed by the dissociation of arrestin (Goodman *et al.*, 1996, Hirsch *et al.*, 1999, Ferguson, 2001, Lefkowitz and Shenoy, 2005, Marchese *et al.*, 2008).

Internalized receptors are transported within endosomal-lysosomal granules. However, endocytic trafficking does not always lead to signal termination of all GPCRs. Recent studies have shown that endocytosed GPCRs may continue to stimulate (Calebiro *et al.*, 2009, Ferrandon *et al.*, 2009) or inhibit (Mullershausen *et al.*, 2009) ADCYs *via* interactions with G_s and G_i, respectively, and thus regulate cAMP levels. It has been postulated that certain GPCRs are able to remain associated with G proteins and ADCYs in the intracellular compartments (Jalink and Moolenaar, 2010).

Many membrane receptors are known to be present in living cells as homo- or heterodimers or higher-order oligomers. This phenomenon may modify their pharmacological and physiological properties in terms of *e.g.* trafficking of receptors, ligand binding, cell signalling and desensitization. Dimerization has not been shown to be necessary for signalling of GPCRs, whereas for tyrosine kinase receptor signalling, dimerization is

a necessity (Agnati *et al.*, 1982, Prinster *et al.*, 2005, Congreve and Marshall, 2010). Dimerization has also been reported to occur for some ARs. For instance, Small and colleagues demonstrated with bioluminescence resonance energy transfer that α_{2A}-ARs and α_{2C}-ARs form heterodimers (Small *et al.*, 2006). Co-immunoprecipitation indicated that the receptors could form both homo- and heterodimers. The α_{2A}-α_{2C}-AR heterodimer seemed to have signalling properties intermediate between the two receptor subtypes. α_{2C}-AR heterodimerization with α_{2A}-AR resulted in lowered agonist-promoted GRK2-mediated phosphorylation of α_{2A}-AR and reduced β-arrestin binding (Small *et al.*, 2006). This may have a regulatory impact on α_{2A}- and α_{2C}-AR functions in presynaptic nerve endings (Hein, 2006) (see 2.2.2).

2.1.4 Tissue distributions

The tissue distributions of the α_2-AR subtypes have been best documented in rodents (Boyajian *et al.*, 1987, MacDonald and Scheinin, 1995), but to some extent also in man (Saunders and Limbird, 1999). The lack of subtype-selective agonists and antagonists has hampered research on the distribution and functions of the α_2-AR subtypes. Studies on genetically engineered mice with α_2-AR subtype gene deletions have helped to elucidate the specific functions of the receptor subtypes (MacDonald *et al.*, 1997, Philipp and Hein, 2004), but current knowledge with regard to the human α_2-AR subtypes is still far from complete.

α_{2A}- and α_{2C}-ARs are the predominant subtypes in the central nervous system (CNS), within the brain and the spinal cord (Nicholas *et al.*, 1993, Scheinin *et al.*, 1994, Nicholas *et al.*, 1996, Wang *et al.*, 1996). In rodents, the α_{2A}-AR subtype is expressed widely throughout the CNS, but α_{2A}-ARs are also present extensively in peripheral organs. α_{2A}-ARs are present in the LC and other noradrenergic cell body aggregates as well as all major noradrenergic projection areas of the brain and spinal cord (Boyajian *et al.*, 1987, Nicholas *et al.*, 1993, Scheinin *et al.*, 1994). α_{2C}-ARs are far less abundantly distributed in the brain, but are present in the striatum, hippocampus and cerebral cortex (Scheinin *et al.*, 1994, Sallinen *et al.*, 1997, Holmberg *et al.*, 2003). Messenger ribonucleic acid (mRNA) for α_{2B}-ARs has been observed in the rat thalamus, while in mice, mRNA for α_{2B}-AR has also been found in the hippocampus, olfactory system and Purkinje cells of the cerebellum (Nicholas *et al.*, 1993, Scheinin *et al.*, 1994, Wang *et al.*, 1996, Winzer-Serhan and Leslie, 1997). The presence of mRNA is not proof of protein expression, and the information provided by mRNA analysis provides only limited information on the significance of a receptor subtype in a given tissue. Indeed, receptor autoradiography experiments with mouse lines with α_2-AR subtype gene deletions have failed to confirm the expression of the α_{2B}-AR gene in the mouse brain (Bucheler *et al.*, 2002).

α_2-AR subtype distributions in peripheral organs have been investigated with radioligand binding assays, *in situ* hybridization and functional assay methods. Radioligand binding assays may not detect the receptor, if the expression levels are low (MacDonald *et al.*, 1997, Saunders and Limbird, 1999). In humans, α_{2A}-ARs have been found in blood platelets, kidney, heart, aorta, liver, spleen, lungs, adrenals, pancreas, mammary gland, placenta, the vasculature, urinary bladder, urethra, prostate and fat tissue. More limited information exists for α_{2B}-ARs and α_{2C}-ARs, but both subtypes have been found in

kidneys and blood vessel walls (Perala *et al.*, 1992, Eason and Liggett, 1993, Berkowitz *et al.*, 1994, MacKinnon *et al.*, 1994, Castan *et al.*, 1995, Lacey *et al.*, 1996, Michel and Vrydag, 2006, Vazquez *et al.*, 2006).

2.1.5 Genetic polymorphisms

The human α_2-ARs are encoded by three intronless genes, approximately 2.8–3.7 kb in length. The α_{2A}-AR gene (*ADRA2A*) is located on 10q24-q26 (www.ncbi.nlm.nih.gov/gene/150), the α_{2B}-AR (*ADRA2B*) gene on 2p13-q13 (www.ncbi.nlm.nih.gov/gene/151), and the α_{2C}-AR gene (*ADRA2C*) on 4p16 (www.ncbi.nlm.nih.gov/gene/152). The α_2-AR subtype genes are well conserved across all mammalian species (Xhaard *et al.*, 2006).

Sequence variations exist within the coding regions of all three human α_2-AR genes. Several polymorphisms alter the structures of the α_2-AR proteins, sometimes resulting in changes in receptor signalling, including alterations in G protein coupling, desensitization, and GRK-mediated phosphorylation (Small and Liggett, 2001, Small *et al.*, 2006). Interesting polymorphisms, single nucleotide polymorphisms (SNPs) and insertion/deletion of amino acids, have been found in the third ICLs of the receptors (Flordellis *et al.*, 2004).

Associations between genetic polymorphisms in the α_2-ARs and physiological and pathological phenotypes have been investigated by several groups. The focus of most studies has been on the CVS, but also lipolysis, diabetes, pain perception, and cognitive functions have been investigated. The large inter-individual variability in the vasoconstriction responses to α_2-AR agonist activation and associations with clinical cardiovascular pathology have inspired clinical experiments with healthy subjects and patients as well as case-control and population-based genetic association studies.

The characteristic effect of the α_{2A}-AR subtype to mediate hypotension in response to agonist activation has prompted research groups to investigate cohorts of patients with hypertension in order to detect possible disease associations with α_{2A}-AR gene polymorphisms. α_{2A}-AR-KO mice exhibit a hyperadrenergic phenotype including elevated plasma NA concentrations, HR and BP. In α_{2A}-AR-KO mice, administration of an α_2-AR agonist does not lead to normal reductions in HR and BP (Altman *et al.*, 1999, Makaritsis *et al.*, 1999). *ADRA2A* could be an important candidate gene also in some other than cardiovascular diseases, since α_{2A}-ARs regulate lipid and glucose metabolism (Castan *et al.*, 1995, Savontaus *et al.*, 2008, Rosmond *et al.*, 2002), body temperature (Hunter *et al.*, 1997), platelet aggregation (Freeman *et al.*, 1995) and cognitive functions, mood and behaviour (Schramm *et al.*, 2001).

Genetic variation of *ADRA2A* and its haplotype structure have been investigated (Small *et al.*, 2006, Kurnik *et al.*, 2006), and some *in vivo* associations between *ADRA2A* genetic variants and receptor expression and function have been reported (Small *et al.*, 2006, Rosengren *et al.*, 2010, Kurnik *et al.*, 2011). Several studies have investigated the involvement of the 3′-UTR SNP rs553668 (G > A), formerly known as the *Dra*I (6.7/6.3 kb) restriction fragment length polymorphism of *ADRA2A* with hypertension. This polymorphism has a minor allele frequency of approximately 15 % in white and

20-30 % in black North American subjects (Small *et al.*, 2006, Kurnik *et al.*, 2006). The results show that there is no association between this polymorphism and hypertension in whites (Sun *et al.*, 1992), but in blacks, an association has been consistently reported (Lockette *et al.*, 1995, Svetkey *et al.*, 1996). However, two large genome-wide association studies (GWAS) did not associate *ADRA2A* with hypertension (Wellcome Trust Case Control Consortium, 2007, Newton-Cheh *et al.*, 2009). Kurnik et al. (Kurnik *et al.*, 2011) recently reported that homozygous carriers of rs553668 and the corresponding haplotype 4, previously associated with increased α_{2A}-AR expression (Small *et al.*, 2006, Rosengren *et al.*, 2010), and carriers of haplotype 3, previously associated with reduced α_{2A}-AR expression (Small *et al.*, 2006), contribute to the inter-individual variability in BP and HR responses to dexmedetomidine. Homozygous carriers of haplotype 3 present with approximately 40 % smaller hypotensive responses to dexmedetomidine, whereas carriers of haplotype 4 display 100 % greater responses (Kurnik *et al.*, 2011).

A recent study reported that rs553668 in *ADRA2A* was also associated with impaired insulin secretion and type 2 diabetes mellitus (Rosengren *et al.*, 2010), and a meta-analysis of GWASs identified a strong association of rs10885122 with higher fasting blood glucose levels (Dupuis *et al.*, 2010). Several *ADRA2A* polymorphisms have been described in the 5´-flanking region of the gene (5´-UTR) and its 3´-UTR. For instance, rs1800544 and rs1800545 have been investigated in several populations, because they can be readily genotyped with restriction enzymes (Kurnik *et al.*, 2006).

There are two common functional polymorphisms in *ADRA2B*. One consists of insertion or deletion (Ins/Del) of nine nucleotides in position +901 of the coding region (901_909del, rs4066772), leading to an Ins/Del of three glutamic acid residues in the third ICL of the receptor (Heinonen *et al.*, 1999). This variation has been subject to multiple investigations. Studies on transfected cells have demonstrated that homozygosity for the deletion results in substantially decreased GRK-mediated agonist-promoted receptor desensitization, but does not affect ligand binding or G protein coupling (Small *et al.*, 2001). The deletion variant has been associated with reduced flow-mediated dilatation of the brachial artery (Heinonen *et al.*, 2002), an increased risk of myocardial infarction and sudden cardiac death (Snapir *et al.*, 2001, Snapir *et al.*, 2003b), decreased myocardial blood flow (MBF) and increased peripheral resistance upon adrenaline infusion (Snapir *et al.*, 2003a), obesity (Heinonen *et al.*, 1999), and an increased risk of early-onset hypertension (von Wowern *et al.*, 2004) in Caucasians. In a Japanese study population, the Del/Del genotype was associated with increased activity of the sympathetic nervous system (Suzuki *et al.*, 2003), and in a Chinese study population, the Ins allele of the *ADRA2B* gene was associated with higher BP, but on the other hand with lower body mass index (BMI) and smaller waist-to-hip ratio, lower serum insulin concentrations and higher insulin sensitivity (Zhang *et al.*, 2005). However, some large GWAS studies that investigated genetic variation associated with hypertension did not identify *ADRA2B* as a risk gene (International Consortium for Blood Pressure Genome-Wide Association Studies *et al.*, 2011, Johnson *et al.*, 2011). Another *ADRA2B* polymorphism was more recently identified, *i.e.* a 12-nucleotide Ins/Del at position −4825 4.8 kb upstream from the *ADRA2B* coding region (Crassous *et al.*, 2010). The previously identified 901_909del polymorphism is in strong linkage disequilibrium with the −4825 Del/Ins. The −4825

Ins allele is associated with markedly reduced transcriptional activity *in vitro*, but its functional impact has not yet been studied *in vivo* (Crassous *et al.*, 2010).

A variant form of the α_{2C}-AR subtype gene (*ADRA2C*) with a 12-bp deletion (loss of Gly-Ala-Gly-Pro in the third ICL) has been found with a minor allele frequency of 46 % in black North Americans and 4 % in whites (Kurnik *et al.*, 2008). In transfected Chinese hamster ovary cells, the variant receptor showed impaired coupling to G_i, with subsequent decreased inhibition of ADCY and reduced stimulation of mitogen-activated protein kinases (Small *et al.*, 2000). This del322-325 variant has been associated in African-American subjects with an increased risk for the development of congestive heart failure (Small *et al.*, 2002). In another study with Caucasian subjects, the frequency of the deletion variant did not differ between heart failure patients (11 %) and healthy controls (11 %). However, the heart failure patients with the deletion variant of the receptor suffered more impaired cardiac function compared to the other patients (Small *et al.*, 2002, Brede *et al.*, 2002).

Very large inter-individual variability exists in the constriction responses of DHVs to α-AR agonist activation. A range of over 1000-fold has been observed between subjects in their sensitivities to respond to local infusions of the α_2-AR agonist dexmedetomidine, as assessed with ED_{50} values (Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a). Genetic factors may to contribute significantly, as DHV sensitivity to constrict after infusion of NA is a familial trait (Luthra *et al.*, 1991, Gupta and Carruthers, 1997). Studies with mono- and dizygotic twin pairs and parents and their children have clearly demonstrated that genetic factors contribute significantly to the inter-individual differences in the responses of DHVs to NA, although these studies did not examine the genes involved (Luthra *et al.*, 1991, Gupta and Carruthers, 1997). Pharmacogenetic association studies of several candidate genes (i.e. *ADRA2B*, 901_909del and other polymorphisms, and *ADRA1A*, the α_{1A}-AR gene, Arg347Cys polymorphism) (Muszkat *et al.*, 2005a, Muszkat *et al.*, 2005b, Sofowora *et al.*, 2004) did not reveal any significant contributions of these gene variants to the inter-individual variability in DHV responses to α-AR activation. In spite of this large inter-individual variability, it has been demonstrated that DHV responses to dexmedetomidine are not significantly different at the group level between black and white North American subjects (Muszkat *et al.*, 2004).

2.2 Haemodynamic effects mediated by α_2-adrenoceptors

2.2.1 Central nervous system

In humans, the most prominent effects of activation of α_2-ARs in the CNS are sedation, bradycardia and hypotension resulting from sympatholysis (Ebert *et al.*, 2000, Talke *et al.*, 2003, Aantaa and Jalonen, 2006). In the CNS, α_{2A}-ARs mediate both postsynaptic inhibitory effects and autoinhibition of NA release from noradrenergic neurones. The role of α_{2C}-ARs in the autoinhibitory system of the CNS is less pronounced (Trendelenburg *et al.*, 1997, Philipp *et al.*, 2002, Trendelenburg *et al.*, 2003,).

Older microinjection studies with cats suggested that hypotension and bradycardia were produced by the activation of α_2-ARs in several brain stem nuclei – especially in a region

that is lateral to the pyramid of the medulla oblongata and is limited rostrally by the pons and caudally by the hypoglossal nerve roots (Unnerstall *et al.*, 1984). The reciprocal connections of the nuclei in this region and the effects produced by α_2-AR agonists are complex. Injection of the α_2-AR agonist clonidine into the nucleus tractus solitarius resulted in bradycardia (Unnerstall *et al.*, 1984), whereas injection of clonidine into the rostral ventrolateral medulla, including the nucleus reticularis lateralis (C1 area) lead to both hypotension and bradycardia (Bousquet *et al.*, 1975, Bousquet *et al.*, 1981, Reis *et al.*, 1988). The reduction in HR was suggested to be a net effect of inhibition of NA release from cardiac sympathetic nerves, central sympatholysis, and facilitation of the baroreceptor reflex (Cavero and Roach, 1980, de Jonge *et al.*, 1982). Bousquet *et al.* reported that clonidine produced its pharmacological effect in the CNS by interactions not only with α_2-ARs but also with imidazoline receptors (Bousquet *et al.*, 1984). Such receptors, or alternatively imidazoline binding sites, I_1 and I_2, are pharmacologically distinct from the α_2-ARs because they are not activated by catecholamines (Karppanen *et al.*, 1976, Michel and Insel, 1989). The pharmacological functions of imidazoline receptors are not completely understood, and few applications of these receptors have emerged: the clinically perhaps most relevant finding is that imidazoline receptors are involved in the control of BP, and certain ligands such as moxonidine and rilmenidine are used to treat hypertension with minimal sedative effects that are mediated by α_2-ARs (Ernsberger *et al.*, 1995, Dardonville and Rozas, 2004). On the other hand, the whole concept of imidazoline receptors has been challenged, *e.g.* it was shown in gene-targeted mice that the hypotensive response to rilmenidine was lost in mice with dysfunctional α_{2A}-ARs (Zhu *et al.*, 1999).

The largest noradrenergic cell body aggregate of the brain, the LC is located in the dorsal wall of the rostral pons in the lateral floor of the fourth ventricle. The LC has a high density of α_{2A}-ARs (Nicholas *et al.*, 1993, Scheinin *et al.*, 1994), but the presence of α_{2C}-ARs has not been confirmed. Agonist activation of α_2-ARs in the LC suppresses the firing of the noradrenergic neurons of the LC (Owesson *et al.*, 2003), and stimulation of α_{2A}-ARs on the noradrenergic neurones in LC has been demonstrated to result in hypotensive and bradycardic effects (Gurtu *et al.*, 1984, Sved and Felsten, 1987). The LC also appears to be an essential site of action for the sedative-hypnotic (Doze *et al.*, 1989, Correa-Sales *et al.*, 1992, Scheinin and Schwinn, 1992, Nelson *et al.*, 2003) and analgesic (Guo *et al.*, 1996) effects of α_2-AR agonists.

2.2.2 Peripheral sympathetic nerves

The peripheral sympathetic noradrenergic neuroeffector junction is the final level of control of sympathetic neurotransmission (Figure 4). α_2-ARs are essential feedback regulators of neurotransmitter release at this site. Activation of α_2-ARs in the presynaptic terminals inhibits the release of NA from the sympathetic nerves as an autoinhibitory negative feedback system (Langer, 1974, Langer *et al.*, 1985, Docherty, 1998). In isolated tissues, α_{2A}-ARs are the major feedback regulators of NA release, but α_{2C}-ARs and possibly also α_{2B}-ARs appear to contribute to this presynaptic control (Altman *et al.*, 1999, Hein *et al.*, 1999, Trendelenburg *et al.*, 2003).

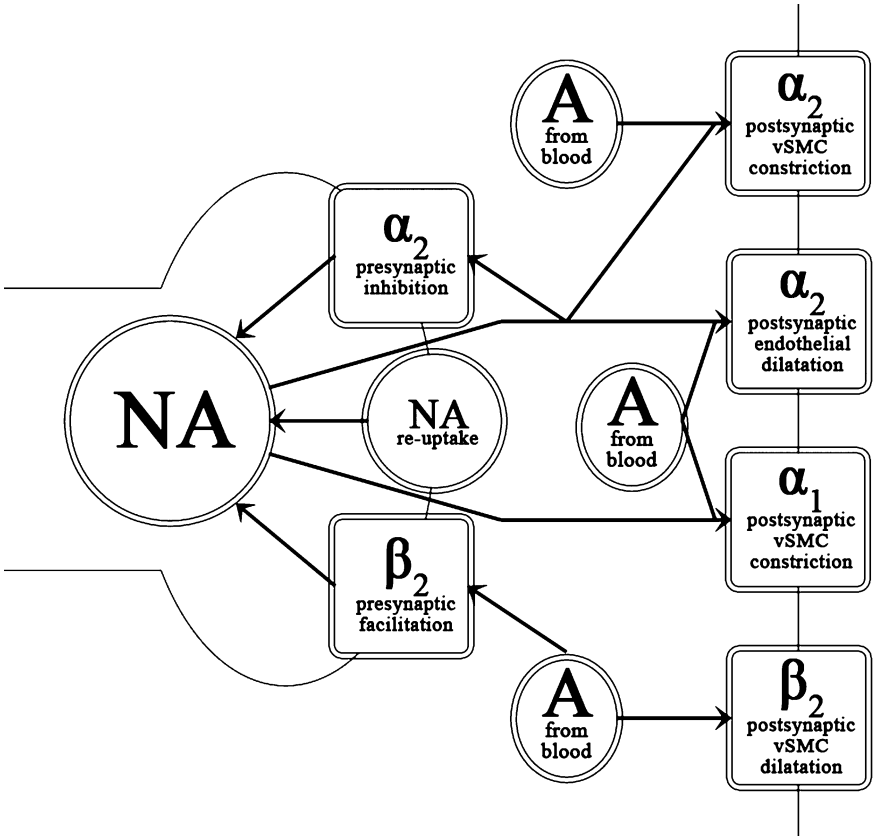

Figure 4. The sympathetic noradrenergic neuroeffector synapse illustrating presynaptic α_2-AR-mediated inhibition of NA release, presynaptic β-AR-mediated facilitation of NA release and neuronal re-uptake of NA into the axon terminal. NA, noradrenaline; A, adrenaline; vSMC, vascular smooth muscle cell. Modified from Docherty, 2002.

Presynaptic α_2-ARs may be partly responsible for the hypotensive effect of activation of α_2-ARs, but central effects appear to be dominant. In human subjects with traumatic spinal cord transection and loss of sympathetic nervous system control by the CNS, infusion of clonidine did not decrease BP, stroke volume (SV) and cardiac output (CO), unlike the situation in healthy subjects (Eisenach *et al.,* 1996). Additionally, resting digital skin blood flow was higher in tetraplegics and fell after clonidine. In normal subjects, however, an increase in digital skin blood flow occurred after clonidine treatment. The inability of clonidine to induce a decline in BP, SV and CO and to cause peripheral vasodilation in subjects with spinal cord injury is consistent with its central sympatholytic effects (Kooner *et al.*, 1988). In mammals, infusion of an α_2-AR agonist causes a biphasic BP response: the initial short-lived increase in BP is followed by a long-lasting decrease below baseline. Studies with gene-targeted (or KO) mice have suggested that α_{2B}-ARs are responsible for the initial hypertensive phase, whereas the long-lasting hypotension is mediated by α_{2A}-ARs (Link *et al.*, 1996, MacMillan *et al.*, 1996, Altman *et al.*, 1999). The initial response is presumably a result of activation of vascular postsynaptic α_2-ARs, this being later overwhelmed by a hypotensive response resulting from the reduction of central sympathetic tone.

In vivo, α_{2A}- and α_{2C}-ARs differentially control the release of NA from sympathetic nerves (α_{2A}-AR and α_{2C}-AR) and adrenaline from the adrenal gland (α_{2C}-AR) (Brede *et al.*, 2003a, Brede *et al.*, 2003b, Hein, 2006,). In sympathetic nerve endings, α_{2A}-ARs inhibit NA release faster and at higher action potential frequencies than α_{2C}-ARs (Hein *et al.*, 1999, Scheibner *et al.*, 2001).

2.2.3 Cardiovascular system: the heart and blood vessels

Part of the effects of α_2-AR activation on the CVS are mediated by postsynaptic α_2-ARs in target organs. Activation of presynaptic α_2-ARs causes sympatholysis, whereas postsynaptic α_2-ARs mediate both vasoconstriction, by contraction of smooth muscle, and vasodilatation by NO release in endothelial cells (Figueroa *et al.*, 2001 Talke *et al.*, 2003, Wong *et al.*, 2010) (Figure 4 and Figure 5).

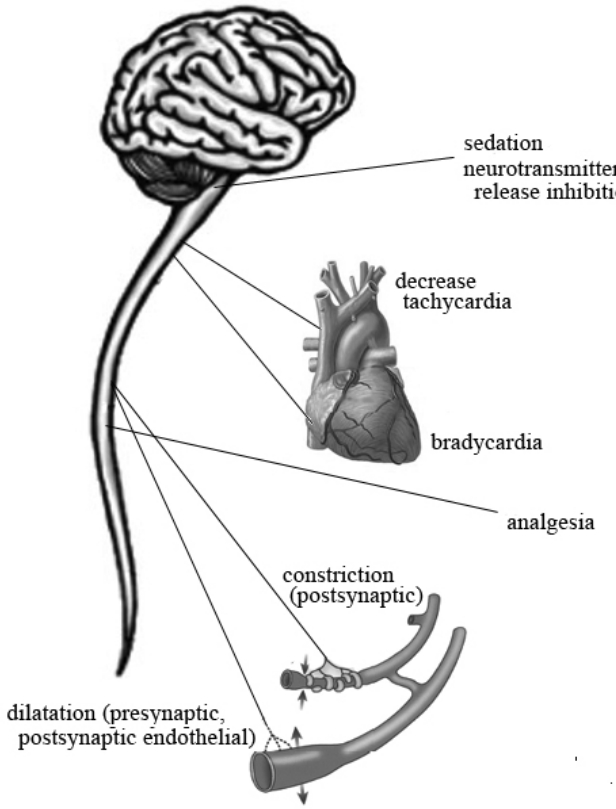

Figure 5. Responses mediated by α_2-ARs. The site of inhibition of neurotransmitter release and sedation is primarily the major noradrenergic nucleus locus coeruleus (LC) that is located in the pons in the brain stem. Analgesic effects are largely mediated by α_2-ARs located in the spinal cord. In the heart, the dominant result of activation of α_2-ARs is a decrease in tachycardia (through block of the cardioaccelerator nerve) and bradycardia (through a vagomimetic action). Activation of α_2-ARs in the CNS and PNS cause sympatholysis, whereas postsynaptic α_2-ARs mediate both vasoconstriction, by contraction of smooth muscle, and vasodilatation by nitric oxide (NO) release in endothelial cells. Modified from Kamibayashi and Maze, 2000 and Quaglia *et al*., 2011.

In vivo and *in vitro* studies have revealed extensive variability in vasoconstriction responses to α_2-AR activation between species (Nielsen *et al.*, 1991, Link *et al.*, 1996), anatomical locations (Hughes *et al.*, 1988), vessel types (Itoh *et al.*, 1987, Figueroa *et al.*, 2001, Talke *et al.*, 2003), vessel sizes (Itoh *et al.*, 1987, Hughes *et al.*, 1988), physiological and pathophysiological conditions (Indolfi *et al.*, 1992, Baumgart *et al.*, 1999, Chotani *et al.*, 2004), agonist concentrations (Wong *et al.*, 2010), and individual human subjects (Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a, Kurnik *et al.*, 2011).

In vitro studies with human vessels have demonstrated that the contractile responses mediated by α_2-ARs are most prominent in small resistance arteries, while larger arteries show minimal, if any, responses (Nielsen *et al.*, 1990, Nielsen *et al.*, 1991, Hughes *et al.*, 1996). In murine vessels *in vitro*, contractile responses to α_2-AR activation have been documented in mesenteric arteries and also in the aorta (Wong *et al.*, 2010), and in canine vessels, activation of α_2-ARs resulted in more potent constriction of mesenteric veins than mesenteric arteries (Itoh *et al.*, 1987). The rat tail artery has also been used as a model system to investigate vasoconstriction evoked by α_2-AR activation (Xiao and Rand, 1989, Chen *et al.*, 1999). In humans, α_2-ARs appear to mediate constriction of small peripheral arteries (Talke *et al.*, 2003), coronary arteries (Baumgart *et al*., 1999), and large superficial veins (Aellig, 1985, Muszkat *et al.*, 2004). In healthy human coronary arteries, α_2-AR activation reduced perfusion by microvascular constriction, this being augmented by atherosclerosis (Indolfi *et al.*, 1992, Baumgart *et al.*, 1999). Endothelial α_2-ARs are known to mediate vasodilatation, and it was recently reported that in small murine arteries, dexmedetomidine evoked endothelium-dependent relaxation at low concentrations, followed by contraction at higher concentrations. It was postulated that endothelial vasodilatation was predominantly evoked by activation of α_{2A}-ARs and to a lesser extent by α_{2B}-ARs (Wong *et al.*, 2010). It is not clear which subtypes of α_2-ARs are responsible for the vasoconstriction seen in humans.

The α_2-ARs signal *via* G_i proteins that inhibit ADCY activity and cAMP formation, but they may also mediate stimulation of extracellular Ca^{2+} influx through voltage-sensitive Ca^{2+} channels resulting in increased availability of intracellular Ca^{2+} and contraction of vascular smooth muscle (Parkinson and Hughes, 1995, ZhuGe *et al.*, 1997). In recombinant cell systems, α_2-ARs *via* G_q-type G proteins activate phospholipase C, resulting in the formation of inositol trisphosphate and Ca^{2+} release from the endoplasmic reticulum (Cotecchia *et al.*, 1990, Chabre *et al.*, 1994, Gesek, 1996). α_2-ARs can also increase the activity of PKC, which has been reported to contribute to membrane depolarization, Ca^{2+} influx, and smooth muscle contraction (Gesek, 1996, Yamboliev and Mutafova-Yambolieva, 2005). However, the exact mechanisms of α_2-ARs in evoking smooth muscle constriction remain incompletely understood.

In dogs, activation of α_2-ARs by dexmedetomidine was associated with a negative inotropic effect in the heart that was accredited to a limitation of the oxygen supply (Flacke *et al.*, 1993), because the CO decreased in the absence of sympatholysis in autonomically denervated dogs (Flacke *et al.*, 1990). However, it has been demonstrated that activation of α_2-ARs with dexmedetomidine does not have direct effects on myocardial cells (Flacke

et al., 1990, Housmans, 1990). Ebert *et al.* (Ebert *et al.*, 2000) reported that plasma concentrations of dexmedetomidine that significantly exceeded the therapeutically recommended level caused progressive increases in systemic and pulmonary vascular resistance and substantial increases in central venous and pulmonary arterial pressures. These changes were accompanied by a progressive increase in systemic BP and a steady decline in HR, SV and CO (Ebert *et al.*, 2000).

The endothelial cells on the interior surface of blood vessels interact with the smooth muscle layer of the vessel wall. The activation of endothelial α_2-ARs leads to release of NO and other vasodilating agents that evoke relaxation of smooth muscle cells (Figueroa *et al.*, 2001, Bruck *et al.*, 2001, Vanhoutte, 2001, Kim *et al.*, 2009). It was reported that endogenous NO attenuated noradrenergic constriction in the forearm skin circulation, and by using the α_2-AR antagonist yohimbine, indirect evidence was obtained that endothelial α_2-ARs are involved in the release of NO (Bruck *et al.*, 2005). Removal of the endothelium augmented α_2-AR-mediated contraction responses (Vanhoutte, 2001); also, in the rat aorta and mesenteric arteries, the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) and the α_2-AR antagonist rauwolscine abolished the endothelium-dependent relaxation evoked by dexmedetomidine (Wong *et al.*, 2010). A study with some relatively α_2-AR subtype-selective antagonists and PTX demonstrated that the endothelium-dependent relaxation of large rat arteries was being mediated mainly by the α_{2A}-AR subtype as well as partly by the α_{2B}-AR subtype (Wong *et al.*, 2010). This is in line with previous findings from studies with α_{2A}-AR-KO mice indicating that the α_{2A}-AR subtype is responsible for the endothelium-dependent relaxation (Vandeputte *et al.*, 2003).

2.3 Drugs acting on α_2-adrenoceptors

2.3.1 Clinical applications of α_2-adrenoceptor agonists

Drugs targeting α_2-ARs have potent hypotensive, sympatholythic and sedative effects and they are used in clinical practice for sedation and as adjuncts of general anaesthesia, for BP reduction, and for attenuation of sympathetic overactivity during substance withdrawal (Khan *et al.*, 1999a, Aantaa and Jalonen, 2006).

NA and adrenaline are metabolized in the gastrointestinal tract when administered orally, and when administered parenterally they do not pass through the blood-brain barrier. Furthermore, NA and adrenaline activate all subtypes of the AR family, therefore selective synthetic α_1- and α_2-AR agonists have become indispensable (Table 2). The clinical indications for the α_2-AR agonists depend not only on the individual affinity between α_1- and α_2-ARs, but also on the subtypes of α_2-ARs and density of the receptors present in the target organ.

Table 2. α_2-AR agonists and their binding affinities and functional properties.

Agonist	Binding affinity	Agonist efficacy	Origin
Adrenaline	$\alpha_{2A} \approx \alpha_{2B} \approx \alpha_{2C}$	Full	Endogenous
Biphenyline	$\alpha_{2A} > \alpha_{2B} \approx \alpha_{2C}$	Partial	Synthetic
Brimonidine (UK14,304)	$\alpha_{2A} \approx \alpha_{2B} > \alpha_{2C}$	Full α_{2A}; Partial α_{2B}, α_{2C}	Synthetic
Clonidine	$\alpha_{2A} \approx \alpha_{2B} \approx \alpha_{2C}$	Partial	Synthetic
Dexmedetomidine	$\alpha_{2A} \approx \alpha_{2B} \approx \alpha_{2C}$	Full α_{2B}; Partial α_{2A}, α_{2C}	Synthetic
Dopamine	$\alpha_{2A} \approx \alpha_{2B} \approx \alpha_{2C}$	Full α_{2A}; n.d. α_{2B}, α_{2C}	Endogenous
Guanfacine	$\alpha_{2A} >> \alpha_{2B} \approx \alpha_{2C}$	Partial	Synthetic
Noradrenaline	$\alpha_{2A} \approx \alpha_{2B} \approx \alpha_{2C}$	Full	Endogenous
Oxymetazoline	$\alpha_{2A} >>> \alpha_{2B} > \alpha_{2C}$	Partial ($\alpha_{2B} >> \alpha_{2A}$)	Synthetic
2-Amino-1-phenylethanol	$\alpha_{2A} > \alpha_{2B} \approx \alpha_{2C}$	Partial	Synthetic

Derived from Lomasney *et al.* 1991, Bylund *et al.* 1992, Devedjian *et al.* 1994, Jasper *et al.* 1998, Peltonen *et al.* 1998, Peltonen *et al.* 2003, Ruuskanen *et al.* 2005, Laurila *et al.* 2011, Quaglia *et al.* 2011

2.3.1.1 Sedation, anaesthesia and analgesia

The sedative effects of α_2-AR agonists are well established. Dexmedetomidine is used as an efficacious sedative agent in some situations. It provides similar sedation in the intensive care setting compared to midazolam and propofol in patients requiring light to moderate sedation for mechanical ventilation (Jakob *et al.,* 2012). There is evidence to suggest that the sedative effect is mediated by the α_{2A}-AR subtype, because α_{2A}-AR-KO mice did not show the sedative response to dexmedetomidine, while in α_{2B}- and α_{2C}-AR-KO mice, the sedative effect was unaltered (Hunter *et al.*, 1997).

The sedative effects of α_2-AR agonists have some unique aspects. Infusion of high doses does not result in clinically significant respiratory depression, but rather in a decreased apnea/hypopnea index (Ebert *et al.*, 2000, Hsu *et al.*, 2004), and α_2-AR agonists mimic some aspects of natural sleep; patients sedated with dexmedetomidine can be aroused by auditory and tactile stimuli (Hall *et al.*, 2000, Venn and Grounds, 2001). The selective α_2-AR agonists possess several useful clinical features, such as opioid-sparing, anaesthetic-sparing, sympatholytic, haemodynamic-stabilizing, anti-nausea and anti-shivering properties, that make them useful adjuncts to general anaesthesia (Jalonen *et al.*, 1997, Aantaa and Jalonen, 2006, Chrysostomou and Schmitt, 2008). In humans, the analgesic effects of α_2-AR agonists are not sufficient to achieve clinically useful analgesia after systemic administration, but clonidine has been used in combination with local anaesthetic agents to evoke potent spinal analgesia (Kamibayashi and Maze, 2000, Aantaa and Jalonen, 2006, Chrysostomou and Schmitt, 2008).

2.3.1.2 Cardio- and renoprotection

It has been reported that α_2-AR agonists decrease myocardial energy requirements and oxygen demand (Lawrence *et al.*, 1996). α_2-AR agonists have been demonstrated to be cardioprotective by diminishing the catecholamine storm that is usually encountered during cardiac and non-cardiac surgery. Evidence from meta-analyses suggests that α_2-AR agonists may reduce perioperative mortality and myocardial ischaemia and infarction (Aantaa and Jalonen, 2006, London, 2008). During both cardiac and non-

cardiac surgery, they have reduced the incidence of perioperative myocardial ischaemia (Oliver *et al.*, 1999, Nishina *et al.*, 2002, Wijeysundera *et al.*, 2003, Wallace *et al.*, 2004) and postoperative deaths (Wallace *et al.*, 2004). Aantaa and Jalonen (Aantaa and Jalonen, 2006) concluded on the basis of the preceding studies that α_2-AR agonists would be beneficial for reducing risk of perioperative death and myocardial complications, with no clear evidence of significant differences between the effects of clonidine, dexmedetomidine and mivazerol (Aantaa and Jalonen, 2006).

Acute kidney injury following surgery significantly increases mortality, and there is no proven therapy to prevent this effect. There is evidence to suggest that α_2-AR agonists can exhibit renoprotective effects. Renal insufficiency is a commonly seen complication after cardiothoracic surgery. It has been demonstrated that preoperative administration of clonidine could result in good maintenance of creatinine clearance (Kulka *et al.*, 1996). Another study showed that patients undergoing thoracic surgery, who received dexmedetomidine, had lower serum creatinine levels for seven postoperative days than the placebo group (Frumento *et al.*, 2006, Chrysostomou and Schmitt, 2008). Gu and colleagues (Gu *et al.*, 2011) reported that prophylactic clonidine treatment prevented renal dysfunction attributable to cardiac surgery and that dexmedetomidine improved renal function following thoracic surgery. The group suggested that dexmedetomidine was activating the α_2-AR-mediated cell survival signal phosphorylated Akt to reduce cell death and high-mobility group protein B1 (HMGB1) release and subsequent inhibition of toll-like receptor 4 (TLR4) signaling as a way to confer renoprotection (Gu *et al.*, 2011).

2.3.1.3 Muscle relaxation

The α_2-AR agonist tizanidine is used clinically as a muscle relaxant to relieve spasticity associated with chronic neurological diseases (stroke, cerebral trauma and multiple sclerosis) and acute painful conditions. It is known to inhibit the presynaptic activity of some excitatory interneurons in the spinal cord (Kita and Goodkin, 2000, Abbruzzese, 2002).

2.3.1.4 Glaucoma

α_2-AR agonists reduce intraocular pressure in patients with glaucoma, and are possibly associated with improved retinal ganglion cell survival (Yoles *et al.*, 1999, Wheeler *et al.*, 2001). α_2-AR agonists reduce intraocular pressure mainly by decreasing aqueous humour production (Toris *et al.*, 1999). The selective α_2-AR agonist brimonidine has been considered by some authors to be more favourable in treating glaucoma than some other α_2-AR agonists, because it is relatively devoid of unwanted central effects as it does not readily enter the brain (Gentili *et al.*, 2007, Weigert *et al.*, 2007). Apraclonidine is an amino derivative of clonidine and a relatively selective α_2-AR agonist with minimal adverse systemic cardiovascular effects. Apraclonidine lowers intraocular pressure by reducing aqueous humour production without altering aqueous outflow. The mechanisms involved in the reduction of aqueous humour production are not completely understood, although it is believed that part of its effects may be secondary to vasoconstriction of

the ciliary body (Torris *et al.*, 1995b). However, while the α_2-AR agonist drugs reduce intraocular pressure, their vasoconstriction properties may affect intraocular blood flow (Costa *et al.*, 2003).

2.3.2 Pharmacology of dexmedetomidine

Dexmedetomidine is a highly selective α_2-AR agonist that has an α_2/α_1-selectivity ratio of 1620:1 in terms of receptor binding affinity, which is 7 to 8 times higher than that of clonidine (Virtanen, 1986, Dyck *et al.*, 1993).

Orion Pharma (Espoo, Finland) has recently conducted a pivotal clinical trial (Jakob *et al.,* 2012). The results indicated that dexmedetomidine provided similar sedation in the intensive care setting compared to midazolam and propofol in patients requiring light to moderate sedation for mechanical ventilation. The safety findings were in line with previous knowledge and no significant new safety concerns were detected (Jakob *et al.,* 2012). On the basis of these results, Orion received European marketing authorisation from EMA (the European Medicines Agency) for dexmedetomidine (Dexdor® 100 µg/ml). According to the label of Dexdor®, the recommended initiation dose is 0.7 µg/kg/h and the maintenance dose is 0.2-1.4 µg/kg/h until the desired state of sedation is achieved. It is not allowed to exceed the maximum dose 1.4 µg/kg/h. Nor is it recommended to continue the Dexdor® administration over 14 days (Dexdor® Prescription Information, Orion Pharma, Espoo, Finland). According to the label of Precedex® (Hospira, Lake Forest, IL, USA), the recommendations for its use in adults include a loading dose of 1 µg/kg intravenously over 10 min (0.1 µg/kg/min) and a maintenance infusion dose of 0.2 – 0.7 µg/kg/h for no more than 24 h. Titration of the infusion rate should be performed to obtain the desired state of sedation (Precedex® Prescription Information, Hospira Inc., Lake Forest, IL, USA, 2004).

Pharmacokinetic (PK) data obtained from continuous intravenous infusions of dexmedetomidine have revealed a half-life (t½) of 9 min for distribution and 2 h for elimination (Khan *et al.*, 1999b), and in another study 1.6 – 2.4 h for elimination, an apparent total plasma clearance of 0.7 to 0.9 l/h/kg, and an apparent volume of distribution of 2.1 to 2.6 l/kg (Scheinin *et al.*, 1992b). No dose-dependence was observed for the t½, clearance, or volume of distribution (Khan *et al.*, 1999b). In a recent study with prolonged infusions of dexmedetomidine (mean, 92 h) in critically ill patients, the mean dexmedetomidine clearance was 39.7 l/h, the elimination t½ was 3.7 h, and the volume of distribution during the elimination phase was 223 l (Iirola *et al.*, 2011a). The metabolites of dexmedetomidine are eliminated mainly in the urine (95 %) and faeces (4 %). Dexmedetomidine is absorbed through the oral mucosa, its buccal bioavailability being as high as 82 %, while its bioavailability after oral administration was only 16 % (Anttila *et al.*, 2003). Dexmedetomidine is metabolized through direct N-glucuronidation and in addition by aliphatic hydroxylation *via* the CYP2A6 metabolic pathway, to form 3-hydroxy-dexmedetomidine, the glucuronide of 3-hydroxy-dexmedetomidine, and 3-carboxy-dexmedetomidine. N-methylation can also occur to form 3-hydroxy N-methyl dexmedetomidine, 3-carboxy-N-methyl dexmedetomidine, and N-methyl dexmedetomidine-O-glucuronide (Chrysostomou and Schmitt, 2008). In addition to CYP2A6, also other cytochrome P450 enzymes, *i.e.* CYP1A1, CYP2C19, CYP2D6 and

CYP2E1 may metabolize dexmedetomidine (Karol and Maze, 2000) Dexmedetomidine is in human blood relatively highly bound (94 %) to serum albumin and α1-acid glycoprotein (Precedex® Prescription Information, Hospira Inc., Lake Forest, IL, USA, 2004). After intravenous infusion of a small dose of radioactive dexmedetomidine, 95 % of the radioactivity was excreted in the urine and 4 % in the faeces (Karol and Maze, 2000). Less than 1 % of the administered dexmedetomidine dose is excreted unchanged in the urine, and 28 % of the urinary metabolites were unidentified minor metabolites of dexmedetomidine (Dexdor® Prescription Information, Orion Pharma, Espoo, Finland).

2.3.3 Cardiovascular effects of dexmedetomidine

In humans, systemic infusion of dexmedetomidine evokes complex direct and indirect effects on the CVS. The effects of α_2-AR agonists on the peripheral nervous system (PNS) (McCallum *et al.*, 1998) and the CNS (Talke *et al.*, 1997) result in sympatholysis, whereas postsynaptic effects on blood vessels include both vasoconstriction (Talke *et al.*, 2005) and NO-dependent dilatation (Figueroa *et al.*, 2001). The sympatholysis leads to hypotension, bradycardia (Ebert *et al.*, 2000, Chrysostomou and Schmitt, 2008, Chrysostomou *et al.*, 2008), and decreased myocardial energy requirements (Lawrence *et al.*, 1996) that are parallel to the reductions in HR and CO (Ebert *et al.*, 2000).

The net effects of dexmedetomidine on haemodynamics depend on the dose and the dosing regimen: supratherapeutic concentrations increase systemic BP, and short-lasting hypertension may also occur when rapid administration of loading doses induces vasoconstriction mediated *via* peripheral α_2-ARs. This is seen before the onset of centrally mediated sympatholysis and the ensuing reduction of BP (Ebert *et al.*, 2000, Aantaa and Jalonen, 2006). Dexmedetomidine reliably controls HR and BP in patients undergoing surgery, *i.e.* haemodynamic stability is augmented, and adverse haemodynamic responses to procedural stimuli, including the emergence from anaesthesia, are attenuated, and whole-body energy and oxygen consumption are reduced (Ebert *et al.*, 2000, Chrysostomou and Schmitt, 2008, Biccard *et al.*, 2008). Reports have emerged on dexmedetomidine-associated bradycardic and hypotensive events, and particular care has to be taken if the patient presents with a cardiac condition (Gerlach and Murphy, 2009).

The safety and suitability of dexmedetomidine for use in patients who have or who are at risk for neurologic injuries, remains a significant unresolved issue. Concerns have been expressed with respect to the effect of dexmedetomidine on the ratio of cerebral oxygen supply to cerebral oxygen demand. In humans, earlier evidence suggested that dexmedetomidine could reduce cerebral blood flow (CBF) (Zornow *et al.*, 1990, Prielipp *et al.*, 2002), while the effects of dexmedetomidine on cerebral metabolic rate (CMR) were less well documented. Based on two studies with dogs, it was reported that administration of 10 μg/kg dexmedetomidine decreased CBF without changing CMR (Zornow *et al.*, 1990, Karlsson *et al.*, 1990). However, a recent study with healthy human subjects demonstrated that dexmedetomidine, at plasma levels of 0.6 ng/ml – 1.2 ng/ml, caused dose-related decreases in both CBF and CMR during normocapnia and hypocapnia, suggesting that adverse events regarding the cerebral oxygen supply-demand relation should not be an issue in humans (Drummond *et al.*, 2008). However,

these results do not guarantee the safe use of dexmedetomidine in patients with acute traumatic neurological injury, given that this group of patients usually has hypercapnia and cerebral hypoxia resulting from increased intracranial pressure and impaired CBF.

2.3.4 Clinical indications of dexmedetomidine

2.3.4.1 Sedation and analgesia

Dexmedetomidine was approved by the US Food and Drug Administration in 1999 for sedation of mechanically ventilated patients for up to 24 h in intensive care unit (ICU) settings (Aantaa and Jalonen, 2006). This time limitation was probably set because of a lack of data regarding longer ICU treatment durations and short-term and long-term complications during and after discharge from the ICU (Carollo *et al.*, 2008). In a recent review (Guinter and Kristeller, 2010), it was reported that dexmedetomidine could maintain sedation in critically ill adult patients over at least 24 hours, but hypotension and bradycardia remained a concern, especially during the administration of loading doses and bolus injections. However, bradycardia usually resolves without treatment in most patients. Several studies have demonstrated that dexmedetomidine treatment in the ICU exceeding 24 h does not impair the safety and efficacy of the drug. Adverse events in long-term treatment have been similar as in patients that received dexmedetomidine for 24 h or less. In addition, in spite of concerns regarding rebound or withdrawal effects, no published studies have reported these kinds of effects, even when the discontinuation has been abrupt (Guinter and Kristeller, 2010).

Dexmedetomidine has shown advantages over propofol sedation in the ICU in several studies. It has been demonstrated that the use of dexmedetomidine as sedative could reduce the requirement of opioids by more than 50 %, when compared to propofol or benzodiazepines (Chrysostomou and Schmitt, 2008). Patients receiving dexmedetomidine during their stay in the ICU exhibited better recall and more pleasant impressions of their intensive care stay, and confusion and delirium were reduced (Venn and Grounds, 2001, Triltsch *et al.*, 2002). Compared to midazolam and propofol, dexmedetomidine was associated with a smaller risk for postoperative delirium (Kobayashi *et al.*, 2007).

2.3.4.2 Anaesthetic adjunct

Dexmedetomidine exhibits both anaesthetic- and opioid-sparing effects. In 1988, Segal *et al.* reported that dexmedetomidine reduced anaesthetic requirements of halothane in rats (Segal *et al.*, 1988). Subsequently, several clinical studies have reported anaesthetic-sparing effects in various surgical procedures and circumstances with a variety of anaesthetics agents during general anaesthesia (Jalonen *et al.*, 1997, Aho *et al.*, 1991, Aho *et al.*, 1992, Scheinin *et al.*, 1992a, Talke *et al.*, 1995). These studies also revealed that dexmedetomidine could help to maintain haemodynamic stability during intubation and induction of anesthesia. This is concordant with a recent study, which demonstrated that dexmedetomidine blunted the haemodynamic response to tracheal intubation (Kunisawa *et al.*, 2009). When administered as a premedication before surgical procedures in a dose range of 0.33 – 0.67 μg/kg, dexmedetomidine reduced thiopental requirements

by approximately 30 % for short procedures and reduced the requirements of volatile anaesthetics by approximately 25 % (Aantaa *et al.*, 1990).

In supratentorial neurosurgical procedures, dexmedetomidine has been shown to improve perioperative haemodynamic stability at target plasma levels of 0.2 – 0.4 ng/ml, and when compared with fentanyl, the patients could be extubated earlier without signs of respiratory depression (Tanskanen *et al.*, 2006). In a recent study, dexmedetomidine provided good control over acute perioperative BP responses and adequate brain relaxation during neurosurgical procedures (Gunduz, 2009). Dexmedetomidine has been shown to prevent perioperative and postoperative myocardial ischaemia in cardiac and non-cardiac surgical patients (Nishina *et al.*, 2002, Wijeysundera *et al.*, 2003, Wallace *et al.*, 2004). In a paediatric study, it was shown that a single postoperative intravenous bolus of dexmedetomidine, 0.5 μg/kg over 3 – 5 min, was effective in the treatment of postoperative shivering (Blaine Easley *et al.*, 2007).

2.3.4.3 Procedural sedation

Dexmedetomidine has been used for procedural sedation because of its ability to produce anxiolysis, sedation and analgesia, the latter reducing the requirements for opioid usage. Dexmedetomidine has been successfully used for procedural sedation during diagnostic procedures including computerized tomography and MRI scans, and EEG analysis in paediatric patients (Tobias, 2002, Koroglu *et al.*, 2006, Mason *et al.*, 2006). When compared with midazolam, children 1 – 7 years old undergoing MRI were more likely to achieve an adequate lack of movement and sedation with dexmedetomidine than with midazolam (Koroglu *et al.*, 2005), whereas the sedation was similar to the propofol treatment group, but the onsets of action, recovery and discharge were faster with dexmedetomidine (Koroglu *et al.*, 2006). Dexmedetomidine has provided well tolerated and effective sedation during EEG analysis in children with autism, without disturbing the EEG examination (Tobias, 2007).

In adults, dexmedetomidine has been used as a procedural sedative for shoulder dislocation reposition with infusions of 75 – 120 μg over 2 – 5 minutes (Jewett and Phillips, 2010). Dexmedetomidine has also been used successfully as a sedative in awake fiberoptic intubation, providing maintenance of an open airway and spontaneous ventilation to avoid possible respiratory depression and aspiration (Bergese *et al.*, 2007). In recent years, many neurosurgical procedures have been developed to be minimally invasive; examples include perioperative imaging, endoscopies, mini-size craniotomies, stereotactic procedures, and functional neurosurgery including deep brain stimulation (Tanskanen *et al.*, 2006, Rozet, 2008, Shinoura *et al.*, 2010). Many neurosurgical procedures require perioperative active patient participation, including assessment of responses following initial deep brain stimulation for treatment of motor disorders including Parkinson's disease, intraparenchymal electrode implantation, surgical management of epilepsy, and surgery near eloquent areas, such Broca's and Wernicke's speech areas (Frost and Booij, 2007, Rozet, 2008). Dexmedetomidine has useful properties in such perioperative situations, as patient participation, adequate anxiolysis and arousability are possible at the same time. In addition, dexmedetomidine enables perioperative neurocognitive and neurologic evaluations with concomitant neuroprotection (Mack *et al.*, 2004, Bekker

and Sturaitis, 2005, Chrysostomou and Schmitt, 2008, Shinoura *et al.*, 2010). Still, since dexmedetomidine has caused dose-related decrease in both CBF and CMR in healthy volunteers (Drummond *et al.*, 2008), and in patients undergoing neurovascular surgery with and without prior neurological lesion-related deficits (Drummond and Sturaitis, 2010), the safety of dexmedetomidine in patients suffering from acute head trauma and neurological injury is not assured.

2.3.4.4 Future applications

There are many ongoing trials that are intended to reveal the future possibilities of dexmedetomidine (see e.g. http://clinicaltrials.gov), and new possible indications have emerged. Dexmedetomidine may provide additional benefits in the management of alcohol withdrawal by offering a different mechanism of action for targeting withdrawal symptoms (Muzyk *et al.*, 2011). According to case reports, dexmedetomidine has been used in treatment of withdrawal symptoms from benzodiazepines and opioids in a dose range of 0.25 – 0.7 μg/kg/h (Baddigam *et al.*, 2005, Tobias, 2006), and dexmedetomidine has shown therapeutic potential in the treatment of perioperative supraventricular and junctional tachyarrhythmias in children (Chrysostomou *et al.*, 2008). It has also been demonstrated that intranasal doses of 1 and 1.5 μg/kg caused sedation onset at 45 min with a peak effect at 90 – 150 min without effects on pain threshold or respiratory function, whereas systolic BP was reduced by 21 % (Yuen *et al.*, 2007). Furthermore, Iirola et al. (Iirola *et al.*, 2011b) recently investigated the pharmacokinetics and pharmacodynamics of intranasal delivery of dexmedetomidine compared with its intravenous administration with a dose of 84 μg. They reported that dexmedetomidine was rather rapidly and efficiently absorbed after intranasal administration, and in comparison to the disadvantages associated with intravenous administration, intranasal delivery may represent a feasible alternative in patients requiring light sedation (Iirola *et al.*, 2011b).

Dexmedetomidine has gained an important place as a research tool in pharmacological research *in vitro* and *in vivo*, and its use in clinical practice is growing rapidly. For all of these reasons, it is important to try to elucidate the mechanisms and significance of α_2-ARs in cardiovascular regulation and in the effects of dexmedetomidine *in vivo.*

3. AIMS OF THE STUDY

The present series of studies was designed to evaluate the roles of α_2-ARs in agonist-induced vasoconstriction responses in healthy human volunteer subjects. The studies were planned with two general aims in mind: to learn more about the roles of α_2-ARs in physiological and pharmacological regulation of cardiovascular functions, and to develop and validate new approaches for the *in vivo* evaluation of drugs active at α_2-ARs. The specific aims of the four individual original studies on which this thesis is based were:

1. To evaluate the effects of therapeutic and high concentrations of the clinically employed, selective α_2-AR agonist, dexmedetomidine, on myocardial perfusion and cardiac function in healthy subjects;

2. To assess the contribution of endothelial NO synthesis to the net responses of digital arteries and DHVs to dexmedetomidine;

3. To examine whether the effects of activation of α_2-ARs are co-regulated in individual healthy volunteer subjects with the effects of activation of α_1-ARs in DHVs; and

4. To try to identify genetic factors contributing to the large inter-individual variability in α_2-AR–mediated DHV constriction responses induced by dexmedetomidine.

4. SUBJECTS AND METHODS

This thesis work is based on four separate original studies that are referred to in the text by the Roman numerals I-IV. The study designs, important variables, and study population characteristics are summarized in Table 3.

4.1 Study populations

A total of 151 healthy non-smoking Finnish Caucasian male and female volunteers participated in the clinical experimental sessions carried out in Turku over the years 2004-2008. Additionally, 68 healthy male and female white and black volunteer subjects from Middle Tennessee, USA, served as a replication study population for a candidate gene approach after performance of the initial GWAS analysis in study IV. All of the Finnish subjects were recruited through on-line advertisements on electronic bulletin boards of the City of Turku and the University of Turku and its student organizations. In studies I and II, after an initial contact by the prospective subject by e-mail, the subject candidates were initially interviewed over the phone, and interested and eligible volunteers were then invited to attend a screening visit after having read the complete study information package. The screening procedures were carried out after voluntary informed consent had been obtained.

For recruitment into studies III and IV, subject candidates first logged in to the study website and answered an on-line questionnaire regarding their health, medications, operations and smoking habits. Next, eligible volunteers were invited to a screening visit after they had read the complete study information package. The screening procedures were carried out after voluntary informed consent had been obtained.

The studies were conducted in accordance with the Declaration of Helsinki (2000) of the World Medical Association. All studies were approved by the Ethics Committee of Southwest Finland Hospital District, Turku, Finland, and the Finnish regulatory agency, Fimea. Study IV had also approval by the Institutional Review Board of Vanderbilt University Medical Center, Nashville, TN, USA. All subjects were informed about the potential risks and concerns that were involved in the studies, lack of personal benefit, and about their right to discontinue their participation at any time without giving any reason and without any resulting detriment. All subjects gave their written informed consent before any actual study procedures were implemented.

For studies I and II, the volunteer subjects had to meet all of the following inclusion criteria: male gender, age 18 – 35 (I) or 18 – 45 years (II), and good general health, *i.e.* American Society of Anesthesiologists physical status classification system (ASA) physical status I. For studies III and IV, both genders were accepted and subjects had to meet all of the following inclusion criteria; age 18 – 45 years and good general health, *i.e.* ASA physical status I. The exclusion criteria were BMI < 19 or > 27.5 (I), BMI <

18 or > 30 (II), or BMI < 18 or > 27 (III and IV), any chronic medication (other than hormonal contraception, III and IV), strong susceptibility to allergic reactions, regular use of nicotine-containing products, history of cardiac arrhythmia (other than innocent supraventricular extrasystoles), clinically significant abnormality in screening laboratory tests, positive result in the drug screening test, blood donation within 90 days prior to the study, active allergic or idiopathic eczema distal from elbow line (II, III), no suitable DHV to investigate (II, III), and doubtful capacity to provide voluntary informed consent. The subjects had to refrain from the use of any drugs or alcohol during the 48 hours preceding the study sessions, ingestion of caffeinated beverages in the 12 h preceding the sessions and involvement in heavy exercise during the 24 h preceding the sessions.

4.1.1 Study I

Twelve healthy, non-smoking Caucasian young men were enrolled in the study. The subjects were 20 – 28 years old (mean, 24 years) with a BMI of 19 to 27 kg/m^2 (mean, 24 kg/m^2). The health of the subjects was assessed by medical history, physical examination, 12-lead electrocardiogram (ECG), bicycle maximal exercise test, blood cell count, and urine drug screening. Peripheral circulation in the hand that was to undergo radial arterial cannulation was tested with Allen's test. Based on the concept of α_{2B}-ARs being involved in vasoconstriction (Link *et al.*, 1996) and on the association of the human α_{2B}-AR 901_909del polymorphism with impaired receptor desensitization (Small *et al.*, 2001) and increased cardiovascular disease risk (Snapir *et al.*, 2001, Snapir *et al.*, 2003c), half of the subjects were selected to have the Del/Del genotype and the other half the Ins/Ins genotype of the *ADRA2B* gene.

4.1.2 Study II

Twenty-one healthy, non-smoking Caucasian men were enrolled in the study. Subjects in the finger blood flow experiment (LTF) (n = 11) were 21 – 36 years old (mean, 26 years) with a BMI of 18 to 27 kg/m^2 (mean, 24 kg/m^2). Subjects in the DHV experiment (n = 10) were 21 – 40 years old (mean, 27 years) with a BMI of 20 – 30 kg/m^2 (mean, 24 kg/m^2). The health of the subjects was assessed by medical history, physical examination, 12-lead ECG, blood cell count, serum lipid concentrations, and urine drug screening.

The number of subjects in the LTF sub-study was calculated to provide about 90 % power to detect a difference of 10 % in light transmission through a finger (LTF) between before and after infusion of L-NMMA, using the previously observed variability in LTF in anaesthetized subjects infused with dexmedetomidine to a target plasma concentration of 0.6 ng/ml (Talke *et al.*, 2003). In the DHV experiment, 10 subjects were calculated to provide about 80 % power to detect a one log unit difference in the ED_{50} of dexmedetomidine between the DHV response with and without L-NMMA using the within-group standard deviation of the ED_{50} of the response to dexmedetomidine as previously reported (Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a). Less variability was expected in the response to dexmedetomidine because of the within-subject paired study design. In both calculations, a type I error probability of 0.05 for a two-sided test was used.

Table 3. Subject demographics and study summary. Data are presented as number or means (S.D.)

Study	N	Gender: male : female	Ethnicity: Caucasian : African American	Age (years)	BMI (kg/m^2)	Study Design	Methods	Main Variables
Study I	12	12 : 0	12 : 0	24.0 (4.0)	23.6 (1.8)	non-randomized, open-label	drug infusions, PET, TTE, BPM, lab	MBF, CVR, cardiac function, BP, SVR, CO, RPP
Study II: LTF	11	11 : 0	11: 0	26.0 (7.5)	23.2 (2.5)	randomized, open-label, placebo-controlled	drug infusions, LTF, BPM, temp, lab	finger blo od flow, finger temp
Study II: DHV	10	10 : 0	10 : 0	27.0 (9.5)	22.2 (2.3)	randomized, open-label, placebo-controlled	drug infusions, LVDT, BPM, temp, lab	DHV constriction, dex ED_{50}
Study III	75	32 : 43	75 : 0	24.3 (4.9)	22.6 (2.4)	randomized, single-blinded	drug infusions, LVDT, BPM, temp, lab	DHV constriction, dex ED_{50}, PE ED_{50}
Study IV	131	61 : 70	100 : 31	26.4 (6.4)	23.9 (4.9)	pharmacogenetic analysis	genotyping, GWAS, candidate gene selection	dex ED_{50}, GWAS hits, candidate gene associations

LTF, light transmission through a finger, (digital blood flow) substudy; DHV, dorsal hand vein substudy; BMI, body mass index; PET, positron emission tomography; TTE, transthoracic echocardiography; BPM, blood pressure measurements; lab, study drug and catecholamine determinations; temp, temperature measurements; GWAS, genome-wide association study; MBF, myocardial blood flow; CVR, coronary vascular resistance; BP, blood pressure; SVR, systemic vascular resistance; CO, cardiac output; RPP, rate-pressure product; finger temp, finger temperature; DHV constriction, constriction of dorsal hand veins; dex ED_{50}, dexmedetomidine ED_{50}; PE $ED_{50,}$ phenylephrine ED_{50}; GWAS hits, genome-wide association study hits.

4.1.3 Study III

118 healthy, non-smoking Caucasian men and women were enrolled in the study carried out in Turku. Their health was assessed by medical history, physical examination, 12-lead ECG, blood count and serum lipid profile. No power calculations were performed, because the hypothesis was that an individual's sensitivity of DHVs to α_2-AR-mediated constriction would be determined to a significant extent by the same subject's sensitivity to phenylephrine-induced α_1-AR-mediated vasoconstriction. It was presumed that a statistically significant positive correlation between the ED_{50} values of these two agonists could be considered as proof of common factors explaining the drug responses. Of the 118 subjects, 19 had to be excluded because of failure in DHV cannulation or inability to warm the hands to at least 33 ºC. Of the 99 subjects that received drug infusions, 24 were excluded from the analysis of study III because ED_{50} estimates for both study drugs (dexmedetomidine and phenylephrine) could not be calculated reliably. The efficacy results of study III were thus collected from 75 subjects.

4.1.4 Study IV

This study comprised two study populations: one from Turku, Finland (discovery phase, GWAS) and one from Middle Tennessee, USA (replication phase, candidate gene approach). The pool of 118 recruited subjects from Turku was the same as that in Study III. Of the 99 subjects that received drug infusions, 64 subjects with the largest and smallest ED_{50} estimates for dexmedetomidine were selected (ED_{50} values greater than 30 ng/min or less than 5 ng/min). The study population for the replication phase was one that had been recruited and investigated at Vanderbilt University Medical Center, TN, USA. Caucasian and African–American subjects of either sex were eligible for the study if they were 18 – 45 years of age and had no clinically significant abnormalities according to medical history, physical examination, and laboratory testing. Ethnicity was determined by self-report. The subjects had taken no medications for at least 2 weeks, and had abstained from alcohol and caffeine for at least 5 days before the investigation.

4.2 Study designs

4.2.1 Study I

This study had a non-randomized, open-label study design. The study was designed to explore the effects of therapeutic (target concentration, 0.5 ng/ml) and high (target, 3.2 ng/ml) steady-state plasma concentrations of dexmedetomidine on myocardial perfusion and cardiac function in healthy young male subjects.

Baseline data were collected before infusion of dexmedetomidine (Baseline) and during low (Low Dex, actual mean concentration, 0.5 ng/ml) and high (High Dex, actual mean, 5 ng/ml) plasma concentrations of dexmedetomidine. The clinical experiments were conducted during the morning hours after an eight-hour fast. The sessions were started with the required cannulations, pulmonary artery catheterization, and other preparations.

This was followed by a stabilization phase, which lasted for at least 30 min. After this, baseline measurements were obtained in the following order: blood sampling; positron emission tomography (PET) measurements; transthoracic echocardiography (TTE) measurements; and a second blood sampling. After the completion of the baseline measurements, the low dexmedetomidine infusion phase was started. Thirteen minutes after the initiation of the infusion, blood samples were drawn, and exactly 15 min from the initiation of the drug infusion, the second PET scan was started, followed by TTE measurements and blood sampling at 30 min from the beginning of the drug infusion. This sequence was then repeated in the High Dex infusion phase. The study design is summarized in Figure 6.

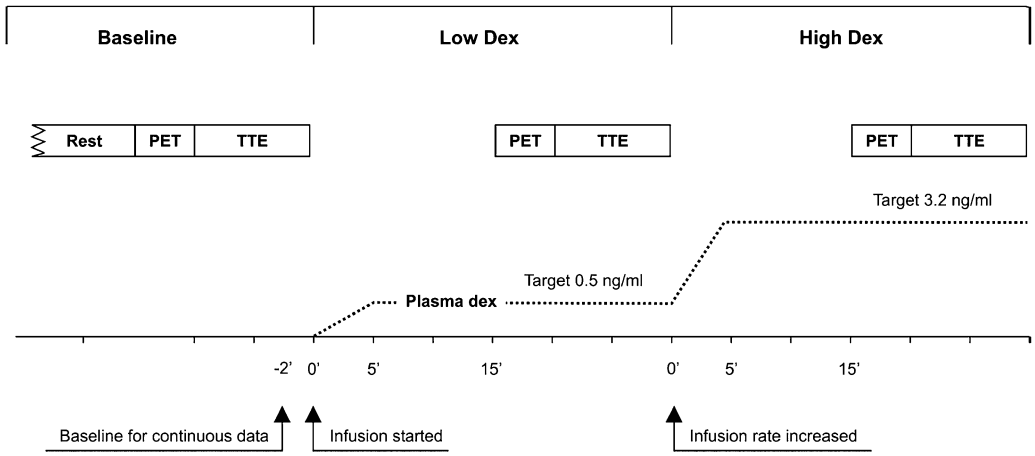

Figure 6. Outline of the design of Study I. After at least 30 min of rest, baseline measurements were obtained. The Low Dex phase started with the infusion of dexmedetomidine (Dex) to target a plasma concentration of 0.5 ng/ml. After completion of the positron emission tomography (PET) and transthoracic echocardiography (TTE) measurements, High Dex was started by increasing the rate of infusion of dexmedetomidine to target a plasma concentration of 3.2 ng/ml. The infusion of the PET tracer was started exactly 15 min after the infusion of dexmedetomidine was initiated or increased. The PET measurements were followed by the TTE measurements.

4.2.2 Study II

Study II had a randomized, open-label, placebo-controlled design. The aim of the study was to investigate the contribution of endothelial NOS to the net responses of human peripheral blood vessels to dexmedetomidine-induced vasoconstriction. Two separate series of experiments with healthy young men were performed; constriction responses of digital arteries to dexmedetomidine with and without NOS inhibition by infusion of L-NMMA were studied using photoplethysmography, and the responses of DHVs to dexmedetomidine with and without NOS inhibition were studied using the linear variable differential transformer method (LVDT).

The finger blood flow sub-study was conducted in two sessions. In both sessions, after induction of axillary nerve blockade with 1 % mepivacaine (35 ml) to eliminate the

effects of the sympathetic nervous system on blood vessels (Talke *et al.*, 2003), a 22 G catheter was inserted into the brachial artery for drug infusion, BP monitoring and blood sampling. A vein in the opposite forearm was cannulated for administration of fluids and dexmedetomidine. The study subject was connected to monitoring devices for recording of LTF, BP, ECG, and skin temperature. In addition, in both sessions, after a 30 min stabilization period of supine rest, blood samples were drawn for analysis of plasma levels of dexmedetomidine, NA and adrenaline.

In one session, after drawing the first blood samples, an intravenous infusion of dexmedetomidine was started to achieve a pseudo steady-state plasma dexmedetomidine concentration of 1.2 ng/ml. 30 min after the beginning of dexmedetomidine infusion, blood samples were collected, and an infusion of saline for 5 min was given into the brachial artery. After the end of the saline infusion, a 5 min L-NMMA infusion was started. Blood samples were drawn at the end of the saline and L-NMMA infusions and 10 min after the end of the L-NMMA infusion. The effect of L-NMMA alone on digital small arteries was studied in another session that was similar to the previously described session – this time with intravenous infusion of saline instead of dexmedetomidine. Blood flow through digital arteries in both hands was assessed by monitoring LTF. The order of these two sessions was randomized and sessions were separated by a minimum of 7 days. Different hands were studied in each session to avoid repeated brachial artery cannulation.

In the DHV study, effects of L-NMMA on responses to dexmedetomidine were investigated by simultaneous infusion of a constant dose of L-NMMA and increasing doses of dexmedetomidine into a dorsal vein in one of the hands, and saline and increasing doses of dexmedetomidine into a vein in the other hand. Graded increases in dexmedetomidine dose were achieved by increasing the drug concentration in the syringes while keeping the infusion rate constant. After cannulation of the radial artery in one hand (for BP monitoring and blood sampling), subjects were prepared for assessment of the diameter of DHVs in both hands by LVDT, and were connected to monitoring devices for recording of BP, ECG, and skin temperature. Infusions of saline from two infusion pumps were started into both DHVs. After at least 25 min of supine rest, baseline measurements were taken. After the first measurements, infusion of L-NMMA replaced the infusion of saline in one of the hands. This L-NMMA infusion lasted until the end of the session. Ten minutes after the initiation of the L-NMMA infusion, the measurements were made. Infusions of eight graded doses of dexmedetomidine were initiated in both hands – in one hand with concurrent infusion of L-NMMA and in the other hand with saline. Each of the eight infusion phases lasted 5 min, with the cuffs inflated in the last 2 min of each phase. The subjects were given 500 mg of acetylsalisylic acid p.o. one hour before the experimental session to inhibit synthesis of prostaglandins. The study subjects were monitored in the study unit for at least 1 h after the termination of the drug infusions. The subjects were released according to modified outpatient criteria of Turku University Hospital. The study design is depicted in Figure 7.

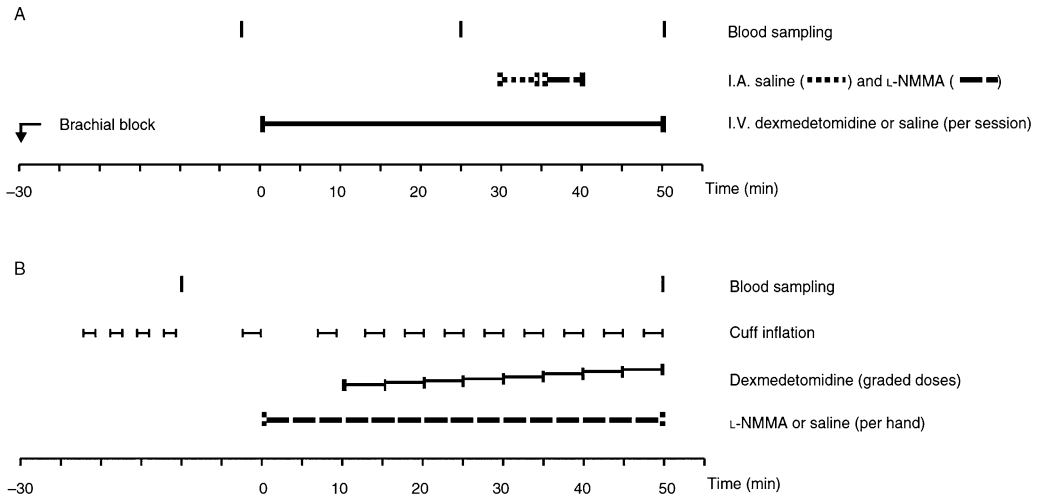

Figure 7. Outline of the design of Study II. (A) The LTF experiment. Subjects were studied in two sessions, one with i.v. infusion of saline and the other with dexmedetomidine, started 30 min after the induction of brachial block. An i.a. saline infusion was started 30 min after the initiation of the i.v. dexmedetomidine infusion, and lasted 5 min. The i.a. saline infusion was followed by a 5 min i.a. infusion of L-NMMA. (B) The DHV experiment. In this experiment, both hands were studied simultaneously with one hand infused with saline and dexmedetomidine (control) and the other hand infused with L-NMMA and dexmedetomidine. The cuffs on both hands were inflated several times until a stable venous distension was observed. Ten minutes after the start of the L-NMMA infusion in one hand, infusion of eight graded doses of dexmedetomidine was started — 5 min of each drug concentration. Venous distension was measured in the last 3 min of each infusion phase. i.a., intra-arterial; i.v., intravenous.

4.2.3 Study III

This was a single-blinded, randomized study that was designed to investigate the effects of activation of α_1-adrenoceptors (with phenylephrine) and α_2-adrenoceptors (with dexmedetomidine) on DHV constriction. DHV responses were measured with LVDT. In this study, increasing doses of dexmedetomidine were infused into a selected DHV in one hand and phenylephrine into a similar vein in the other hand to determine to what extent the potencies of the α_1-AR and α_2-AR agonists to induce venous constriction are associated within a subject. The treatments were randomly allocated to the left and right hands of the subjects.

After placement of BP recording cuffs on both arms, the subjects were prepared for assessment of the diameter of dorsal veins in both hands and were connected to monitoring devices for recording of BP and skin temperature. Graded increases in drug dose rates were achieved by increasing the drug concentrations in the syringes while keeping the infusion rates constant. Infusions of saline from 2 infusion pumps for each hand (at a rate of 0.1 ml/min from each) were started into both DHVs. The subjects were prepared for assessment of the diameter of DHVs in both hands by LVDT. Twenty min after completion of all preparations, the cuffs were inflated for 2 min at 3-min intervals

until reproducible plateau distensions was attained. Thirty minutes from the beginning of the session, the cuffs were inflated for 3 min, and baseline measurements were taken. After vein distension recording and the release of the cuffs, an infusion of increasing doses of dexmedetomidine replaced the infusion of saline in one of the hands (rate of 0.1 ml/min). At the same time, an infusion of increasing doses of phenylephrine replaced the infusion of saline in the other hand. These graded dexmedetomidine and phenylephrine infusions continued until the end of the session. Each of the eight infusion phases lasted 5 min, with the BP cuffs inflated to 45 mmHg in the last 3 min of each phase. The study subjects were monitored in the study unit for at least 30 min after the termination of the drug infusions. The subjects were released according to modified outpatient criteria of Turku University Hospital. The study design is depicted in Figure 8.

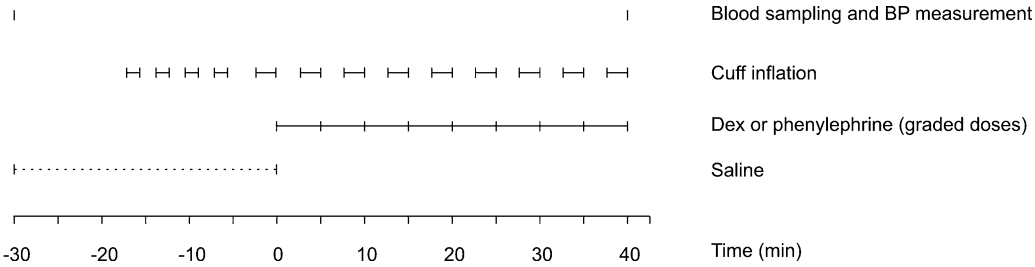

Figure 8. Outline of the design of Study III. Dorsal hand veins were infused with saline before infusion of the study drugs started. The cuffs were inflated for 2 min at 3-min intervals until reproducible plateau distensions were attained. Just before the beginning of infusion of the study drugs, the cuffs were inflated for 3 min, and baseline measurements were taken. An infusion of increasing doses of dexmedetomidine and phenylephrine replaced the infusion of saline. Each of the 8 infusion phases lasted 5 min and venous distension was measured in the last 3 min of each phase. dex, dexmedetomidine; BP, blood pressure.

4.2.4 Study IV

This study investigated possible associations of common genetic variants with responsivity of DHV constriction to dexmedetomidine. The study consisted of a discovery phase and a replication phase. In the discovery phase, we performed a GWAS of 433,378 polymorphic gene loci with the sensitivity of DHV responses in 64 healthy Finnish subjects selected to represent the low end and the high end of sensitivity to dexmedetomidine, as assessed in a quantitative manner in terms of ED_{50} values for drug-induced DHV constriction. In the replication phase, we selected 20 SNPs identified in the GWAS and tested their associations with the ED_{50} of dexmedetomidine in an independent North American study population of 68 healthy individuals. The *in vivo* experiments of the discovery (Turku, Finland) and replication (Vanderbilt, TN, USA) phases of the study were performed independently by the group in Turku and by Prof. C. Michael Stein's research group. In the discovery phase, the experiments were conducted in the Clinical Pharmacology Laboratory of the Department of Pharmacology, Drug Development and Therapeutics, University of Turku. The clinical experiments of the replication phase were conducted at Vanderbilt University Clinical Research Center, TN, USA. α_2-AR-mediated DHV constriction was elicited by graded infusions of increasing

concentrations of dexmedetomidine (Precedex®, Hospira, Lake Forest, IL, USA) into the investigated vein.

In Turku and Vanderbilt, the subjects were placed in a supine position on a bed and their arms were placed on padded supports. The veins to be investigated were cannulated, and after placement of low-pressure tourniquet cuffs on both arms the subjects were prepared for measurement of the diameters of the cannulated hand veins. Responses of DHVs to dexmedetomidine were analysed in both study populations and ED_{50} values of dexmedetomidine were determined. Genotyping and data analysis were performed at the Technology Centre of the Finnish Institute for Molecular Medicine (FIMM), University of Helsinki, Finland. The 64 discovery-phase study samples were genotyped, and one pair of siblings (estimated genome-wide Identity by Descent (IBD) > 0.2) was identified and the individual with fewer successful genotype calls was removed from the study. A total of 433,378 SNPs passed the quality control and were included in the analysis. Twenty SNPs from the GWAS were selected for the replication phase by including the top 5 loci of the GWAS probability ranking list and the top 15 loci from a pre-defined candidate gene set (n = 256) that was compiled based on an extensive literature review. The replication samples were genotyped at FIMM. The study design is illustrated in Figure 9.

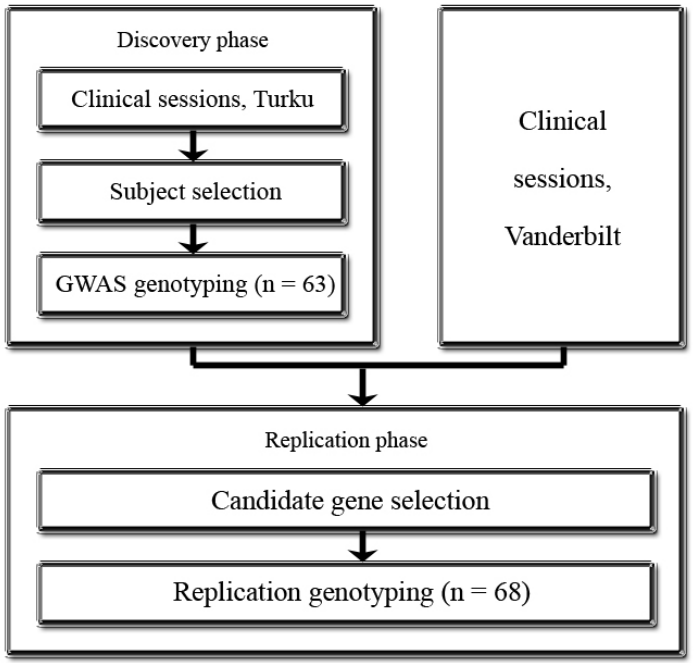

Figure 9. Outline of the design of Study IV. The clinical DHV sessions were conducted in Turku, Finland and Vanderbilt University, TN, USA. The pool of 118 recruited subjects from Turku was the same as that in Study III. Of the 99 subjects that received drug infusions, 64 subjects with the largest and smallest ED_{50} estimates for dexmedetomidine were selected. 20 SNPs were selected based on the GWAS results and a pre-defined candidate gene set (n = 256) that was compiled based on an extensive literature review and their associations with the ED_{50} of dexmedetomidine were tested in an independent North American study population of 68 subjects.

4.3 Methods

4.3.1 Drug infusions (I, II, III)

In Study I, dexmedetomidine (Precedex®) was diluted in normal saline (2 ml of Precedex into 48 ml of 0.9 % NaCl solution, final concentration 4 μg/ml) , and was administered as a continuous i.v. infusion using a computer-driven (STANPUMP® software) infusion pump (Harvard 22®, Harvard Apparatus, Holliston, MA, USA) aiming at pseudo steady-state plasma drug concentrations of 0.5 ng/ml (Low Dex) and 3.2 ng/ml (High Dex). The software that controlled the pump was supplied with the appropriate pharmacokinetic parameters of dexmedetomidine (Talke *et al.*, 2003). The maximum infusion rate was set to 0.3 μg/kg/min. With these infusion settings, it was expected that the targeted plasma concentration of 3.2 ng/ml would be reached in about 5 min from the start of the High Dex infusion. The total average dexmedetomidine dose that was infused during the study was 4.6 μg/kg.

In the finger blood flow sub-study of Study II, the same procedures and equipment were used, this time aiming at a pseudo steady-state plasma concentration of 1.2 ng/ml (Talke *et al.*, 2003). The total average dexmedetomidine dose was 2.1 μg/kg. With these parameters, the target plasma concentration of 1.2 ng/ml was expected to be reached in about 3 min from the initiation of the infusion. Infusion of an 8 μmol/min dose of L-NMMA was initiated i.a. (1 ml/min, Perfusor ED2, B. Braun, Melsungen, Germany) 30 minutes after the initiation of the saline (one session) or dexmedetomidine (another session) i.v. infusion. In this sub-study, the average total infused dexmedetomidine dose was 2.2 μg/kg, and the total infused L-NMMA dose was 40 μmol.

In the DHV sub-study of Study II, dexmedetomidine (Precedex®) was diluted in saline to prepare eight concentrations that when delivered at a constant rate of 0.2 ml/min resulted in doses from 0.01 to 164 ng/min (0.01, 0.04, 0.16, 0.64, 2.6, 10.2, 41 and 164 ng/min) into the investigated DHVs on both hands (Module PDS, Fresenius Vial, Brezins, France). A similar method has been earlier employed by Muszkat *et al.* (Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a). This infused dose was not expected to have systemic effects (changes in HR or BP). In clinical use, the recommended dosage of dexmedetomidine consists of an initial bolus dose of 1 μg/kg over 10 minutes followed by a constant-rate infusion from 0.2 to 0.7 μg/kg/h. Each of the eight infusion phases lasted 5 min. To avoid lengthy interruptions in the infusion protocol, two infusion pumps were used to infuse dexmedetomidine - the switching time between the infusion steps was only a few seconds. L-NMMA infusion of 0.5 μmol/min with a constant infusion rate of 0.1 ml/min was initiated before dexmedetomidine into one of the hands; a constant infusion rate of 0.3 ml/min was maintained by a saline infusion until the dexmedetomidine infusion was started. In this sub-study, the total infused dose of L-NMMA was about 35 μmol, and the total infused dose of dexmedetomidine was only about 2.2 μg per subject.

In Study III, eight increasing dose rates of dexmedetomidine were infused at a constant infusion volume rate (0.1 ml/min) into a dorsal vein in one hand. Dexmedetomidine (Precedex®) was diluted in physiological saline to prepare 8 concentrations that were delivered at dose rates of 0.0128 to 1000 ng/min (0.0128, 0.064, 0.32, 1.6, 8, 40, 200

and 1000 ng/min). The total infused dexmedetomidine dose was about 6.25 µg over 40 minutes. This infused dose was not expected to exert systemic effects. Phenylephrine was diluted in physiological saline to prepare eight concentrations that when delivered at a constant rate of 0.1 ml/min ranged in dose from 3.66 to 8000 ng/ml (3.66, 10.97, 32.92, 98.77, 296.3, 888.9, 2667 and 8000 ng/ml). Similar doses of phenylephrine have been used by Landau *et al.* (Landau *et al.*, 2004) and no systemic haemodynamic effects were noted during the drug infusions. The total infused phenylephrine dose was about 60.0 µg over 40 min. In clinical anaesthetic practice when treating hypotension, the usual intravenous bolus dose is 0.2 mg with a range from 0.1 mg to 0.5 mg.

In study IV, the first group of subjects was selected from the study population of Study III. For the second group of subjects investigated at Vanderbilt University, dexmedetomidine (Precedex®; 0.01 – 100 ng·min) was administered in increasing doses, with each dose infused for 7 min and with the DHV diameter recorded during the last 2 min of each infusion phase.

4.3.2 Measurement of cardiac function (I)

4.3.2.1 PET assessments

Study I was performed at Turku PET Centre using a GE Advance (General Electrics, Milwaukee, WI, USA) scanner. MBF can be directly assessed with [^{15}O] labeled water ([^{15}O]H_2O) and PET. [^{15}O]H_2O is chemically inert, freely diffusible, and has a short half-life of approximately 2 min, and is therefore ideal for perfusion assessments. [^{15}O]O_2 gas (radiochemical purity 97 %) was produced with a low-energy deuteron accelerator Cyclone 3 (IBA, Ion Beam Applications Inc., Louvain-la-Neuve, Belgium) at Turku University Hospital. The [^{15}O]O_2 was recovered at the target output and purified as described earlier (Strijckmans *et al.*, 1985). [^{15}O]H_2O was manufactured by using a diffusion membrane technique in a continuously working water module (Sipilä *et al.*, 2001).

MBF was measured by administering [^{15}O]H_2O over 20 s with an automated infusion system (infused mean [SD] doses during Baseline, Low Dex, and High Dex were 991 [141], 968 [102], and 956 [89] MBq) and acquisition of serial transaxial tomographic images of the heart was performed with the PET scanner. The image acquisition was undertaken during approximately 5 min (14 · 5, 3 · 10, 3 · 20 and 4 · 30 s frames). The arterial blood radioactivity was simultaneously assessed by obtaining arterial blood from the radial artery using a peristaltic roller pump. The radioactivity concentration was measured using a two-channel coincidence detection system (GEMS, Uppsala, Sweden), which was cross-calibrated with the PET scanner. All data were corrected for dead time, decay, and measured photon attenuation as described before (Iida *et al.*, 1988). The acquired data sets were processed by factor analysis to enable accurate localization of myocardial tissue. The PET image analyst was blind to all study variables. Quantitative analysis of MBF was based on four myocardial mid-ventricular slices from the acquired transaxial images. Regions of interest that were first defined on images taken at Baseline were used to analyze the images taken during Low and High Dex. Time–activity curves

of myocardial tissue were created based on these regions of interest. Coronary vascular resistance (CVR) was calculated by dividing mean systemic arterial BP with MBF. The method for the calculation of regional MBF, which is presented in milliliters per gram of tissue per minute, has been described elsewhere (Iida *et al.*, 1988, Schiller *et al.*, 1989).

4.3.2.2 Transthoracic echocardiography

Parameters of left ventricle function were measured with TTE. All measurements were performed by the same investigator using an Acuson Sequoia C 512 (Acuson Inc., Mountain View, CA, USA) instrument with a 3.5 MHz transducer. Ultrasound scans were recorded on videotape for later analysis. Standard and modified subcostal, apical, and parasternal imaging windows were used. Results are the averages of the three measurements. Early (E´) and late (A´) myocardial relaxation velocities were measured using tissue Doppler imaging with an 8 mm gate basally in the lateral wall. Peak velocity of early filling (E) was measured with pulsed-wave Doppler with a 5 mm gate at the level of the mitral leaflets in the four-chamber view. Longitudinal contraction of the left and right ventricles was measured as displacement of the lateral atrial valve annulus in M-mode using the apical imaging window. The ratios of E´ to A´ and E to E´ were used to assess parameters of myocardial relaxation. The ratio between the pre-ejection period and the left ventricular ejection time (the contractility index, pre-ejection period divided by the left ventricle ejection time) was measured using phonocardiography and pulsed-wave Doppler–derived outflow of the left ventricle. Ejection fraction and left ventricular diameter were based on M-mode measurements. SV was calculated as the product of the cross-sectional area of the left ventricular outflow tract and the velocity time integral of the left ventricular outflow measured approximately 5 mm from the aortic valve. CO (SV · HR) was calculated as the mean of the CO measured in the right and the left ventricles. Motion of the ventricle walls was monitored with TTE for recognition of ischaemic events.

4.3.2.3 Cardiac output and mixed venous oxygen saturation

CO and mixed venous oxygen saturation were assessed by a fiberoptic pulmonary artery flotation catheter (Swan-Ganz CCOmbo, CCO/SVO2, Catheter model 744HF75, 7.5 French; Edwards Lifesciences LLC, Irvine, CA, USA), which was introduced into a pulmonary artery during pressure monitoring *via* an 8.5-French introducer inserted into the right internal jugular or the left subclavian vein. The catheter was connected to an Edwards Vigilance® Monitor (Edwards Lifesciences LLC). Data from the Vigilance® monitor was stored on a personal computer using Vigilance Monitor Driver 2.1 program (Technical Services Division, Department of Medical Physics, Royal Perth Hospital, Perth, Australia). Systemic vascular resistance was calculated as 80 · mean systemic arterial BP/CO.

4.3.2.4 ECG and respiratory rate

12-lead ECG was monitored for ischaemic episodes (ST-segment deviation ≥ 1 mm (0.1 mV) below the ST-segment baseline or ≥ 2 mm above ST-segment baseline and

lasting for at least 1 min) throughout the study. ECG data were collected with a personal computer running S/5 Collect software (version 4; Datex-Ohmeda) connected to an S/5 patient monitoring system (Datex-Ohmeda, Helsinki, Finland). Saved TTE and ECG data were later analyzed for ischaemic events, the former according to a five-point scale that defines the severity and the extent (by standard 16 area segmentation) of wall motion abnormality (Schiller *et al.*, 1989).

Respiratory rate was measured with a PowerLab system (PowerLab/4SP and Chart version 5.02, ADInstruments, Castle Hill, New South Wales, Australia) using a nasal air temperature probe (model MLT415, connected to a thermistor pod model ML309, ADInstruments).

4.3.3 Measurement of blood pressure (I, II, III)

In study I, systemic BP was measured and monitored continuously with a 20 G intra-arterial transducer (left radial artery, Truwave PX-600F 3X; Edwards Lifesciences LLC, Irvine, CA, USA) Pulmonary arterial BP was monitored with a transducer in the pulmonary artery. The transducers were connected to an S/5 patient monitoring system (Datex-Ohmeda, Helsinki, Finland) that was connected to a personal computer running S/5 Collect software (version 4; Datex-Ohmeda).

In Study II, arterial BP was measured and monitored in a similar fashion as in Study I. In Study III, BP was measured non-invasively with an automated measurement device (Omron 7051T, Berner Ltd., Helsinki, Finland) with the pressure cuff positioned around the left ankle.

4.3.4 Measurement of digital artery constriction (II)

LTF was measured with infrared photoelectric probes attached to the middle fingers of both hands. The probes (Oximeter Sensor OxyTip+ OXY-AF, GE Healthcare, Helsinki, Finland) were connected to patient monitoring systems (Datex-Ohmeda S/5 Anaesthesia Monitor with E-PRESTN haemodynamic module, GE Healthcare) that were connected to a personal computer equipped with an S/5 iServer 4.2 network package and two data acquisition software packages (S/5 iCollect version 5.0, GE Healthcare). The iCollect systems were set to collect composite plethysmographic waveform data from both patient monitors at a frequency of 100 Hz. Photoelectric plethysmography measures the transmission of infrared light through the tissues of the finger (LTF). The LTF magnitude reflects the finger blood volume.

4.3.5 Measurement of dorsal hand vein constriction (II, III)

The DHV technique was first described by Aellig (Aellig, 1981). This LVDT technique has been evaluated and found to be highly reproducible as a means of studying venous responses repeatedly within subjects (Aellig, 1985, Alradi and Carruthers, 1985, Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a).

Venous tone and constriction responses were measured and recorded using the LVDT device (type CD-375-100, connected to an LVC-2500 signal conditioner, Macro Sensors, Pennsauken, NJ, USA) placed over a vein on the dorsum of the hand while the subjects rested supine on a standard patient bed (Figure 10). The arm was placed on a rigid support sloping upwards at an angle of 25˚ from the horizontal plane, allowing complete emptying of the superficial veins. Movement of the hand and fingers was restricted by lightweight straps and tapes that were attached to the padded support. A BP cuff was placed on the upper arm and inflated to a pressure of 45 mmHg to help to identify suitable veins for the study. Veins that had no tributaries over a segment of 2 to 3 cm, and neither over- nor under-crossed by other veins within about the same distance were considered suitable (Aellig, 1994). A 24 G butterfly needle was inserted into the distal part of the selected vein. With the aid of a small tripod, a measurement transducer was placed over the vein with the central movable core resting exactly on the summit of the vein about 10 mm proximal from the tip of the inserted needle, and with aid of a signal conditioner, output was registered continuously on a chart recorder (PowerLab/4SP and Chart version 5.5.1 software package, ADInstruments). It has been previously demonstrated that these responses are very reproducible within individuals over time and also between the right and left hand (Alradi and Carruthers, 1985, Luthra *et al.*, 1991, Schindler *et al.*, 2003, Muszkat *et al.*, 2010).

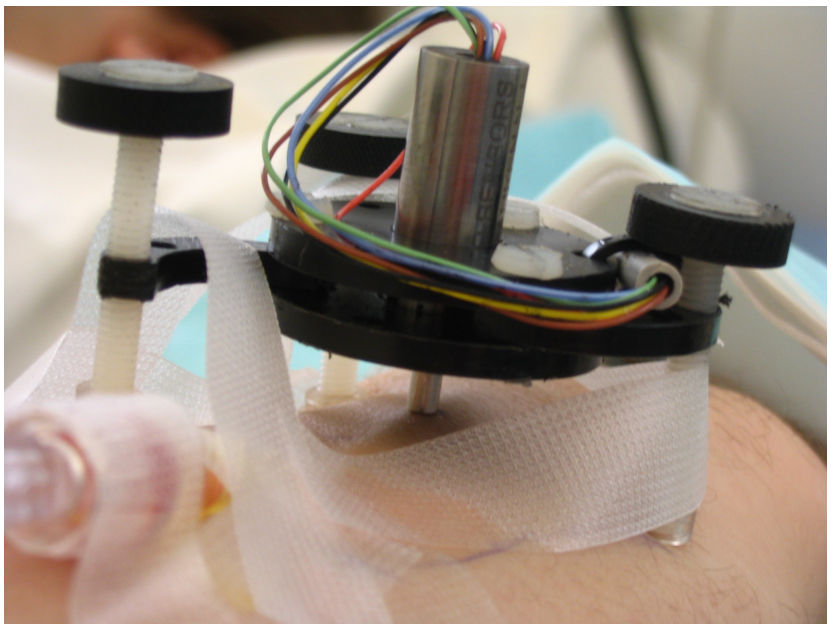

Figure 10. The LVDT tripod and transducer placed on the studied vein. The transducer contains a central movable core resting on the summit of the vein about 10 mm proximally from the tip of the venous catheter. The tripod position is secured with tape and the subject's arm is placed on padded supports and elevated to the same level above the heart, at 25º above horizontal.

After the preparations and stabilization period, baseline measurements of vein distension were recorded by inflating at intervals the cuff to a pressure of 45 mmHg until reproducible plateau distensions were attained. After recording of baseline vein distension and cuff release, the drug infusions were initiated. In studies II and III, each of the infusion phases

lasted 5 min, with the tourniquet cuffs inflated to 45 mmHg during the last 3 min of each phase. In data collection for the replication phase of study IV (Vanderbilt), each dose of dexmedetomidine was infused for 7 min and the DHV diameter was recorded during the last 2 min of each infusion phase.

4.3.6 Skin temperature measurements (II, III)

In Study II, a skin temperature probe (400 series, GE Healthcare) was placed on the distal phalanx of the middle finger of the studied hand and connected to an S/5 patient monitoring system (Datex-Ohmeda, Helsinki, Finland). For data acquisition, the monitor was connected to a personal computer running S/5 Collect software (version 4, Datex-Ohmeda).

In study III, finger tip temperature was monitored with temperature probes (MLT409/A, ADInstruments) placed on the distal phalanx of the middle fingers of both hands. The probes were connected to thermistor pods (ML309, ADInstruments) that were connected to a data acquisition system (PowerLab 4/30, ML866/P, ADInstruments). The data were collected on a personal computer using the data acquisition system Chart5 (Ver 5.5.1 for Windows, ADInstruments).

4.3.7 Genetic analysis (IV)

4.3.7.1 Genome-wide association study genotyping and quality assurance

Genotyping and data analysis were performed at FIMM, University of Helsinki, Finland. The 64 discovery-phase study samples were genotyped using Illumina's Human660W-Quad BeadChips, iScan System, and with standard reagents and protocols provided by Illumina Inc. (San Diego, CA, USA). The genotypes were read and confirmed with Illumina's GenomeStudio v. 2009.1 software, in-house developed database tools, and the PLINK v1.07 toolset (http://pngu.mgh.harvard.edu/~purcell/plink/) (Purcell *et al.*, 2007).

SNPs with a genotyping success rate of < 0.95, minor allele frequency of < 0.10 or p value of $< 10^{-6}$ in an exact test for Hardy-Weinberg equilibrium were removed. Relatedness between the study subjects was assessed by estimating the pairwise identity-by-descent for all subject pairs in the sample with PLINK. One pair of related individuals (estimated genome-wide identity-by-descent > 0.2) was identified and the individual with fewer successful genotype calls was removed from the study. A total of 433,378 SNPs passed the quality control and were included in the analysis.

4.3.7.2 Replication sample genotyping, quality assurance and single nucleotide polymorphism selection

The replication samples were genotyped at FIMM for 20 SNPs selected on the basis of the discovery phase results in two multiplex reactions using the iPlex assay on the MassARRAY System (Sequenom, San Diego, CA, USA) with standard reagents and protocols. The primer sequences are listed in Table 4. Each individual sample was genotyped in duplicate. The concordance rate for all samples was 100 %.

Table 4. The primer sequences and locations used in Study IV

SNP	2nd-PCRP	1st-PCRP	UEP
rs1285441	ACGTTGGATGGAACCTAGATTTGTAAGCAG	ACGTTGGATGCTCATTTGCTTCAGAAAGCC	TGTAAGCAGAAAGCAGA
rs10189339	ACGTTGGATGCAACGCAGATTAACACCGTC	ACGTTGGATGCACGCCCTGCCTGGGATTT	TTCCCCGCCATTGTTAGT
rs4677889	ACGTTGGATGCACCCCCTGCTAGGATATAG	ACGTTGGATGAGGCAGAGAAGCAGACCTTG	AGGATATAGGAGAGCACT
rs815815	ACGTTGGATGACACATTTTCTCTAGGGCAC	ACGTTGGATGCAAAAATTGTTGAGCAGGG	CAAGAAGGAATCCAGAAAG
rs1017437	ACGTTGGATGTTTTGTTTGTTTTTCACCG	ACGTTGGATGGGGAATGTATCATAGCTTTC	TGTTTTTCACCGATCTTTTA
rs10989249	ACGTTGGATGTGGATGTCTGTTATGGCCTC	ACGTTGGATGGTCATAGTGGGAGTTAAGGC	CTGTTATGGCCTCAATCTTG
rs7100726	ACGTTGGATGTTTTCATGACTGTGCCTTGC	ACGTTGGATGCCTGGCCTGACTCTTTCATT	agAGTTGTGGTGTCTCAAAC
rs9922316	ACGTTGGATGCTTTTCTGCCAATCTTCACC	ACGTTGGATGTCTCATTTTGAGGGCCAGAG	ccTCTTCACCTAGTTTTTGTTT
rs9562057	ACGTTGGATGCCCTACAGATTACTGATGTC	ACGTTGGATGAAATGGCTCTAAAGGCAGTC	TGATGTCAAGTTATCCTTTTTC
rs2514897	ACGTTGGATGTTTCCCCAGAAGGGTCTTAG	ACGTTGGATGCCTCTTTGCATATTTGTACC	ggatGGTCTTAGAAGCCCAGTA
rs2859136	ACGTTGGATGCTAATCTCATCAGCTCCCAC	ACGTTGGATGTTACGGTGGATCCCAGGAC	CCACTGCCTCCCCTAGCTAGTCCTTA
rs11130254	ACGTTGGATGTCACTATTCCCGACCCAAAG	ACGTTGGATGCAGTGACATTTTCCAAAGTG	CCAAAGGGTGACTTTTC
rs1540293	ACGTTGGATGATTCTCCCTAGTGGCTGAAG	ACGTTGGATGCACCCAAGGTGTTGAGCTTC	GACTGCAGGCATGTGTC
rs6691456	ACGTTGGATGAACAGATGCTGGCAAGGC	ACGTTGGATGGCTTTCCACAGTGGCTAAAA	AGGCTGTGGAAGAAAGG
rs7893279	ACGTTGGATGGACAGATGAATGTACATGAC	ACGTTGGATGCACCGTGCCTGGCCAAGAT	CCTCTCCCTTAAGAAACT
rs9347479	ACGTTGGATGCCGGGAAGCTTTTCTGGTAG	ACGTTGGATGGTGAGTAAATGGTGTGAGAG	GAATCCTTGTGCTTCTGAA
rs6796205	ACGTTGGATGCTGGGATTTGAACCCAGTC	ACGTTGGATGCAGTCCACTTCTAAAGGCAG	ACCCAGTCAATCTAACACCA
rs10435634	ACGTTGGATGGCATTATACTTACCAGTTG	ACGTTGGATGCAAAGGAAATGCTCATTGG	ACTTACCAGTTGAACATCCCAA
rs7144087	ACGTTGGATGCTGAGAGACAAGTTCTTCAC	ACGTTGGATGACTCTCTTTCAAAGTGACCC	TCTTCACATGTAAATCAATTGTC
rs851006	ACGTTGGATGGTCAGTGTTACCACAATGTC	ACGTTGGATGCACTCTAATTCAGCTTCTAC	CAACCTAAAGAGGTATTTTTTTC

SNPs, sequences of the pre-amplification PCR primers (PCRP) and single nucleotide extension primers (UEP) designed for multiplex genotyping

A total of 20 SNPs from the GWAS were selected for the replication phase by including the top 5 loci of the GWAS probability ranking list (p value range, $7.1 \cdot 10^{-6}$ to $5.2 \cdot 10^{-5}$) with no consideration of gene identity, and the top 15 loci (p value range, 0.00011 to 0.018) from a pre-defined candidate gene set. The candidate gene set was based on an extensive literature review and included altogether 256 genes implicated in α_2-AR-mediated signalling (see Table 5). The coordinates of the genes were retrieved from the BioMart database (http://www.biomart.org) and converted them from the GRCh37 to the NCBI36 reference genome coordinates with the Ensembl assembly converter. For each gene on the candidate gene list, SNPs within 2000 base pairs of the gene of interest and SNPs annotated as putative expression quantitative trait loci for the gene in the eQTL browser (http://eqtl.uchicago.edu/cgi-bin/gbrowse/eqtl/) were considered. The candidate genes were ranked and one SNP was selected from each of the top 15 candidate genes for association analysis (see Table 6). For each gene, the most strongly associated SNP was selected. However, for *PRKCB* and *RGS20*, these were not accessible for the genotyping technology and the second-best SNPs were used in their place.

4.3.8 Analytical laboratory methods (I, II, III)

Concentrations of dexmedetomidine in plasma were determined using reversed-phase high-performance liquid chromatography with tandem mass spectrometric detection (PE Sciex API365 or API4000 instrument, PE Sciex, Foster City, CA, USA). The method was developed in-house by modifying a previously published procedure (Ji *et al.*, 2004). In study I and in studies II and III, the lower limits of reliable quantitation of the assay were 0.1 ng/ml and 0.02 ng/ml, respectively. The within- and between-run precision of the assay (coefficient of variation) was within 8 % in the relevant concentration range. Concentrations of catecholamines in plasma were determined using high-performance liquid chromatography with coulometric electrochemical detection (Coulochem 5100A, ESA Inc., Bedford, MA, USA) (Scheinin *et al.*, 1991). In studies I, II and III, blood samples were drawn by the study personnel and the screening and safety assessments (blood count, lipid profile and drug screening) were performed at TYKSLAB (Turku University Hospital).

4.3.9 Data handling (I, II, III)

4.3.9.1 Haemodynamic responses (I)

In study I, for analysis of the effects of dexmedetomidine at discrete time points, BP, CO, HR, respiratory rate, and mixed venous oxygen saturation were reduced from continuous 10-s measurements to 1-min median values that corresponded with the non-continuous measurements (PET, TTE and blood tests).

4.3.9.2 Peripheral vasoconstriction (II, III)

In the LTF sub-study of study II, the effects of L-NMMA on peripheral vasoconstriction in the presence and absence of dexmedetomidine were assessed by two methods. In

Table 5. The candidate gene set used in Study IV. The list is based on a literature review and includes 256 genes implicated in α_2-AR-mediated signalling.

Gene	Protein	Location
ADCY1	adenylate cyclase 1 (brain)	7p13-p12
ADCY2	adenylate cyclase 2 (brain)	5p15.3
ADCY3	adenylate cyclase 3	2p24-p22
ADCY4	adenylate cyclase 4	14q11.2
ADCY5	adenylate cyclase 5	3q13.2-q21
ADCY6	adenylate cyclase 6	12q12-q13
ADCY7	adenylate cyclase 7	16q12.1
ADCY8	adenylate cyclase 8 (brain)	8q24
ADCY9	adenylate cyclase 9	16q13.3
ADORA2A	adenosine A2a receptor	22q11.23
ADORA2B	adenosine A2b receptor	17p12
ADORA2BP1	adenosine A2b receptor pseudogene 1	1q32
ADRA2A	adrenergic, alpha-2A-, receptor	10q24-q26
ADRA2B	adrenergic, alpha-2B-, receptor	2p13-q13
ADRA2C	adrenergic, alpha-2C-, receptor	4p16
AKR1C3	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	10p15-p14
ARRB1	arrestin, beta 1	11q13
ARRB2	arrestin, beta 2	17p13
ATP6V1A	ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A	3q13.31
ATP6V1B1	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B1	2p13
CACNA1A	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	19p13
CACNA1B	calcium channel, voltage-dependent, N type, alpha 1B subunit	9q34
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	12p13.3
CACNA1D	calcium channel, voltage-dependent, L type, alpha 1D subunit	3p14.3
CACNA1E	calcium channel, voltage-dependent, L type, alpha 1E subunit	1q25-q31
CACNA1F	calcium channel, voltage-dependent, L type, alpha 1F subunit	Xp11.23
CACNA1S	calcium channel, voltage-dependent, L type, alpha 1S subunit	1q32
CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	3p21.3
CACNA2D3	calcium channel, voltage-dependent, alpha 2/delta subunit 3	3p21.1
CACNA2D4	calcium channel, voltage-dependent, alpha 2/delta subunit 4	12p13.33
CACNB1	calcium channel, voltage-dependent, beta 1 subunit	17q21-q22
CACNB2	calcium channel, voltage-dependent, beta 2 subunit	10p12
CACNB3	calcium channel, voltage-dependent, beta 3 subunit	12q13
CACNB4	calcium channel, voltage-dependent, beta 4 subunit	2q22-q23
CACNG1	calcium channel, voltage-dependent, gamma subunit 1	17q34
CACNG2	calcium channel, voltage-dependent, gamma subunit 2	22q13.1
CACNG3	calcium channel, voltage-dependent, gamma subunit 3	16p12.1
CACNG4	calcium channel, voltage-dependent, gamma subunit 4	17q24
CACNG5	calcium channel, voltage-dependent, gamma subunit 5	17q24
CACNG6	calcium channel, voltage-dependent, gamma subunit 6	19q13.4
CACNG7	calcium channel, voltage-dependent, gamma subunit 7	19q13.4
CACNG8	calcium channel, voltage-dependent, gamma subunit 8	19q13.4
CALM1	calmodulin 1 (phosphorylase kinase, delta)	14q24-q31
CALM2	calmodulin 2 (phosphorylase kinase, delta)	2p21.3-p21.1
CALM3	calmodulin 3 (phosphorylase kinase, delta)	19q13.2-q13.3
CALML3	calmodulin-like 3	10pter-p13
CALML4	calmodulin-like 4	15q22.31
CALML5	calmodulin-like 5	10p15.1
CALML6	calmodulin-like 6	1p36.33
CAMK1	calcium/calmodulin-dependent protein kinase I	3p25.3

Gene	Protein	Location
CAMK1D	calcium/calmodulin-dependent protein kinase ID	10p13
CAMK1G	calcium/calmodulin-dependent protein kinase IG	1q32-q41
CAMK2A	calcium/calmodulin-dependent protein kinase II alpha	5
CAMK2B	calcium/calmodulin-dependent protein kinase II beta	7p14.3-p14.1
CAMK2D	calcium/calmodulin-dependent protein kinase II delta	4q26
CAMK2G	calcium/calmodulin-dependent protein kinase II gamma	10q22
CAMK2N1	calcium/calmodulin-dependent protein kinase II inhibitor 1	1p36.12
CAMK2N2	calcium/calmodulin-dependent protein kinase II inhibitor 2	3q27.1
CAMK4	calcium/calmodulin-dependent protein kinase IV	5q21-q23
CAMKK1	calcium/calmodulin-dependent protein kinase kinase 1, alpha	17p13.3
CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta	12q24.2
CHGA	chromogranin A (parathyroid secretory protein 1)	14q32
CHGB	chromogranin B (secretogranin 1)	20pter-p12
COMT	catechol-O-methyltransferase	22q11.21
CSK	c-src tyrosine kinase	15q24.1
CYB561	cytochrome b-561	17q23.3
CYB561D1	cytochrome b-561 domain containing 1	1p13.2
CYB561D2	cytochrome b-561 domain containing 2	3p21.3
DAGLA	diacylglycerol lipase alpha	11q12.3
DAGLB	diacylglycerol lipase beta	7p22.1
DDC	dopa decarboxylase (aromatic L-amino acid decarboxylase)	7p11
DBH	dopamine beta-hydroxylase (dopamine beta-monooxygenase)	9q34
FMO3	flavin containing monooxygenase 3	1q24.3
GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	19p13.3
GNAI1	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	7q21
GNAI2	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	3p21
GNAI3	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	1p13
GNAO1	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O	16q13
GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	9q21
GNAS	GNAS complex locus	20q13.2-q13.3
GNAT1	guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 1	3p21
GNAT2	guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 2	1p13
GNAT3	guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 3	7q21.11
GNAZ	guanine nucleotide binding protein (G protein), alpha z polypeptide	22q11.1-q11.2
GNB1	guanine nucleotide binding protein (G protein), beta polypeptide 1	1p36.33
GNB2	guanine nucleotide binding protein (G protein), beta polypeptide 2	7q21.3-q22.1
GNB3	guanine nucleotide binding protein (G protein), beta polypeptide 3	12p13
GNB4	guanine nucleotide binding protein (G protein), gamma 4	3q27.1
GNB5	guanine nucleotide binding protein (G protein), gamma 5	15q21.1
GNG2	guanine nucleotide binding protein (G protein), gamma 2	14q21
GNG3	guanine nucleotide binding protein (G protein), gamma 3	11p11
GNG4	guanine nucleotide binding protein (G protein), gamma 4	1q42.3
GNG5	guanine nucleotide binding protein (G protein), gamma 5	1p22
GNG7	guanine nucleotide binding protein (G protein), gamma 7	19p13.3
GNG8	guanine nucleotide binding protein (G protein), gamma 8	19q13.32
GNG10	guanine nucleotide binding protein (G protein), gamma 10	9q32
GNG11	guanine nucleotide binding protein (G protein), gamma 11	7q31-q32
GNG12	guanine nucleotide binding protein (G protein), gamma 12	1p31.2
GNG13	guanine nucleotide binding protein (G protein), gamma 13	16p13.3
GNGT1	guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 1	7q21.3
GNGT2	guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2	17q21
GPR44	prostaglandin D2 receptor (DP2)	11q12-q13.3

Gene	Protein	Location
GRK1	G protein-coupled receptor kinase 1	13q34
GRK4	G protein-coupled receptor kinase 4	4p16.3
GRK5	G protein-coupled receptor kinase 5	10q24-qter
GRK6	G protein-coupled receptor kinase 6	5q35
GRK6PS	G protein-coupled receptor kinase 6 pseudogene	13q12.11
GRK7	G protein-coupled receptor kinase 7	3q24
HPGDS	hematopoietic prostaglandin D synthase	4q22.2
ITPKA	inositol-trisphosphate 3-kinase A	15q15.1
ITPKB	inositol-trisphosphate 3-kinase B	1q42.12
ITPKC	inositol-trisphosphate 3-kinase C	19q13.1
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1	3p26.1
ITPR2	inositol 1,4,5-triphosphate receptor, type 2	12p11.23
ITPR3	inositol 1,4,5-triphosphate receptor, type 3	6p21.31
ITPRIP	inositol 1,4,5-triphosphate receptor-interacting protein	10q25.1
MAOA	monoamine oxidase A	Xp11.4-p11.3
MAOB	monoamine oxidase B	Xp11.4-p11.3
MAP2K1	mitogen-activated protein kinase kinase 1	15q22.1-q22.33
MAP2K3	mitogen-activated protein kinase kinase 3	17q11.2
MAP2K5	mitogen-activated protein kinase kinase 5	15q22.31
MAP2K6	mitogen-activated protein kinase kinase 6	17q
MAP3K1	mitogen-activated protein kinase kinase kinase 1	5q11.2
MAP3K3	mitogen-activated protein kinase kinase kinase 3	17q
MAP3K4	mitogen-activated protein kinase kinase kinase 4	6q25.3
MAP3K5	mitogen-activated protein kinase kinase kinase 5	6q22.33
MAP3K6	mitogen-activated protein kinase kinase kinase 6	1p36.11
MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3	2p22.3
MAPK1	mitogen-activated protein kinase 1	22q11.2
MAPK10	mitogen-activated protein kinase 10	4q22-q23
MAPK11	mitogen-activated protein kinase 11	22q13.33
MAPK12	mitogen-activated protein kinase 12	22q13.3
MAPK13	mitogen-activated protein kinase 13	6p21
MAPK14	mitogen-activated protein kinase 14	6p21.3-p21.2
MAPK15	mitogen-activated protein kinase 15	8q24.3
MAPK1IP1L	mitogen-activated protein kinase 1 interacting protein 1-like	14q22.1
MAPK3	mitogen-activated protein kinase 3	16p11.2
MAPK4	mitogen-activated protein kinase 4	18q21.2
MAPK6	mitogen-activated protein kinase 6	15q21
MAPK6PS1	mitogen-activated protein kinase 6 pseudogene 1	8q11.23
MAPK6PS2	mitogen-activated protein kinase 6 pseudogene 2	21q11.2
MAPK6PS3	mitogen-activated protein kinase 6 pseudogene 3	13q14.13
MAPK6PS4	mitogen-activated protein kinase 6 pseudogene 4	8q11.21
MAPK6PS5	mitogen-activated protein kinase 6 pseudogene 5	8q23
MAPK6PS6	mitogen-activated protein kinase 6 pseudogene 6	10q11.21
MAPK7	mitogen-activated protein kinase 7	17p11.2
MAPK8	mitogen-activated protein kinase 8	10q11
MAPK8IP1	mitogen-activated protein kinase 8 interacting protein 1	11p11.2
MAPK8IP2	mitogen-activated protein kinase 8 interacting protein 2	22q13.33
MAPK8IP3	mitogen-activated protein kinase 8 interacting protein 3	16p13.3
MAPK8IPP	mitogen-activated protein kinase 8 interacting protein, pseudogene	17q21.31
MAPK9	mitogen-activated protein kinase 9	5q35
MAPKAP1	mitogen-activated protein kinase associated protein 1	9q34.11
MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2	1q32

Gene	Protein	Location
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	3p21.3
MAPKAPK5	mitogen-activated protein kinase-activated protein kinase 5	12q24.13
MAPKBP1	mitogen-activated protein kinase binding protein 1	15q15.1
MAPKSP1 (LAMTOR3)	late endosomal/lysosomal adaptor, MAPK and MTOR activator 3	4q24-q26
MGLL	monoglyceride lipase	3p13-q13.33
NOS1	nitric oxide synthase 1 (neuronal)	12q14-qter
NOS3	nitric oxide synthase 3 (endothelial cell)	7q36
PKIA	protein kinase (cAMP-dependent, catalytic) inhibitor alpha	8q21.11
PKIB	protein kinase (cAMP-dependent, catalytic) inhibitor beta	6q21-q22.1
PLA2G10	phospholipase A2, group X	16p13.1-p12
PLA2G12A	phospholipase A2, group XIIA	4q25
PLA2G12B	phospholipase A2, group XIIB	10q22.1
PLA2G1B	phospholipase A2, group IB (pancreas)	12q23-qter
PLA2G2A	phospholipase A2, group IIA (platelets, synovial fluid)	1p35
PLA2G2C	phospholipase A2, group IIC	1p36.12
PLA2G2D	phospholipase A2, group IID	1p36.12
PLA2G2E	phospholipase A2, group IIE	1p36.13
PLA2G2F	phospholipase A2, group IIF	1p35
PLA2G3	phospholipase A2, group III	22q11.2-q13.2
PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)	19q13.32
PLA2G4B	phospholipase A2, group IVB (cytosolic)	15q11.2-q21.3
PLA2G4C	phospholipase A2, group IVC (cytosolic, calcium-independent)	1q25
PLA2G4D	phospholipase A2, group IVD (cytosolic)	15q14
PLA2G4E	phospholipase A2, group IVE	15q15.1
PLA2G4F	phospholipase A2, group IVF	15q15.1
PLA2G5	phospholipase A2, group V	1p36-p34
PLA2G6	phospholipase A2, group VI (cytosolic, calcium-independent)	22q13.1
PLCB1	phospholipase C, beta 1 (phosphoinositide-specific)	20p12
PLCB2	phospholipase C, beta 2	15q15
PLCB3	phospholipase C, beta 3 (phosphatidylinositol-specific)	11q13
PLCB4	phospholipase C, beta 4	20p12
PLCD1	phospholipase C, delta 1	3p22-p21.3
PLCD3	phospholipase C, delta 3	17q21.31
PLCD4	phospholipase C, delta 4	2q35
PLCG1	phospholipase C, gamma 1	20q12-q13.1
PLCG2	phospholipase C, gamma 2	16q24.1
PNMT	phenylethanolamine N-methyltransferase	17q
PRKACA	protein kinase, cAMP-dependent, catalytic, alpha	19p13.1
PRKACB	protein kinase, cAMP-dependent, catalytic, beta	1p36.1
PRKACG	protein kinase, cAMP-dependent, catalytic, gamma	9q13
PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	17q23-q24
PRKAR1AP	protein kinase, cAMP-dependent, regulatory, type I, alpha pseudogene	1p31-p21
PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta	7pter-p22
PRKAR2A	protein kinase, cAMP-dependent, regulatory, type II, alpha	3p21.3-p21.2
PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	7q22.3
PRKCA	protein kinase C, alpha	17q22-q24
PRKCB	protein kinase C, beta	16p12
PRKCD	protein kinase C, delta	3p21.31
PRKCDBP	protein kinase C, delta binding protein	11p15.4
PRKCE	protein kinase C, epsilon	2p21
PRKCG	protein kinase C, gamma	19q13.4

Gene	Protein	Location
PRKCH	protein kinase C, eta	14q23.1
PRKCI	protein kinase C, iota	3q26.3
PRKCQ	protein kinase C, theta	10p15
PRKCZ	protein kinase C, zeta	1p36.33-p36.2
PTGDR	prostaglandin D2 receptor (DP)	14q22.1
PTGDS	prostaglandin D2 synthase 21kDa (brain)	9q34.2-q34.3
PTGER1	prostaglandin E receptor 1 (subtype EP1), 42kDa	19p13.1
PTGER2	prostaglandin E receptor 2 (subtype EP2), 53kDa	14q22
PTGER3	prostaglandin E receptor 3 (subtype EP3)	1p31.2
PTGER4	prostaglandin E receptor 4 (subtype EP4)	5p13.1
PTGES	prostaglandin E synthase	9q34.3
PTGES2	prostaglandin E synthase 2	9q34.12
PTGES3	prostaglandin E synthase 3 (cytosolic)	12q13.13
PTGFR	prostaglandin F receptor (FP)	1p31.1
PTGIR	prostaglandin I2 (prostacyclin) receptor (IP)	19q13.3
PTGIS	prostaglandin I2 (prostacyclin) synthase	20q13
PTGS1	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	9q32-q33.3
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	1q25.2-q25.3
PTK2B	PTK2B protein tyrosine kinase 2 beta	8p21.1
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	3p25
RGS1	regulator of G-protein signaling 1	1q31
RGS2	regulator of G-protein signaling 2	1q31
RGS3	regulator of G-protein signaling 3	9q32
RGS4	regulator of G-protein signaling 4	1q23.3
RGS5	regulator of G-protein signaling 5	1q23.1
RGS6	regulator of G-protein signaling 6	14q24.3
RGS7	regulator of G-protein signaling 7	1q43
RGS7BP	regulator of G-protein signaling 7 binding protein	5q12.3
RGS8	regulator of G-protein signaling 8	1q25
RGS9	regulator of G-protein signaling 9	17q24
RGS10	regulator of G-protein signaling 10	10q25
RGS11	regulator of G-protein signaling 11	16p13.3
RGS12	regulator of G-protein signaling 12	4p16.3
RGS13	regulator of G-protein signaling 13	1q31.2
RGS14	regulator of G-protein signaling 14	5q35.3
RGS16	regulator of G-protein signaling 16	1q25-q31
RGS17	regulator of G-protein signaling 17	6q25-q26
RGS18	regulator of G-protein signaling 18	1q31.2
RGS19	regulator of G-protein signaling 19	20q13.33
RGS20	regulator of G-protein signaling 20	8q11.23
RGS21	regulator of G-protein signaling 21	1q31.1
RGS22	regulator of G-protein signaling 22	8q22.2
RGS9BP	regulator of G protein signaling 9 binding protein	19q13.11
RGSL1	regulator of G protein signaling like 1	1q25
SLC18A1	solute carrier family 18 (vesicular monoamine), member 1	8p21.3
SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	10q25
SLC6A2	solute carrier family 6 (neurotransmitter transporter, noradrenaline), member 2	16q12.2
SLCO2A1	solute carrier organic anion transporter family, member 2A	3q21
TBXA2R	thromboxane A2 receptor	19p13.3
TBXAS1	thromboxane A synthase 1 (platelet)	7q34-q35
TH	tyrosine hydroxylase	11p15.5

Gene and protein names obtained from The HUGO Gene Nomenclature Committee (HGNC) homepage (http://www.genenames.org)

Table 6. Candidate genes for the replication phase of Study IV. The replication analysis included the top 5 loci from the GWAS (smallest *p* values) and top 15 loci (smallest *p* values) from a pre-defined candidate gene list of 256 α_2-AR-associated genes

Gene	Protein	SNP	Source
HS6ST3	heparan sulfate 6-O-sulfotransferase 3	rs9562057	GWAS
intergenic	-	rs1017437	GWAS
intergenic	-	rs1285441	GWAS
intergenic	-	rs2514897	GWAS
intergenic	-	rs7144087	GWAS
ADCY5	adenylate cyclase 5	rs4677889	candidate
CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	rs1540293	candidate
CACNB2	calcium channel, voltage-dependent, beta 2 subunit	rs7893279	candidate
CALM2	calmodulin 2 (phosphorylase kinase, delta)	rs815815	candidate
CAMK1D	calcium/calmodulin-dependent protein kinase ID	rs7100726	candidate
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1	rs6796205	candidate
MAP3K4	mitogen-activated protein kinase kinase kinase 4	rs9347479	candidate
MAPK13	mitogen-activated protein kinase 13	rs2859136	candidate
MAPK14	mitogen-activated protein kinase 14	rs851006	candidate
MAPK4	mitogen-activated protein kinase 4	rs10989249	candidate
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	rs11130254	candidate
PRKCB	protein kinase C, beta	rs9922316*	candidate
PRKCE	protein kinase C, epsilon	rs10189339	candidate
RGS20	regulator of G-protein signaling 20	rs10435634*	candidate
RGS5	regulator of G-protein signaling 5	rs6691456	candidate

* PRKCB and RGS20 were not directly assessable by the employed genotyping technology and the second-best SNPs were used in their place. Gene and protein names obtained from The HUGO Gene Nomenclature Committee (HGNC) homepage (http://www.genenames.org)

the first method, the area under the photoplethysmographic time–response curve (AUC) was calculated during the 5 min i.a. saline and L-NMMA infusions. The baseline for AUC was defined as the value at the start of the segment. Net effects of L-NMMA on digital vasoconstriction for each session (saline and dexmedetomidine) were calculated by subtracting the AUC during the saline infusion from the AUC during the L-NMMA infusion. In the second method, the relative significance of NO production on finger blood vessel tone in the presence and absence of dexmedetomidine was evaluated with 1 min means of LTF for three segments being calculated: *i.e.* just before initiation of the i.v. dexmedetomidine infusion (baseline), at the end of the i.a. saline infusion (100 % of response to dexmedetomidine), and at the highest response to L-NMMA.

In the DHV sub-study of study II, responses of DHVs to L-NMMA and dexmedetomidine were analyzed by calculating the area under the LVDT-time curve. The AUC was calculated for the last 3 min of each infusion phase (the period of inflated cuff), with the value at the time when cuff inflation was turned on defined as baseline. Integral data were then transformed into per cent, with 100 % defined as the AUC of the phase just before the first dexmedetomidine infusion was started. The effect of L-NMMA alone on vein distension was analyzed by comparing the AUC of the phase just before time 0 and the next phase (*i.e.* the phase before dexmedetomidine infusion). The dexmedetomidine ED_{50} value was determined using a sigmoidal dose–response model with variable slope.

In study III, responses of DHVs to phenylephrine and dexmedetomidine were analysed by calculating AUC in a similar manner as in study II. ED_{50} values of phenylephrine and dexmedetomidine were determined using a sigmoidal dose-response model with variable slope.

4.3.10 Statistical analysis (I, II, III, IV)

Descriptive data are presented as means and 95 % confidence intervals or means and SD.

In study I, statistical significance was assessed with paired-samples *t* tests, or with repeated-measures analysis of variance with Tukey's *post hoc* tests. Multivariate correlates of MBF were assessed by multivariate stepwise linear regression modeling (forward manner, $p < 0.05$ to enter, $p \geq 0.10$ to remove). Variables of interest were first assessed with Pearson's bivariate correlation analysis, and those that were correlated with MBF with $p < 0.1$ were inserted into the multivariate regression model. For presentation of relative changes in systemic mean arterial pressure, HR, CO, systemic vascular resistance, and rate–pressure product (RPP, where RPP = systolic BP · HR), the median of 1-min continuous measurements that started 2 min before the initiation of the dexmedetomidine infusion was determined as baseline (100 %). Statistical analyses were performed with SPSS for Windows (version 12.0.1, SPSS Inc., Chicago, IL, USA) and with GraphPad Prism for Windows (version 4.03; GraphPad Software, San Diego, CA, USA).

In the DHV sub-study of Study II, the curve-fit models were accepted only when the goodness of fit (R^2) was > 0.95. Statistical analyses were performed with SPSS for Windows (version 12.0.1, SPSS Inc.) and with GraphPad Prism for Windows (version 4.03, GraphPad Software).

In study III, data were included in the statistical efficacy analysis if the curve-fit models of both hands fitted the AUC data points with a goodness of fit (R^2) of 0.95 or greater, and if the ratio between the upper or lower limit of the ED_{50} 95 % confidence interval and the ED_{50} estimate was less than six-fold. This was done to ensure that only reliable ED_{50} estimates were included in the analysis. ED_{50} values were log-transformed before analysis. Pearson's correlation analysis was used to assess bivariate relationships. Linear regression modelling was used to calculate an estimate of the extent to which the included independent factors explained the ED_{50} of dexmedetomidine. Three models were used: in Model 1, the independent factor was $\log(ED_{50})$ of phenylephrine. In Model 2, the independent factors were $\log(ED_{50})$ of phenylephrine, age, BMI and finger-tip temperature in the dexmedetomidine infusion hand (mean temperature from the last 25 min of the drug infusion). Model 3 included the covariates of Model 2 and the interaction between age and finger temperature. Possible associations between DHV responses to dexmedetomidine and blood lipid levels, haemoglobin concentration, BP, HR, plasma NA and dexmedetomidine concentrations, and in women also hormonal contraception and menstrual cycle phase were assessed with single covariate linear regression models. Student's *t*-test was used to assess the difference between genders in the time (in minutes) that passed from the beginning of the preparation of the subject for the experimental session until the hands were warm enough to start the drug infusion. Differences in

systolic and diastolic BP and HR before and after the infusions were assessed with paired samples *t* tests. Statistical analyses were performed with SPSS program for Windows (versions 16.0.1, SPSS Inc.) and with GraphPad Prism for Windows (version 4.03, GraphPad Software).

In Study IV, the statistical tests for association were done with PLINK using linear regression and an additive genetic model. Log-transformed dexmedetomidine ED_{50} was set as the dependent variable with sex, age and temperature of the infusion hand finger tip (mean temperature from the last 25 min of the drug infusion (discovery phase) or sex and age (replication phase) used as covariates. The results from the two phases of the study were combined with PLINK in a fixed-effects meta-analysis.

5. RESULTS

5.1 Effects of therapeutic and high dexmedetomidine plasma levels on myocardial perfusion, cardiac function and haemodynamics (I)

In study I, dexmedetomidine exerted a biphasic effect on haemodynamics. Low dexmedetomidine concentrations (0.5 ng/ml) reduced BP and HR, and high concentrations (5 ng/ml) produced substantial increases in systemic arterial, pulmonary arterial and central venous BP and peripheral, coronary and pulmonary vascular resistance in parallel with reductions in HR, mixed venous oxygen saturation, and CO. The results are presented for the entire study population of 12 subjects, because the *ADRA2B* 901_909del polymorphism of the α_{2B}-AR had no apparent effects on MBF or CVR at baseline or during the two drug infusion phases. The effects of dexmedetomidine on haemodynamics are summarized in Table 7, and the results with regard to systemic, pulmonary arterial and central venous BP are illustrated in Figure 11.

Table 7. Effects of dexmedetomidine on myocardial function and haemodynamics in Study I. *Low Dex* and *High Dex* correspond to two drug infusion phases with target plasma concentrations of 0.5 ng/ml and 3.2 ng/ml, respectively. Data at *Baseline* are presented as means (SD). Data in the other columns are differences between the means, and their 95 % confidence intervals. The corresponding *p* values are from repeated measures ANOVA with Tukey's multiple comparisons *post-hoc* test. The ANOVA *p* value for all comparisons was less than 0.001 for all variables. *n* = 12 or 11 (marked with #). Time-point data are 1-min medians extracted from continuous data (except for the measurements by PET and TTE). ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

	Baseline	Low Dex vs. Baseline	High Dex vs. Baseline	High Dex vs. Low Dex
Myocardial blood flow (ml/g·min)	0.86 (0.16)	-0.23 [-0.33, -0.13] ***	-0.26 [-0.37, -0.16] ***	-0.03 [-0.14, 0.07] ns
Coronary resistance (mmHg·g·min/m)	83 (13)	+16 [2, 30] *	+58 [44, 72] ***	+43 [29, 57] ***
Systolic systemic BP (mmHg)	140 (18)	-25 [-33, -17]***	+5 [-4, 13] ns	+30 [22, 38] ***
Diastolic systemic BP (mmHg)	70 (6)	-10 [-14, -6] ***	+14 [10, 18] ***	+24 [20, 28] ***
Systolic pulmonary BP# (mmHg)	20 (4)	-3 [-6, 0] *	+7 [5, 10] ***	+10 [8, 12] ***
Diastolic pulmonary BP# (mmHg)	9 (2)	-2 [-3, -1] **	+1 [0, 2] *	+4 [3, 5] ***
Systemic vascular resistance (dyn·s·cm^{-5})	1014 (258)	-139 [-300, 22] ns	+595 [434, 756] ***	+735 [574, 896] ***
Rate-pressure product (mmHg/min)	9342 (2088)	-2261 [-3195, -1327] ***	-1852 [-2786, -918] ***	+408 [-526, 1342] ns
Central venous oxygen saturation# (%)	77 (4)	-1 [-4, 1] ns	-7 [-9, -5] ***	-6 [-8, -3] ***
Heart rate (beats/min)	66 (10)	-4 [-8, 0] *	-14 [-18, -10] ***	-10 [-14, -6] ***
Respiratory rate (breaths/min)	14 (3)	-0 [-2, 2] ns	+5 [3, 7] ***	+5 [3, 7] ***
Cardiac output (Vigilance®) (l/min)	7.1 (1.6)	-0.2 [-1.2, 0.8] ns	-2.2 [-3.2, -1.2] ***	-2.0 [-3.0, -1.0] ***
Cardiac output (TTE) (l/min)	5.4 (1.2)	-0.4 [-0.9, 0.1] ns	-1.7 [-2.2, -1.2] ***	-1.3 [-1.8, -0.8] ***
Stroke volume (TTE) (ml)	78 (14)	-2 [-8, 4] ns	-11 [-17, -5] ***	-9 [-15, -3] **

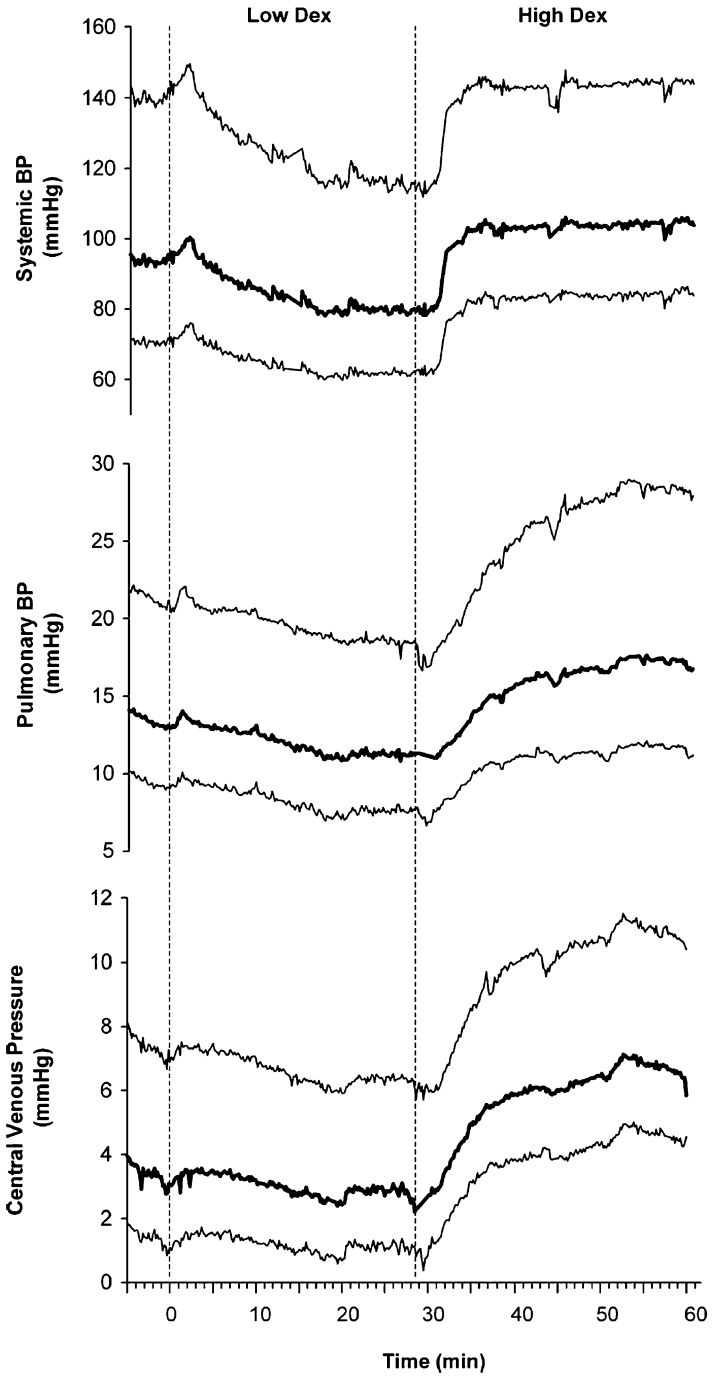

Figure 11. Systemic, pulmonary and central venous BP during low (mean, 0.5 ng/ml) and high (mean, 5 ng/ml) plasma levels of dexmedetomidine. The upper and lower thinner lines represent systolic and diastolic BP. The thicker lines represent mean arterial pressure ([systolic − diastolic BP]/3 + diastolic BP). Data are means of all subjects (systemic BP, n = 12; central venous pressure and pulmonary BP, n = 11).

The dexmedetomidine infusions caused substantial changes in CVR and MBF as assessed with [^{15}O]H_2O and PET. In the Low Dex infusion phase, the average (95 % CI) increase in CVR elicited by the low dexmedetomidine concentration was 20 % (11 to 29 %, $p < 0.001$); at the same time, an average 26 % (-31 to -23 %, $p < 0.001$) decrease in MBF with a concomitant average reduction of 23 % (-28 to -18 %, $p <$

0.01) in RPP was seen, reflecting decreased myocardial oxygen demand and workload. The high dexmedetomidine concentration in the High Dex infusion phase produced no appreciable further decrease of MBF (mean, -3 %; -12 % to +6 %, $p > 0.05$) from Low Dex. In the High Dex phase, the decrease of MBF was 29% (-39 to -18%, $p < 0.001$) from baseline. There was quite extensive inter-individual variation in the responses to dexmedetomidine in the High Dex phase. The change in RPP during Low Dex was the only variable that was significantly associated with the reduction in MBF from baseline ($r^2 = 0.45$, $p = 0.017$). The effects of dexmedetomidine on MBF and CVR are presented in Figure 12.

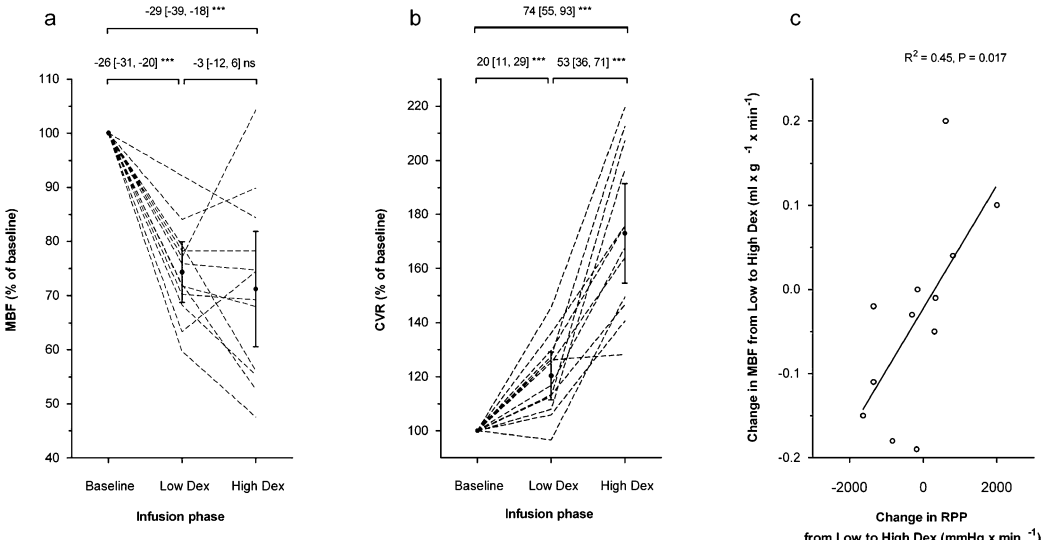

Figure 12. Effects of low and high plasma concentrations of dexmedetomidine on MBF (A) and CVR (B), and the relationships between changes in the rate–pressure product (RPP) and MBF during the two infusion phases (C). Effects of dexmedetomidine on MBF and CVR are presented for individual subjects and the means and their 95 % confidence intervals for all subjects are shown (presented as per cent of baseline values). Differences between means, their 95 % confidence intervals, and the corresponding p values from paired t tests are shown. Changes in RPP between the Low Dex and High Dex phases were associated with the corresponding changes in MBF. The regression line, r^2, and the corresponding p value are from a linear regression model. *** $p < 0.001$, ns = not significant ($p > 0.05$)

Systolic myocardial function was only slightly affected by dexmedetomidine administration. Changes in ejection fraction, contractility index (preejection period divided by the left ventricle ejection time), and displacements of the mitral and tricuspid valve annuli indicated mildly depressed myocardial function during the Low Dex and High Dex phases as compared to baseline. The diastolic function of the heart was not altered. As assessed with TTE and ECG, no signs of myocardial ischaemia were observed during the experimental sessions. The effects of dexmedetomidine on myocardial function are presented in Table 8 and Figure 13.

Table 8. Effects of dexmedetomidine on myocardial function in Study I. *Low Dex* and *High Dex* correspond to dexmedetomidine target plasma concentrations of 0.5 ng/ml and 3.2 ng/ml, respectively. Data at *Baseline* are presented as means (SD). Data in the other columns are differences between the means, and their 95 % confidence intervals. The corresponding *p* values are from repeated measures ANOVA with Tukey's multiple comparisons *post-hoc* test. $n = 12$ or 11 (marked with #). A' = late diastolic blood flow velocity through the mitral valve annulus; E = peak velocity of early filling; E' = early diastolic blood flow velocity through the mitral valve annulus; MV = mitral valve; PEP = the time from the Q-wave in the ECG to the second heart sound minus left ventricular ejection time; LVET = left ventricular ejection time; TCV = tricuspid valve; TDI = tissue Doppler imaging; ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

	Baseline	**Low Dex vs. Baseline**	**High Dex vs. Baseline**	**High Dex vs. Low Dex**	**Overall p**
Diastolic function					
E'/A' #	2.0 (0.5)	+0.2 [-0.3, 0.7] ns	+0.1 [-0.4, 0.6] ns	-0.1 [-0.6, 0.4] ns	0.66
E/E' #	3.1 (0.6)	-0.4 [-0.9, 0.1] ns	-0.5 [-1.0, 0] ns	-0.0 [-0.5, 0.5] ns	0.054
Systolic function					
TDI lateral wall (m/s)	0.19 (0.04)	+0.02 [-0.02, 0.06] ns	-0.00 [-0.04, 0.04] ns	-0.03 [-0.07, 0.01] ns	0.14
M-mode MV lateral annulus (cm)	1.5 (0.3)	-0.1 [-0.2, 0] ns	-0.2 [-0.3, -0.1] ***	-0.1 [-0.2, 0] ns	< 0.001
M-mode TCV lateral annulus (cm)	2.4 (0.3)	-0.3 [-0.5, -0.1] **	-0.3 [-0.5, 0.1] **	-0.0 [-0.2, 0.2] ns	0.001
PEP/LVET	0.33 (0.06)	+0.07 [0.01, 0.13] **	+0.10 [0.04, 0.16] ***	+0.02 [-0.04, 0.08] ns	< 0.001
Ejection fraction (%)	64 (9)	-2 [-7, 3] ns	-8 [-10, -6] **	-6 [-8, -4] *	0.004
Left ventricle diameter# (cm)	5.4 (0.4)	0.0 [-0.2, 0.2] ns	+0.2 [0, 0.4] ns	+0.2 [0, 0.4] *	0.019

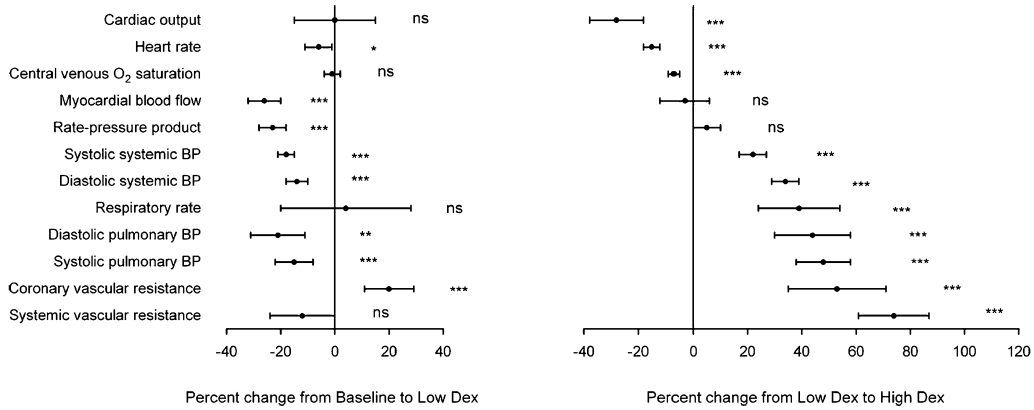

Figure 13. Summary of the effects of dexmedetomidine (Dex) on the cardiovascular system. The data are differences between means and their 95 % confidence intervals between Low Dex (mean plasma dexmedetomidine, 0.5 ng/ml) and Baseline (plasma dexmedetomidine 0 ng/ml) and between High Dex (mean plasma dexmedetomidine, 5 ng/ml) and Low Dex. *p* values are from paired t tests. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. BP = blood pressure; ns = not significant ($p > 0.05$).

5.2 Involvement of endothelial nitric oxide synthesis in peripheral arterial and venous responses to dexmedetomidine (II)

In the LTF sub-study of study II, inhibition of endothelial NO synthesis by L-NMMA significantly augmented the vasoconstriction induced by dexmedetomidine. One subject was excluded from the analysis, because he experienced adverse effects of local anaesthesia. L-NMMA had detectable effects on vascular tone only in the presence of dexmedetomidine-induced vasoconstriction. L-NMMA caused further increases in light transmission through a finger (LTF) (*i.e.*, vasoconstriction) in all of the subjects during the dexmedetomidine infusion. The mean (95 % CI) difference in AUC of the LTF signal between the dexmedetomidine and saline sessions was 26 units (17 to 35 units, $n = 9$, $p < 0.001$; the data from one subject were lost because of technical failure). During the dexmedetomidine session, the mean change in LTF (the difference between the AUC during the 5 min i.a. infusion of L-NMMA and saline) was 26 units (20 to 32 units), while, in the saline session, the mean LTF value remained stable (Figure 14). In the dexmedetomidine session, the mean maximum increase in LTF values during the i.a. L-NMMA infusion was 19 % (14 to 24 %, $p > 0.0001$) (Figure 15). In contrast, there were no statistically significant changes in LTF values during the i.a. L-NMMA infusion during the saline session.

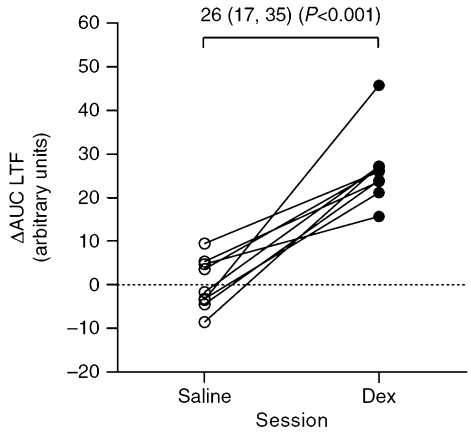

Figure 14. Responses to an intra-arterial (i.a.) infusion of L-NMMA in the absence (saline session) or presence of dexmedetomidine (dex session). Data points are results of subtraction of the area under the time–response curve during a 5-min i.a. saline infusion from the area under the curve during a 5-min i.a. L-NMMA infusion. Dex, dexmedetomidine.

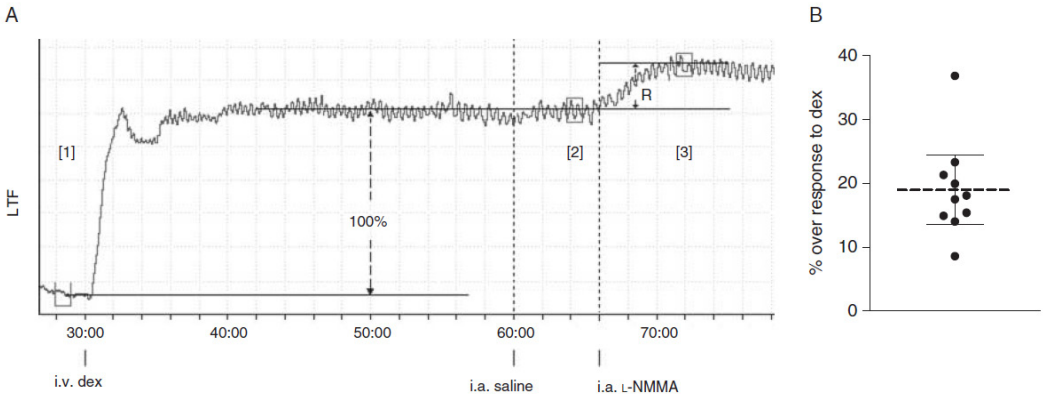

Figure 15. Effects of dexmedetomidine on finger blood flow in the absence and presence of L-NMMA. (A) One minute mean of the composite plethysmograph waveform was calculated for three segments: [1] just before initiation of i.v. dexmedetomidine infusion, [2] at the end of the i.a. saline infusion, and [3] at the highest response to L-NMMA. The first value [1] was determined as baseline, and the second value [2] as 100 % of the dexmedetomidine response. (B) The effect size of NOS inhibition on vessel responses to dexmedetomidine was evaluated by calculating the per cent change between [2] and [3] (denoted as R in A). Data are presented for each subject, and for the study population as the mean and its 95 % confidence interval. Dex, dexmedetomidine; i.a., intra-arterial; i.v., intravenous.

Dexmedetomidine decreased the concentrations of NA and adrenaline in plasma. The mean differences from baseline until the end of the infusion were -0.65 nmol/l (-0.93 to -0.38 nmol/l, $p < 0.001$) and -0.15 nmol/l (-0.22 to -0.08 nmol/l, $p < 0.001$) for NA and adrenaline, respectively. Mean (SD) plasma dexmedetomidine concentrations were 1.76 (0.25) ng/ml at 25 min after the initiation of the dexmedetomidine infusion and 1.77 (0.29) ng/ml at the end of the session. In accordance with expectations and the observed dexmedetomidine concentrations in plasma, all subjects fell asleep before the initiation of the arterial saline infusion. During the dexmedetomidine session, the mean difference (95 % CI) in fingertip temperature between the i.a. infusion of saline and L-NMMA

was -0.20º C (-0.32 to -0.08 ºC), reflecting drug-induced reductions in blood flow. The fingertip temperature data are shown in Figure 16.

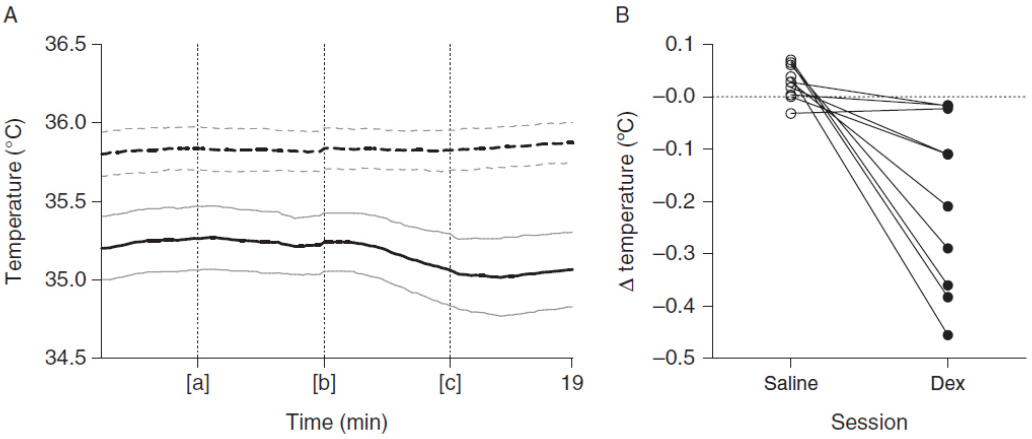

Figure 16. Effects of L-NMMA on fingertip temperature in the absence and presence of dexmedetomidine. (A) Mean fingertip temperature (and its 95 % CI in grey lines) data of all subjects (n = 10) are presented for the control session (dashed line) and the dexmedetomidine session (solid line). [a] Initiation of i.a. saline infusion, [b] initiation of i.a. L-NMMA infusion, and [c] stop of i.a. L-NMMA. (B) Changes in fingertip temperature in response to i.a. infusion of L-NMMA in the absence (Saline) or presence of dexmedetomidine (Dex). Data points are results of subtraction of a 1-min mean of the fingertip temperature during the last minute of i.a. saline infusion from the 1-min mean at the maximal L-NMMA effect calculated at 90 s after the end of the i.a. L-NMMA infusion.

In the DHV sub-study of study II, inhibition of NO synthesis by L-NMMA appeared to have inconsistent effects on dexmedetomidine-induced vasoconstriction. Three subjects were excluded from the analysis of the effect of L-NMMA on dexmedetomidine-induced DHV constriction, because their dose-response curves could not be reliably fitted into a sigmoidal model. The responses of the included subjects are presented in Figure 17. The individual dexmedetomidine ED_{50} estimates were not statistically significantly different in the presence and absence of L-NMMA infusion in 7 subjects. The range of individual dexmedetomidine ED_{50} estimates in the control hand (n = 7) was from 0.3 to 30 ng/min, and from 0.02 to 42 ng/min in the presence of L-NMMA (n = 10) (Figure 18). Arterial BP, HR and plasma concentrations of NA remained stable during the dexmedetomidine infusions. The mean (SD) concentration of dexmedetomidine in the systemic circulation at the end of the low-dose local drug infusion was 0.09 ng/ml (0.05).

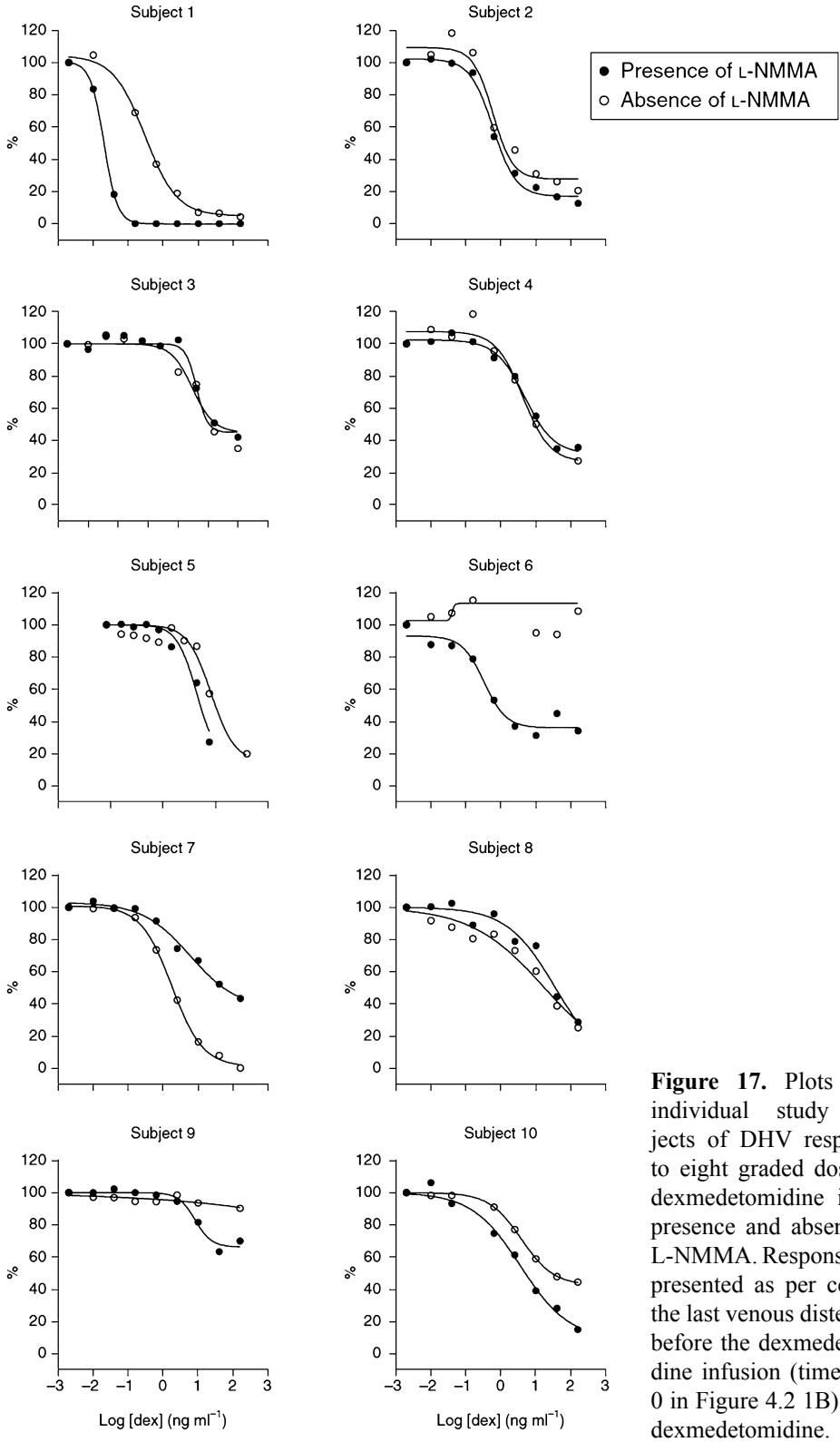

Figure 17. Plots from individual study subjects of DHV responses to eight graded doses of dexmedetomidine in the presence and absence of L-NMMA. Responses are presented as per cent of the last venous distension before the dexmedetomidine infusion (time -3 to 0 in Figure 4.2 1B). Dex, dexmedetomidine.

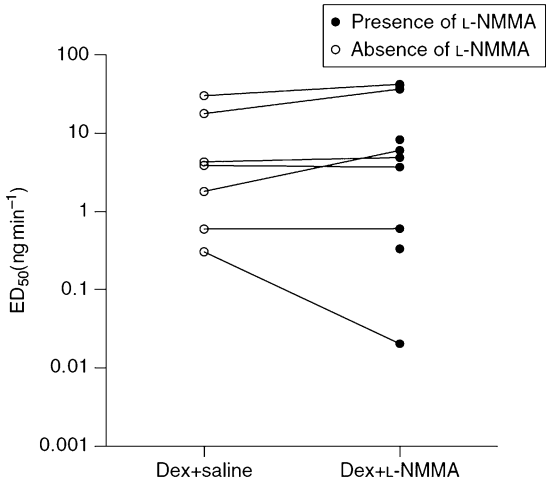

Figure 18. Responses to i.a. infusion of L-NMMA in the absence (saline session) or presence of dexmedetomidine (dex session). Data points are results of subtraction of the area under the time–response curve during a 5-min i.a. saline infusion from the area under the curve during a 5-min i.a. infusion of L-NMMA. In Dex+saline sessions, two values are missing. Dex, dexmedetomidine.

5.3 Clinical determinants of responses to α_2-adrenoceptor activation in dorsal hand veins (III)

In study III, the DHV constriction responses to both dexmedetomidine and phenylephrine showed substantial inter-individual variability. The ranges of the individual ED_{50} estimates were from 0.06 to 412 ng/min and from 14.2 to 7450 ng/min for dexmedetomidine and phenylephrine, respectively. The distributions of these estimates in the efficacy population are presented in Figure 19. A weak positive association between individual ED_{50} estimates of dexmedetomidine and phenylephrine was observed ($r^2 = 0.074$, $p = 0.018$, Figure 20). Together, finger temperature, BMI, age and log(ED_{50}) of phenylephrine explained almost one third of the variation in the sensitivity to dexmedetomidine, in terms of ED_{50} ($r^2 = 0.315$, adjusted $r^2 = 0.275$, $p < 0.001$). A detailed description of the statistical model and analysis results is given in Table 9.

Table 9. Linear regression models and effects of covariates in models for dexmedetomidine ED_{50} in Study III. Results are from linear regression models. SE, standard error; Dex log(ED_{50}), log-transformed ED_{50} of dexmedetomidine; PE log(ED_{50}), log-transformed ED_{50} of phenylephrine; age, age of the subject; BMI, body mass index, dex temp, finger temperature of the dexmedetomidine infusion hand (mean temperature from the last 5 out of 8 infusion dose phases, total 25 min); x denotes an interaction, P represents the statistical significance of individual variables, and *p* shows the statistical significance of each of the three models.

Model	Dependent covariate	Independent covariates	Beta (SE)	P	r^2	p
1	Dex log(ED_{50})	PE log(ED_{50})	0.405 (0.167)	0.018	0.074	< 0.018
2	Dex log(ED_{50})	PE log(ED_{50})	0.366 (0.149)	0.017	0.316	< 0.001
		age	-0.051 (0.018)	0.007		
		dex temp	0.296 (0.117)	0.014		
		BMI	-0.057 (0.037)	0.121		
3	Dex log(ED_{50})	PE log(ED_{50})	0.447 (0.153)	0.005	0.348	< 0.001
		age	1.083 (0.443)	0.017		
		dex temp	0.948 (0.543)	0.085		
		BMI	-0.061 (0.036)	0.095		
		age x dex temp	-0.029 (0.016)	0.070		

Beta coefficients exemplify the effect sizes; they represent the magnitude of an independent variable's effect on the dependent variable in multiple regression analysis.

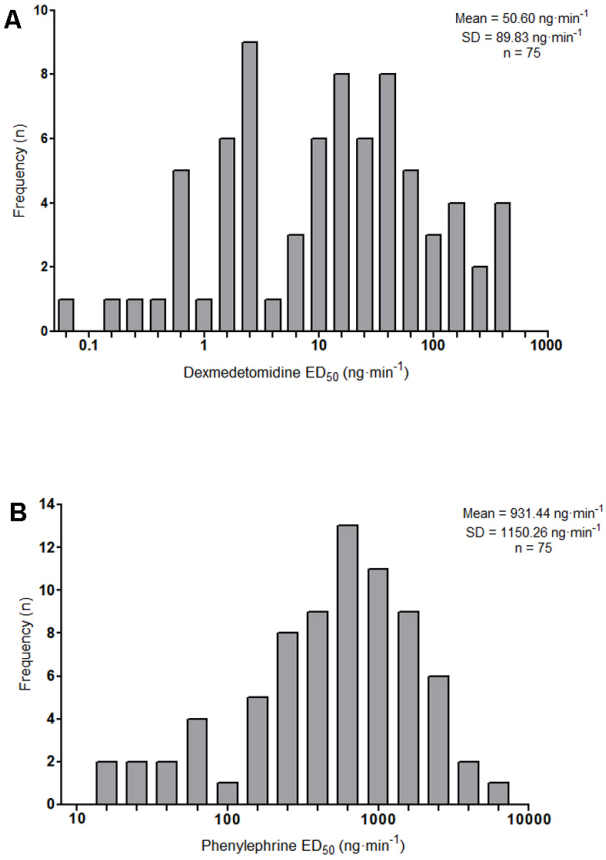

Figure 19. The distributions of the study drug ED_{50} estimates in the per-protocol study population. (A) dexmedetomidine; (B) phenylephrine.

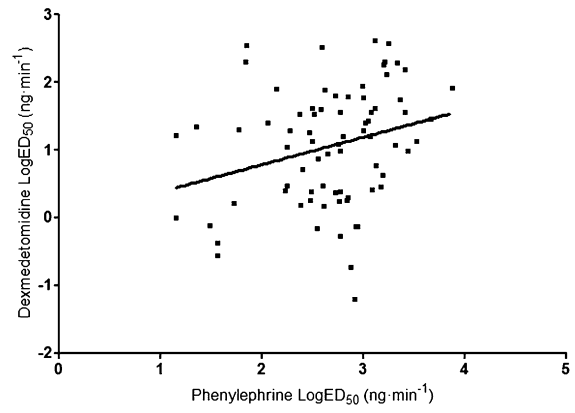

Figure 20. Scatter plot of individual ED_{50} estimates of dorsal hand vein responses to phenylephrine and dexmedetomidine. Log-transformed ED_{50} values and the linear regression line are shown for the equation $y = 0.405\ x - 0.025$, where $y = \log ED_{50}$ for dexmedetomidine and $x = \log ED_{50}$ for phenylephrine.

Gender, blood lipids, BP, plasma NA and dexmedetomidine concentrations, hormonal contraception, blood haemoglobin concentration, HR and menstrual cycle phase were not statistically significant determinants of dexmedetomidine ED_{50}. These results suggested that the large inter-individual variability in the responses of DHVs to both α_1- and α_2-

AR agonists was not explained by some common factors; instead, DHV responsiveness appeared to be separately determined for both receptor mechanisms.

5.4 Genetic factors contributing to the inter-individual variability in α_2-adrenoceptor–mediated vascular constriction induced by dexmedetomidine (IV)

The discovery and replication phases of study IV were performed in succession. In the discovery phase, GWAS was performed with the dexmedetomidine ED_{50} value for DHV constriction as the discriminatory variable. The 63 included subjects represented the top and bottom tertiles of the dexmedetomidine ED_{50} range (ED_{50} values above 5 ng/min or below 30 ng/min) of our material of 99 healthy subjects investigated in Study III. On the basis of the genetic association data for these 63 DNA samples, 20 SNPs were selected for replication analysis in another set of 68 similar DNA samples derived from an experiment carried out in Vanderbilt Medical Center, USA. Five SNPs were the top hits from the entire GWAS dataset and another 15 candidate gene loci were selected from the top (smallest *p* values) of a list of 256 human genes considered to be biologically plausible to be involved in α_2-AR-mediated cellular signalling. In the discovery phase, the association of 433,378 SNPs was correlated with dexmedetomidine ED_{50} for DHV constriction. Associations with unadjusted *p* values smaller than 10^{-4} were found for 46 SNPs (see Table 10). Many of the strongest GWAS association signals were relatively distant from known genes, the most significantly associated SNP rs1285441 ($p = 5.2 \cdot 10^{-6}$) being located more than 400 kb away from the nearest gene, NXPH1. None of these associations remained statistically significant after correction for multiple testing (see Table 11). The 5 top hits from the unselected GWAS result list and the top 15 hits from the candidate gene list were included in the next phase of the study. These 20 SNPs were subjected for replication by association analysis in an independent sample set, and the results were combined in a fixed-effects meta-analysis. In this analysis, none of the associations remained statistically significant after correction for multiple testing. For example, rs1285441 yielded a $p_{combined} = 0.009$.

One of the 20 selected SNPs, rs9922316 that is a marker for the candidate gene PKC type beta (*PRKCB*), was consistently associated with dexmedetomidine ED_{50} for DHV constriction, both in the discovery sample and in the replication sample ($p_{combined} = 0.00016$, see Figure 21). The combined results from the Turku and Vanderbilt samples show that the gene locus for PKC beta exhibited the strongest combined evidence for being a genetic variant associated with inter-individual variation in dexmedetomidine ED_{50} for DHV constriction. The beta coefficients of *PRKCB*, an indicator of the effect size in genetic association analysis, were rather similar in the materials from Turku and Vanderbilt, implying uniform effects in both study populations in the regression analysis. Loci representing PKC epsilon (*PRKCE*, rs10189339), calcium/calmodulin-dependent protein kinase type 1D (*CAMK1D*, rs7100726) and inositol 1,4,5-trisphosphate receptor type 1 (*ITPR1*, rs6796205) also had the same direction of association in both study populations, but these associations failed to reach the statistical significance level of $p < 0.05$ in the replication sample (see Table 11, and Figures 21 and 22).

Table 10. In study IV, in the discovery phase, the association of 433,378 SNPs with dexmedetomidine ED_{50} value for DHV constriction was tested. Associations with unadjusted *p* values less than 10^{-4} were found for 46 SNPs.

Rank	SNP	Chromosome	*p*
1	RS2514897	11	$1.72 \cdot 10^{-6}$
2	RS9562057	13	$5.52 \cdot 10^{-6}$
3	RS1017437	3	$7.24 \cdot 10^{-6}$
4	RS1285441	7	$7.39 \cdot 10^{-6}$
5	RS4546375	5	$7.95 \cdot 10^{-6}$
6	RS7720014	5	$7.95 \cdot 10^{-6}$
7	RS1431034	14	$9.20 \cdot 10^{-6}$
8	RS10483528	14	$9.20 \cdot 10^{-6}$
9	RS2844043	8	$1.16 \cdot 10^{-5}$
10	RS715544	22	$1.58 \cdot 10^{-5}$
11	RS7155420	14	$1.66 \cdot 10^{-5}$
12	RS2415675	14	$1.66 \cdot 10^{-5}$
13	RS10129425	14	$1.66 \cdot 10^{-5}$
14	RS7144087	14	$1.97 \cdot 10^{-5}$
15	RS8142586	22	$2.31 \cdot 10^{-5}$
16	RS764742	1	$2.65 \cdot 10^{-5}$
17	RS17272228	15	$2.94 \cdot 10^{-5}$
18	RS4566595	4	$2.99 \cdot 10^{-5}$
19	RS8086522	18	$3.13 \cdot 10^{-5}$
20	RS8109388	19	$3.32 \cdot 10^{-5}$
21	RS7250696	19	$3.32 \cdot 10^{-5}$
22	RS2479960	13	$3.81 \cdot 10^{-5}$
23	RS1885593	14	$5.07 \cdot 10^{-5}$
24	RS1866226	14	$5.07 \cdot 10^{-5}$
25	RS9579292	13	$5.22 \cdot 10^{-5}$
26	RS1499772	5	$5.39 \cdot 10^{-5}$
27	RS10989249	9	$5.42 \cdot 10^{-5}$
28	RS161163	16	$5.43 \cdot 10^{-5}$
29	RS1558250	12	$5.62 \cdot 10^{-5}$
30	RS6028003	20	$5.91 \cdot 10^{-5}$
31	RS2154637	8	$5.94 \cdot 10^{-5}$
32	RS1465643	14	$5.99 \cdot 10^{-5}$
33	RS3734665	6	$6.95 \cdot 10^{-5}$
34	RS13427732	2	$7.23 \cdot 10^{-5}$
35	RS1025637	10	$7.32 \cdot 10^{-5}$
36	RS4338502	11	$7.38 \cdot 10^{-5}$
37	RS10400960	16	$7.42 \cdot 10^{-5}$
38	RS9579299	13	$7.47 \cdot 10^{-5}$
39	RS4304904	13	$7.77 \cdot 10^{-5}$
40	RS1880143	9	$7.77 \cdot 10^{-5}$
41	RS6578086	8	$8.24 \cdot 10^{-5}$
42	RS501981	11	$8.92 \cdot 10^{-5}$
43	RS6027999	20	$8.95 \cdot 10^{-5}$
44	RS2208299	20	$9.23 \cdot 10^{-5}$
45	RS7746417	6	$9.51 \cdot 10^{-5}$
46	RS1925637	10	$9.68 \cdot 10^{-5}$

Colours denote shared locations in the human genome.

Table 11. Results from the discovery and replication phases and their combined association outcome. The genes and SNPs are in descending order according to their combined *p* values.

			Discovery (*n* = 63)			Replication (*n* = 68)			Combined (*n* = 131)	
Gene	**SNP**	**Source**	**Beta**	**SE**	**P**	**Beta**	**SE**	**P**	**Beta**	**P**
PRKCB	rs9922316*	candidate	0.8205	0.2524	0.001921	0.4295	0.1889	0.02635	0.5699	0.00016
intergenic	rs1017437	GWAS	-1.492	0.3024	$7.12 \cdot 10^{-6}$	0.01319	0.2779	0.9623	-0.676	0.00095
intergenic	rs2514897	GWAS	1.052	0.2181	$1.05 \cdot 10^{-5}$	0.05636	0.1785	0.7532	0.4557	0.00097
intergenic	rs1285441	GWAS	-1.27	0.253	$5.20 \cdot 10^{-6}$	0.3024	0.2445	0.2205	-0.4569	0.00935
ITPR1	rs6796205	candidate	-0.7034	0.2234	0.002591	-0.1482	0.1653	0.3734	-0.3446	0.0095
RGS5	rs6691456	candidate	1.061	0.2998	0.0008001	-0.05814	0.2863	0.8397	0.4757	0.0216
CACNA2D2	rs1540293	candidate	1.151	0.36	0.002249	-0.3216	0.5021	0.5241	0.651	0.02607
CAMK1D	rs7100726	candidate	-0.747	0.2274	0.001728	-0.03011	0.1802	0.8678	-0.3066	0.02992
CALM2	rs815815	candidate	-1.053	0.2822	0.0004346	0.09441	0.2467	0.7033	-0.4026	0.03018
MAP3K4	rs9347479	candidate	-0.7513	0.2368	0.002414	0.09124	0.2538	0.7204	-0.3592	0.03803
HS6ST3	rs9562057	GWAS	-1.286	0.2664	$1.08 \cdot 10^{-5}$	0.2075	0.1937	0.288	-0.309	0.04856
PRKCE	rs10189339	candidate	-0.7297	0.2449	0.004211	-0.0253	0.1885	0.8937	-0.2874	0.05439
MAPKAPK3	rs11130254	candidate	0.9444	0.3225	0.004868	-0.09886	0.3325	0.7672	0.4387	0.05809
MAPK13	rs2859136	candidate	0.8121	0.2496	0.0019	-0.026	0.1874	0.8901	0.2761	0.0654
MAPK4	rs10989249	candidate	-0.8571	0.2069	0.0001128	0.1817	0.1719	0.2944	-0.2425	0.06661
CACNB2	rs7893279	candidate	-0.9722	0.3204	0.003609	0.4617	0.4054	0.2589	-0.4209	0.09405
MAPK14	rs851006	candidate	0.6999	0.229	0.003386	-0.2691	0.247	0.2802	0.252	0.1335
intergenic	rs7144087	GWAS	-1.109	0.2261	$7.99 \cdot 10^{-6}$	0.2719	0.1609	0.09583	-0.1923	0.1424
ADCY5	rs4677889	candidate	-0.7503	0.2361	0.002378	0.1292	0.1805	0.477	-0.1952	0.1734
RGS20	rs10435634*	candidate	0.5487	0.2263	0.01846	-0.09789	0.1706	0.5682	0.1364	0.3166

Beta, beta coefficient; SE, standard error

Beta coefficients describe the effect sizes of genes in the analysis; they represent the magnitude of an independent variable's effect on the dependent variable in multiple regression analysis.

* PRKCB and RGS20 were not directly assessable by the employed genotyping technology and the second-best SNPs were used in their place. Names of genes were obtained from the HUGO Gene Nomenclature Committee (HGNC) homepage (http://www.genenames.org).

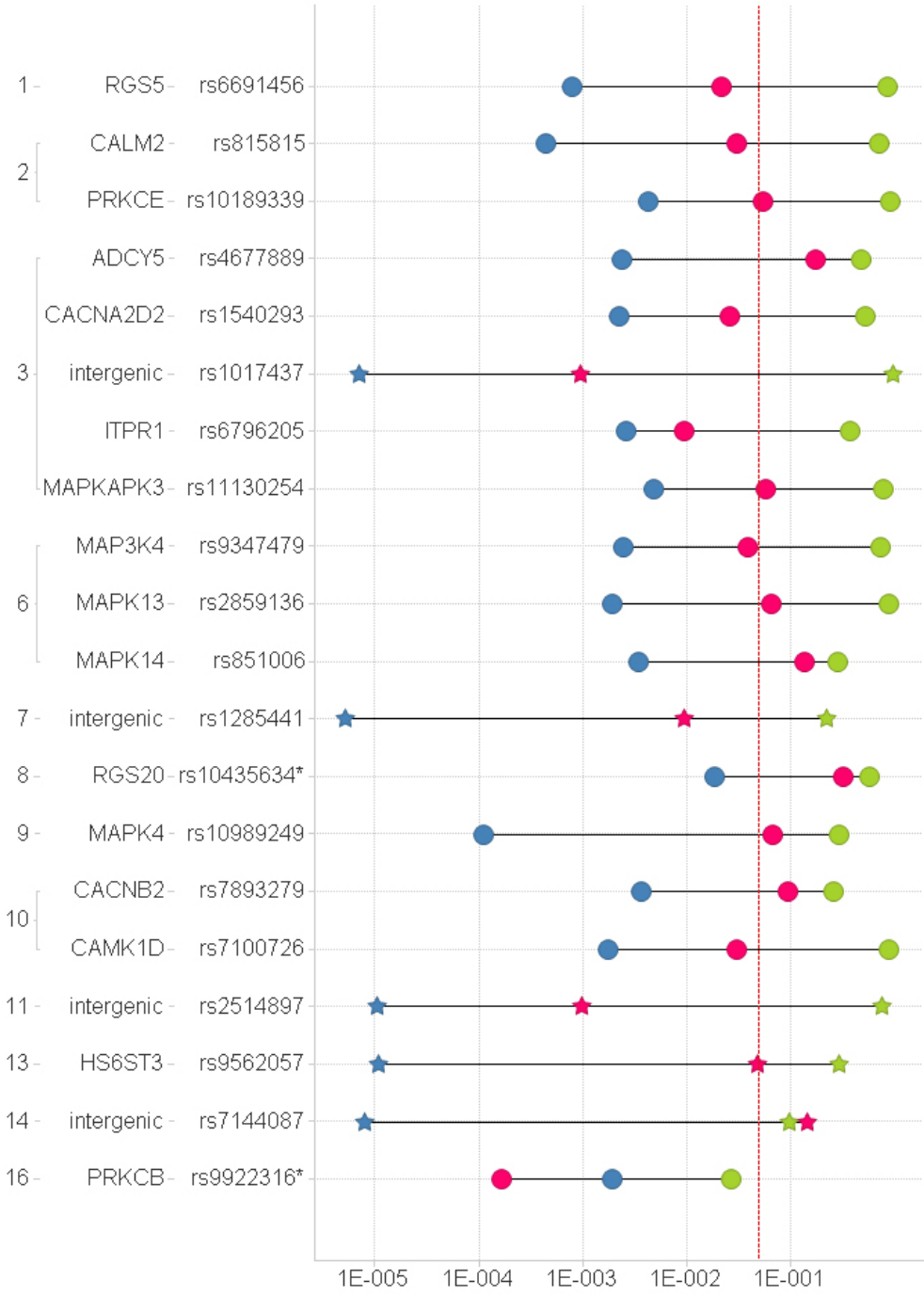

Figure 21. *p* values for genes. Green – replication (Vanderbilt); Blue – GWAS (discovery, Turku); Red – combined; Circle – candidate gene list; Star - GWAS list.

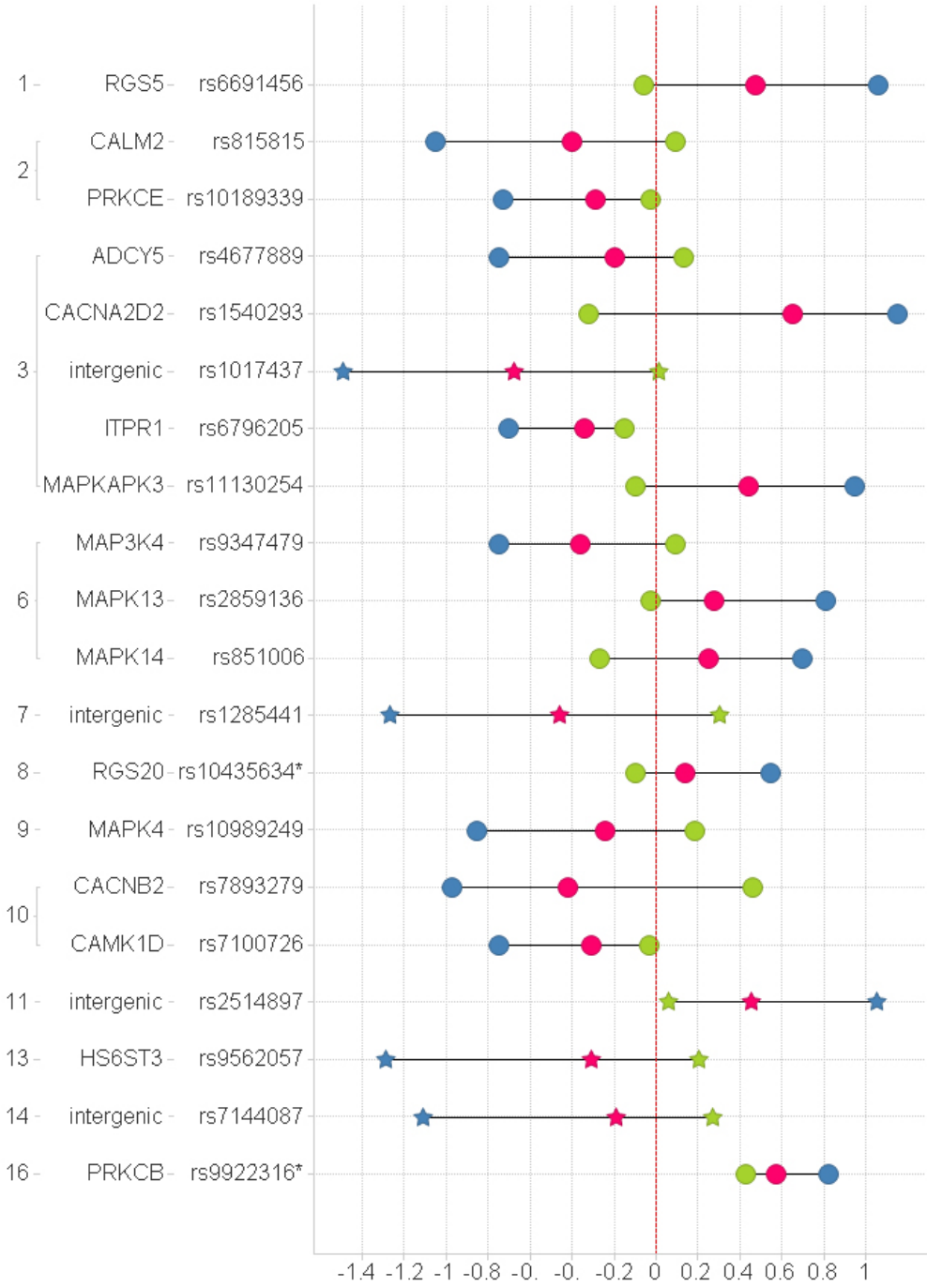

Figure 22. Beta coefficients. Beta coefficients exemplify the effect sizes of genes in the analysis; they represent the magnitude of an independent variable's effect on the dependent variable in the multiple regression analysis. Green – replication (Vanderbilt); Blue – GWAS (discovery, Turku); Red - combined; Circle - candidate gene list; Star - GWAS list.

6. DISCUSSION

6.1 Methodological aspects

6.1.1 Study designs and protocols

The present thesis is based on a series of four original studies that were designed to investigate the role of α_2-ARs in dexmedetomidine-induced effects on cardiovascular functions and to explore clinical and genetic determinants of the responses to dexmedetomidine. Three of these studies were clinical pharmacological experiments with healthy subjects and one was a pharmacogenetic investigation that made use of the results of one of the clinical experiments (Study III) and an another set of data and genetic material from another study population that had been collected in a similar clinical pharmacological setting.

6.1.1.1 Study I

Study I had a non-randomized, open-label, sequential design. Twelve healthy male subjects were enrolled into the study from a pool of 38 volunteers. The effects of therapeutic (0.5 ng/ml) and high concentrations (5 ng/ml) of dexmedetomidine on MBF and cardiac function were investigated. MBF was assessed by PET, myocardial function by TTE, and haemodynamic data were collected using standard clinical monitoring methods. Activation of α_2-ARs in the CNS mediates decreased sympathetic tone leading to decreased vascular resistance, while activation of postsynaptic α_2-ARs mediates both vasoconstriction *via* receptors that reside on vascular smooth muscle cells (Talke *et al.*, 2003) and endothelium-dependent vasodilatation resulting from NO release (Figueroa *et al.*, 2001). These counteracting effects of α_2-AR agonists complicate the interpretation of results from *in vivo* studies aiming at understanding the cardiovascular actions of postsynaptic α_2-ARs. In other words, the physiological and pharmacological roles of α_2-ARs in the regulation of cardiovascular functions in healthy humans are not completely understood, and clinical research papers on this topic are relatively few in number. Study I was designed to examine the postsynaptically mediated cardiovascular effects of α_2-AR activation by dexmedetomidine, and to investigate whether high circulating concentrations of dexmedetomidine could lead to a mismatch between cardiac oxygen demand and supply.

Based on previous evidence derived from animal experiments, it has been suggested that α_2-AR–mediated vasoconstriction is mainly mediated by the α_{2B}-AR subtype (Link *et al.*, 1996). In humans, the *ADRA2B* 901_909del polymorphism (rs4066772) has been associated with altered receptor desensitization (Small *et al.*, 2001, Muszkat *et al.*, 2010) and an increased risk for cardiovascular disease (Snapir *et al.*, 2001, Snapir *et al.*, 2003b). Six of the subjects in Study I were therefore selected to represent carriers of the Del/Del genotype and 6 subjects represented the Ins/Ins genotype. It was observed that this gene polymorphism did not have statistically significant effects on the study results,

and the entire material of 12 subjects was therefore treated as a single study population in the analysis.

The experimental sessions consisted of three sequential phases, including two drug infusion phases, Low Dex and High Dex. All cannulations and catheterizations (see 6.1.3.1) were performed by a specialist in anaesthesiology who was present throughout the experimental sessions to monitor the safety of the study subjects. TTE assessments were performed by an experienced clinical physiologist. The assessment of MBF was performed using $[^{15}O]H_2O$ as tracer and PET scanning, and was conducted according to qualified PET imaging protocols. The subjects were investigated at Turku PET Centre in a quiet, temperature-controlled room. All PET data were corrected for dead time, decay, and measured photon attenuation according to previous experience (Sundell *et al.*, 2002). The calculation of regional MBF was performed with a method published earlier (Iida *et al.*, 1988, Iida *et al.*, 1992). All of the employed methods had been optimized and standardized in their respective fields and the clinical investigators were experienced in their use; it may thus be considered that there were no relevant sources of investigator-dependent bias.

Based on the seminal investigation of the cardiovascular pharmacology of dexmedetomidine conducted by Ebert *et al.* (Ebert *et al.*, 2000), who administered increasing doses aiming to reach plasma concentrations of dexmedetomidine from 0.5 to 8.0 ng/ml, it was decided to choose target plasma concentrations of 0.5 ng/ml for Low Dex and 3.2 ng/ml for High Dex. Low Dex was predicted to produce near-maximal inhibition of the sympathetic nervous system, and High Dex was expected to result in significant vasoconstriction. The recommended therapeutic concentration range of dexmedetomidine is 0.4 – 1.2 ng/ml (Precedex® SPC; Abbott Laboratories, Abbott Park, IL, USA). We chose to use the same pharmacokinetic parameters of dexmedetomidine as Talke *et al.* (Talke *et al.*, 2003) and set the maximum infusion rate to 0.3 μg/kg/min. The clinical safety of the infusions was assured by the presence of an experienced anaesthesiologist. All of the above-mentioned assessments had previously been evaluated for clinical use and the whole complex protocol was tested in one pilot study session.

6.1.1.2 Study II

The study had a randomized, open-label, placebo-controlled cross-over design. This study was undertaken to explore involvement of NOS activation and inhibition in dexmedetomidine-induced digital artery and DHV constriction. It had been previously demonstrated that activation of vascular α_2-ARs leads to dual responses involving smooth muscle-dependent vasoconstriction (Talke *et al.*, 2003) and endothelium-dependent vasodilatation (Figueroa *et al.*, 2001). In congruence with these findings, previous studies with L-NMMA had implied that α_2-AR mediated vasoconstriction is opposed by α_2-AR mediated NO release (Lembo *et al.*, 1997, Bruck *et al.*, 2001).

There are some protocol- and study design-related considerations regarding the measurement of vascular α_2-AR responses to dexmedetomidine *in vivo*. Systemic administration of dexmedetomidine first decreases and then increases BP and also

evokes cardiovascular reflex responses that confound the measurement of local vascular responses. The activation of α_2-ARs in the CNS and PNS leads to sympatholysis, which tends to mask the direct vasoconstriction mediated by peripheral postsynaptic α_2-ARs. In the LTF substudy, this masking phenomenon was avoided by pharmacological sympathetic blockade of the axillary plexus with a local anaesthetic. The sympathetic nerves were silenced with mepivacaine – dexmedetomidine was now not capable of suppressing their activity, and vasoconstriction could be reliably monitored. In the DHV substudy, the solution was to employ the LVDT method (see 6.1.4) that enables the direct infusion of very small doses of dexmedetomidine directly into the investigated vein. The small doses were expected to act locally on vascular α_2-ARs and to exert no systemic effects. This LVDT method (Aellig, 1981, Aellig, 1985, Aellig, 1994) had previously been used in several clinical pharmacological studies (Schindler *et al.*, 2003, Landau *et al.*, 2004, Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a), and we tested its utility in pilot sessions.

The experimental sessions were carried out in a quiet temperature-controlled room. In both sub-studies, the subjects served as their own controls. In the LTF substudy, the responses of digital arteries to brachial i.a. infusion of L-NMMA were investigated during systemic i.v. infusion of dexmedetomidine (target concentration 1.2 ng/ml) in one session, and i.v. infusion of saline in another session. Responses to L-NMMA were recorded with photoplethysmography. The results demonstrated that inhibition of NOS by L-NMMA had a substantial augmenting effect on dexmedetomidine-induced constriction of digital arteries.

L-NMMA has been used to study human endothelial function in many clinical trials (Alderton *et al.*, 2001) (see 6.1.2.2). Our research group had already gained experience in monitoring healthy subjects during systemic dexmedetomidine infusions in Study I. The clinical advantage of the LTF substudy was that the subjects received infusions that aimed at lower dexmedetomidine plasma concentrations (1.2 ng/ml) than used in Study I, which made the subjects' post-infusion recovery faster. The photoplethysmography method is considered as validated and has been employed in many clinical trials (Kollai, 1983).

In the DHV substudy, the DHV responses to increasing local doses of dexmedetomidine were investigated in the presence and absence of L-NMMA. Activation of α_2-ARs may affect the local production of prostanoids, which can modulate vascular tone (Callow *et al.*, 1998, Dinenno and Joyner, 2004, Hermann *et al.*, 2005). The subjects were therefore given 500 mg of acetylsalicylic acid to inhibit cyclo-oxygenases 1 and 2 before the experimental sessions, which should inhibit the formation of all vasoactive prostanoids, but the completeness of this inhibition was not ensured by direct measurements.

The effects of α_2-AR activation on DHV constriction were investigated with gradually increasing local dosing of dexmedetomidine and recording with the technically straightforward LVDT method. The doses were small to avoid systemic pharmacological effects. We adopted and modified the dexmedetomidine infusion doses from an earlier publication (Muszkat *et al.*, 2004). Based on the acquired LVDT data, individual ED_{50} estimates for dexmedetomidine were calculated. L-NMMA appeared to shift the dose–

response curve of dexmedetomidine for constriction to the left in three subjects (ED_{50} reduced by at least 10-fold), whereas in seven subjects, the effect of L-NMMA on the dexmedetomidine ED_{50} was small or non-existing (less than five-fold difference in ED_{50}). It can be concluded that dexmedetomidine evokes constriction of large superficial veins, but the significance of vasodilatation mediated by activation of endothelial NOS remains unclear. It appears that NOS activation also opposes the venous constriction evoked by dexmedetomidine, but the variability between subjects was too large to allow definitive conclusions. The test-retest variability of the method was not tested by us, so it remains unknown how much of the observed variability can really be attributed to inter-subject differences. As expected, no systemic effects related to dexmedetomidine infusion were detected.

6.1.1.3 Study III

Study III had a randomized, single-blind design. At least some components of the α_1- and α_2-AR signalling cascades involved in vascular smooth muscle contraction can be assumed to be shared by both systems (see 2.1.3). Hence, it was plausible to propose that the sensitivity of DHVs to constrict in response to α_1- and α_2-AR agonists would be positively correlated at least to some extent.

Simultaneous α_1- and α_2-AR activation in both hands of individual subjects by potent and selective α_1- and α_2-AR agonists was tested to investigate the possible positive correlation between the constriction potencies of these two receptor mechanisms within the same individual. Phenylephrine and dexmedetomidine were selected, since they are the most selective α_1- and α_2-AR agonists that are registered for clinical use (Sofowora *et al.*, 2004, Aantaa and Jalonen, 2006). The experimental sessions were carried out in a quiet temperature-controlled room to avoid unwanted activation of the sympathetic nervous system. Both hands of the subjects were warmed up carefully with blankets before the experimental session was initiated. In accordance with evidence from earlier publications (see 6.1.4), we had noted that those hands where the fingertip temperature does not exceed 34 ºC show incomplete and inconsistent DHV filling patterns. The drugs were administered locally in gradually increasing doses into DHVs of equal size in the two hands. The doses were kept small in order to avoid systemic pharmacological effects and to guarantee that no cross-activation would occur at the receptor level. The dosing regimens were adapted from previous studies where no systemic haemodynamic effects were noted during the drug infusions (Landau *et al.*, 2004, Muszkat *et al.*, 2005a); see also II. Based on the acquired dose-response data, the ED_{50} estimates were determined for both agonists from regular sigmoidal dose-response curves. Linear regression models demonstrated that the sensitivity of a DHV to phenylephrine was a weak determinant of the response of another similar vein in the same individual to dexmedetomidine. Only weak correlations emerged between the individual dexmedetomidine responses and the tested clinical variables that included age, BMI, and hand temperature. However, the preceding individual variables and the phenylephrine ED_{50} estimate together explained about 30 % of the individual DHV responsiveness variation.

The α_1- and α_2-AR sensitivity was assessed using highly selective agonists. Numerous previous studies have used similar doses of dexmedetomidine (Muszkat *et al.*, 2010) and phenylephrine (Sofowora *et al.*, 2004, Muszkat *et al.*, 2011) that are considered to be the agonists of choice for the purposes of Study III because of their selectivity. The dose-response curves obtained exhibited regular sigmoidal patterns that further indicated the involvement of just one type of α-AR in the responses to each agonist. Furthermore, non-selective α-AR activation by either agonist would have been expected to strengthen the correlation between the responses to dexmedetomidine and phenylephrine, whereas the current results demonstrated only a weak positive association between the responses.

6.1.1.4 Study IV

Study IV was a pharmacogenetic investigation of DHV constriction responses to dexmedetomidine. The large inter-individual variability in DHV constriction responses to α_2-AR agonist activation was assumed to be to a significant extent determined by genetic factors. This combined whole-genome / candidate gene investigation was designed to identify genetic factors contributing to the inter-individual variability in α_2-AR-mediated vascular constriction induced by dexmedetomidine.

In the discovery phase, we performed a GWAS of 433,378 polymorphic gene loci with the sensitivity of DHV responses in 64 healthy Finnish subjects selected to represent the low and high ends of the sensitivity range to dexmedetomidine, as assessed in a quantitative manner in terms of ED_{50} values for drug-induced DHV constriction. In the replication phase, 20 SNPs from the GWAS were selected for testing by including the top 5 loci of the GWAS probability ranking list with no consideration of gene identity, and the top 15 loci from a pre-defined candidate gene set and their associations with the ED_{50} of dexmedetomidine in an independent North American study population of 68 healthy individuals.

There are certain strengths in this unconventional study approach. The Turku and Vanderbilt study populations were investigated under similar controlled clinical pharmacological settings previously found to be appropriate for examination of DHV constriction responses (Alradi and Carruthers, 1985, Luthra *et al.*, 1991, Gupta and Carruthers, 1997, Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a).The subjects were healthy and in most respects similar, apart from their ethnic origin, and the experimental sessions were conducted with similar equipment, which permits a controlled examination of genetic and non-genetic determinants of DHV constriction without the confounding effects of disease, concomitant medications and age-related changes. The advantages of the LVDT method and local administration of small doses of the study drug are discussed in sections 6.1.1.3 and 6.1.4.

GWAS was performed with Finnish Caucasian volunteers, which helps to avoid false positive results due to variations in the population substructure, as it is known that the frequency of alleles for many SNPs vary across ethnic groups. In the final combined analysis, the study included a relatively large ethnically diverse study material from two

different continents; nonetheless, the main finding regarding the rs9922316 marker in *PRKCB* was concordant in both subpopulations.

6.1.2 Dexmedetomidine and other study drugs

6.1.2.1 Dexmedetomidine

Clinical cardiovascular α_2-AR research used to be hampered by a lack of selective and potent α_2-AR agonists. Several groups of investigators have previously used clonidine to study vascular α_2-AR responses (Blochl-Daum *et al.*, 1991, Haefeli *et al.*, 1993, Figueroa *et al.*, 2001). However, the relatively low α_2-/α_1-AR selectivity ratio of clonidine causes unwanted α_1-AR partial agonist effects that confound the measurement of α_2-AR responses. Dexmedetomidine has high selectivity for α_2-ARs vs. α_1-ARs; in receptor binding assays, it is eight times more selective for α_2-ARs than clonidine (Virtanen, 1986, Virtanen *et al.*, 1988).

Contractile responses have been observed in various vessels of several species including coronary arteries of dogs (Coughlan *et al.*, 1992), goats (Lawrence *et al.*, 1997) and pigs (Jalonen *et al.*, 1995); pial arterioles of rats (Asano *et al.*, 1997) and dogs (Iida *et al.*, 1999); cerebral arteries of dogs (Iida *et al.*, 1999) and humans (Zornow *et al.*, 1993); and in human digital (Talke *et al.*, 2005), brachial (Masuki *et al.*, 2005), internal mammary (Yildiz *et al.*, 2007) and gastroepiploic arteries (Hamasaki *et al.*, 2002). There is pharmacological evidence that vasoconstriction is produced by activation of α_2-AR on vascular smooth muscle cells. However, the agonists may also induce contraction by activating α_1-AR at higher concentrations. In a study with isolated canine coronary vessels, it was observed that in the presence of the selective α_2-AR antagonist atipamezole, contractions of proximal coronary arteries were still elicited by high concentrations of dexmedetomidine, whereas atipamezole abolished the contractions at lower dexmedetomidine concentrations (Coughlan *et al.*, 1992). Another *in vitro* study reported that contractions of isolated human internal mammary arteries to low concentrations of dexmedetomidine were inhibited by the α_2-AR antagonist yohimbine, while at higher dexmedetomidine concentrations, contractions were inhibited by the α_1-AR antagonist prazosin (Yildiz *et al.*, 2007).

The possible actions of dexmedetomidine on α_1-ARs at higher concentrations are not likely to represent a serious confounding factor in the present series of studies. The studies that have reported that dexmedetomidine may evoke contractile responses mediated by α_1-ARs at higher concentrations were performed *in vitro* with isolated arteries and very high drug concentrations, and the evidence cannot therefore be directly applied to contractions induced by dexmedetomidine *in vivo*. For example, Yildiz and colleagues observed α_1-AR activation indirectly by using the α_1-AR antagonist prazosin in the presence of high dexmedetomidine concentrations, 10^{-6} to $3 \cdot 10^{-5}$ mol/l (Yildiz *et al.*, 2007). Coughlan and colleagues reported that dexmedetomidine-induced contractions could not be abolished by atipamezole at a very high dexmedetomidine concentration of $1.3 \cdot 10^{-4}$ mol/l (Coughlan *et al.*, 1992). The concentrations of dexmedetomidine in the systemic circulation employed in this series of studies were 0.5 to 5 ng/ml ($2.5 \cdot 10^{-9}$

to 2.5 $\cdot 10^{-8}$ mol/l, Study I) and 1.2 ng/ml (6.0 $\cdot 10^{-9}$ mol/l, Study II, LTF sub-study); the drug concentrations in the DHV studies are not known. The recommended therapeutic concentration range of dexmedetomidine in clinical practice is from 0.4 to 1.2 ng/ml.

The current series of studies employed dexmedetomidine to investigate the effects of α_2-AR activation on vascular tone, since dexmedetomidine possesses well-defined advantages compared to clonidine. As compared to dexmedetomidine, clonidine has a longer systemic duration of action and a longer elimination half-life, resulting in a longer duration of CNS side effects, such as sedation, and thereby limiting its practicality in vascular clinical pharmacological studies, where the subjects should be discharged at the end of the experimental session. Additionally, clonidine has a smaller α_2-/α_1-AR selectivity ratio compared to dexmedetomidine, and its vascular responses might represent the sum of its effects on both α_1-ARs and α_2-ARs.

6.1.2.2 L-NMMA

L-NMMA is an inhibitor of all NOS isoforms (Alderton *et al.*, 2001). It has been used to study endothelial function of human blood vessels in hundreds of clinical trials (Aellig, 1994, Vallance *et al.*, 1989, Mayer *et al.*, 1999, Halcox *et al.*, 2001). Endogenous NO opposes noradrenergic vasoconstriction, and endothelial α_2-ARs are capable of stimulating the release of NO (Bruck *et al.*, 2001). In addition, it has been reported that L-NMMA can enhance α_2-AR agonist-induced reductions in forearm blood flow (Lembo *et al.*, 1997). In Study II, local intravascular infusions of L-NMMA were applied to evoke optimal local inhibition of endothelial NOS, with minimal systemic effects. To achieve this aim, L-NMMA was administered directly into the brachial artery in the LTF sub-study and into the investigated vein in the DHV sub-study at doses that were sufficient to block endothelial NOS in the investigated vessels, but were small enough to avoid any measurable systemic effects of the drug.

The dose-rate of L-NMMA that is needed to inhibit endothelial NOS activity in the DHV has been established in previous studies. It has been determined that an L-NMMA dose-rate of 0.1 μmol/min substantially inhibited acetylcholine (1 nmol/min) and bradykinin (1.25 to 5 pmol/min) –mediated dilatation of veins pre-constricted with NA (Vallance *et al.*, 1989). There are reports that an L-NMMA dose rate of 0.1 μmol/min was not sufficient to block NO production in human DHVs, but a dose rate of 6.3 μmol/min, in addition to effective inhibition of NOS, also promoted generation of endothelial-derived hyperpolarizing factors (Schindler *et al.*, 2004). Based on these results, a dose rate of 0.5 μmol/min was selected to be used in Study II to inhibit NOS in DHVs.

An L-NMMA infusion rate of 32 μmol/min into the brachial artery resulted in maximal reductions in forearm blood flow, but it evoked an increase in systemic BP after five minutes (Veldman *et al.*, 2004). Doses of 4 μmol/min into the brachial artery for five minutes have caused significant reductions in forearm blood flow without affecting systemic haemodynamics (Halcox *et al.*, 2001). Furthermore, doses of 8 μmol/min for five minutes resulted in effective inhibition of NOS, but it was not reported whether they had effects on systemic BP (Huvers *et al.*, 1999). It was considered reasonable to employ an L-NMMA dose rate of 8 μmol/min for five minutes with the possibility that it might

cause small changes in BP. However, it was found in Study II that this dose did not affect systemic haemodynamics.

6.1.2.3 Phenylephrine

Phenylephrine is the most selective α_1-AR agonist available for clinical use (Lee *et al.*, 1995). Hence, it has often been used in pharmacological studies to investigate the effects of α_1-AR activation on vascular tone (Terzic and Vogel, 1991, Hussain and Marshall, 1997, Crassous *et al.*, 2009). Additionally, phenylephrine is commonly employed to preconstrict vessels in pharmacological experiments (Schindler *et al.*, 2003, Landau *et al.*, 2004, Briones *et al.*, 2005), because prolonged exposure does not lead to receptor desensitization (Dishy *et al.*, 2001). It has been demonstrated in rats *in vivo* that pressor responses to phenylephrine are mediated by vascular α_{1A}- and α_{1D}-ARs (Guimares and Moura, 2001), but the roles of the distinct α_1-AR subtypes in human vasoconstriction remain unknown (Guimares and Moura, 2001, Sofowora *et al.*, 2004).

Several investigators have reported that phenylephrine exhibits weak agonist activity on α_2-ARs and β-ARs (van Meel *et al.*, 1981, Crassous *et al.*, 2009). It has been suggested that phenylephrine has an α_1-/α_2-AR selectivity ratio of 10-100:1 (Flavahan and McGrath, 1981, Guimares *et al.*, 1987, Brown *et al.*, 1988). Since phenylephrine is the most selective α_1-AR agonist that is registered for clinical use, it was reasonable to employ this drug in Study III. Phenylephrine was infused at local dose rates of 3.660 to 8000 ng/min without any signs of changes in BP. Similar and slightly higher doses have been used in similar settings and no systemic haemodynamic effects have been noted during the drug infusions (Harada *et al.*, 2000, Landau *et al.*, 2004, Muszkat *et al.*, 2011). In Study III, selectivity was not directly confirmed by the experiments carried out with simultaneous phenylephrine and dexmedetomidine administration, but the selectivity of both agents is supported by two lines of evidence, the regular sigmoidal dose-response curves obtained in most subjects and the most significant result of Study III, the lack of a strong association between the DHV responses evoked by the two agents.

6.1.3 Haemodynamic measurements

6.1.3.1 Pulmonary artery catheterization

The ideal technique to provide estimates of CO should be non-invasive, accurate, reliable, continuous and reproducible. At present, no single technique meets all of these criteria. We chose to employ the classic technique introduced already 40 years ago by Swan, Ganz and colleagues (Swan *et al.*, 1970), since haemodynamic variables such as CO (RPP) and mixed venous saturation cannot be assessed reliably and continuously by less invasive means. In Study I, CO and mixed central venous oxygen saturation were measured using a fiberoptic pulmonary artery flotation catheter, which was inserted by an experienced anaesthesiologist into the right internal jugular vein or the left subclavian vein, depending on the individual anatomy. Evans and colleagues (Evans *et al.*, 2009) divided the complications associated with pulmonary artery catheterization into four broad groups: (A) complications of central venous puncture; (B) complications related

to pulmonary artery catheter insertion and manipulation; (C) complications associated with short- or long-term presence of the pulmonary artery catheter in the CVS; and (D) those resulting from incorrect interpretation/use of acquired data. We minimized risks A and B by employing a skilled specialist in anaesthesiology and intensive care, with vast experience with this procedure. Additionally, risks A-C were minimized by using healthy volunteers in a short (2 – 3 h) experimental session. Risk D was not critical, because the study protocol and the haemodynamic measurements were carefully designed to focus on the desired haemodynamic data. One subject had a short burst of ventricular tachycardia, which is not uncommon in this procedure (Shah *et al.*, 1984). As suggested in the literature, the event was resolved by minor catheter position adjustment. No other adverse events occurred during the experimental sessions.

6.1.3.2 Blood pressure measurements

In Study I, BP was measured directly from the radial artery, in the LTF sub-study of Study II from the brachial artery (or in three cases, from the radial artery), and in the DHV sub-study, from the radial artery. It was considered ethically and clinically justified to monitor BP invasively in order to gain continuous BP data. All arterial cannulations were performed by an experienced specialist in anaesthesiology.

In Study III, BP was measured non-invasively from the left ankle before and after the drug infusions. We did not want to move the subjects' arms after the initial positioning and stabilization, and therefore tested various sphygmomanometers and ended up using a regular automated instrument with a standard arm cuff. We measured several subject's BP from the arm and the ankle and found the readings to be in good agreement. Based on the experience from our previous investigations and published data from other groups (Muszkat *et al.*, 2004, Landau *et al.*, 2004), we were able to estimate the impact of the infused dexmedetomidine and phenylephrine doses on haemodynamics. Since no significant alterations in BP, HR and plasma catecholamines were expected, it was reasonable to assess BP non-invasively, and consequently, make the experimental sessions less stressful for the subjects. Additionally, the availability of both hands for simultaneous drug infusions and DHV measurements enabled us to carry out up to three clinical sessions per day; this was important when taking into account that we investigated a total of 99 subjects.

6.1.4 Linear variable differential transformer method

In studies II and III, DHV responses to dexmedetomidine, L-NMMA, and phenylephrine were measured by the LVDT technique. There are several methods to investigate the responsiveness of human DHVs to vasoactive drugs. The LVDT technique, introduced by Aellig in 1981 (Aellig, 1981, Aellig, 1994) is the simplest and most feasible method for the measurement of venous contractile responses in humans. The LVDT technique allows the measurement of vascular responses independent of systemic reflexes, because extremely low doses of drugs that act mainly on the investigated segment of a vessel can be employed. The technique has been previously used to investigate the vascular pharmacology of a wide range of drugs including some adrenergic agonists. It allows

the construction of complete dose-response curves without any confounding systemic effects. The LVDT technique has been proven to be highly reproducible as a means to study venous responses repeatedly within subjects (Alradi and Carruthers, 1985, Aellig, 1994, Schindler *et al.*, 2003). The DHV responses as assessed with the LVDT method were found to be consistent in different veins of both hands (Alradi and Carruthers, 1985).

However, there are certain restrictions regarding the LVDT technique. The technique is associated with inter-individual variability, whereas the intra-individual variability has been found to be minor when it is employed in the assessment of DHV constriction (Alradi and Carruthers, 1985, Luthra *et al.*, 1991). The results from Study III as well the results from earlier studies (Alradi and Carruthers, 1985, Sofowora *et al.*, 2004) did not provide any evidence that age, menstrual cycle phase, or subjects' physical properties would affect the α_2-AR responsiveness of DHVs. In accordance with earlier observations of four-fold inter-individual variability in NA clearance (FitzGerald *et al.*, 1979), Luthra *et al.* reported a four-fold range in the estimated individual local NA clearance rates, while a 35-fold range in the ED_{50} estimates of DHV responses to NA was detected, suggesting that local pharmacokinetic factors did not have a major role in the observed inter-individual adrenergic response variability (Luthra *et al.*, 1991, Muszkat *et al.*, 2011). It has been suggested that the inter-individual variability is due to a large extent to genetic factors. A study performed with parents and their children using the LVDT technique found that the proportion of the phenotypic variance caused by genetic variation (the heritability) was 0.88 (Gupta and Carruthers, 1997). Since the reasons for the inter-individual response variability were not known, the protocols of the current studies were designed to minimize the possibility of technique-related factors.

In the DHV sub-study of Study II, it was estimated that a total of 10 subjects would provide about 80 % power to detect a one log unit difference in the ED_{50} of dexmedetomidine between the DHV response with and without L-NMMA, using the within-group standard deviation of the ED_{50} of the response to dexmedetomidine that had been previously reported (Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a). In study III, no power calculations were performed, because the hypothesis was that an individual's sensitivity of DHVs to α_2-AR-mediated constriction would be determined to a significant extent by the same subject's sensitivity to phenylephrine-induced α_1-AR-mediated vasoconstriction. In study IV, dexmedetomidine ED_{50} estimates of 99 subjects were used. Of these, 33 were excluded because their ED_{50} estimates were intermediate between the low- and high-responder groups (ED_{50} values above 5 ng/min and below 30 ng/min). One subject was excluded because it was found out (in the genetic analysis) that he was a sibling of another included subject. The pharmacogenetic analysis included data collected from 63 subjects, representing the low- and high-responder tertiles of the material of 99 subjects. Power simulation results showed that a sample size of 75 seemed to provide sufficient statistical confidence for the detection of a single locus determining the majority of the ED_{50} variation.

The responses of DHVs do not entirely resemble those of typical veins. They are markedly reactive to external temperature because of their role in thermoregulation. Cooling results in redistribution of blood flow from cutaneous veins to muscular vessels. Therefore,

the precision of the LVDT method requires rigid control of the room temperature. Studies with anaesthetized dogs have demonstrated that increases in the core or surface temperature of a hind limb skin flap significantly reduce venous resistance and increase venous compliance (Deschamps and Magder, 1990). In line with our experience from pilot study sessions with humans, studies with dogs have shown that cooling augments and warming attenuates venous constriction responses to adrenergic stimulation (Webb-Peploe and Shepherd, 1968, Abdel-Sayed *et al.*, 1970). As reviewed by Pang, *in vitro* studies have demonstrated that the augmentation of constriction responses of cutaneous veins induced by cooling results from increased reactivity of vascular smooth muscle cells rather than increased neurotransmitter release, and cooling seems to enhance the excitation-contraction coupling but not the entire contraction process of the vascular smooth muscle cells (Pang, 2000). Physiological antagonism exists between venous congestion-induced dilatation and NA-evoked constriction. It has been reported that as the congestion pressure of a DHV increases, its apparent sensitivity to the constrictor effect of NA decreases (Abdelmawla *et al.*, 1996). Therefore, it is recommended to use standardized congestion pressures when employing the LVDT technique. In the literature, a pressure of 45 mmHg is commonly employed with low-pressure tourniquet cuffs. Our studies were conducted in a temperature-controlled room and the possibility of draught was eliminated with closed doors. The subjects were carefully acclimatized to the experimental setting by letting them rest for 30-60 minutes before any preparations were initiated. Most of the experimental sessions of Study III were performed during the winter and spring. The hands of the subjects were meticulously warmed before the experimental sessions to ensure the quality of the response data acquired with the LVDT technique. Hand temperature problems were sometimes encountered, most often in female subjects who had a history of cold hands and/or increased reactivity to cold weather. In Study III, it was necessary to exclude 19 subjects because of the inability to warm their hands to at least 33 ºC or failure to cannulate the study vein atraumatically, leading to hyper-sensitization of the vein, which resulted in unreliable contractile responses.

6.1.5 Whole-genome analysis and candidate gene selection and approach

Genetic research to establish the loci contributing to various complex traits has been driven forward at an impressive pace in recent years by numerous GWAS projects. This has revealed a multitude of genetic associations that have frequently been replicated by other groups, often leading to consensus views for the first time in the history of human complex disease genetics. With the application of this recent revolution in SNP genotyping technology across large cohorts of patients and controls, GWAS of complex diseases or traits provides a critically valid, comprehensive, and unbiased strategy to identify causal genes (Hakonarson and Grant, 2011).

The extensive inter-individual variability in α_2-AR-mediated vascular responses has been revealed in previous studies - and in addition, in all experiments that are included in this thesis. The mechanism of α_2-AR-mediated vascular constriction can be considered as a complex phenomenon involving receptors, G proteins, protein kinases and other enzymes, of which many probably still remain unidentified. This was the starting point

for the pharmacogenetic Study IV, and on this premise, a two-phase study was designed. A wide spectrum of different subcellular signalling pathways has been proposed for α_2-ARs at least in recombinant cell systems (see 2.1.3). The canonical α_2-AR pathway of G_i-mediated ADCY inhibition does not reliably account for constriction of different vessel types, and direct and indirect interactions of α_2-ARs with voltage-sensitive Ca^{2+} channels have been postulated. However, there is no consensus regarding the α_2-AR signalling pathways leading to vasoconstriction. On this basis, it was decided to utilize GWAS in the search for the genetic determinants of inter-individual responsiveness of vascular α_2-ARs. The hypothesis of Study IV was that genetic variation in the α_2-AR signalling pathways would contribute to the large inter-individual variability in vasoconstriction responses to α_2-AR agonists. Genotyping and data analysis were performed at FIMM, University of Helsinki, using state-of-the-art technology. The 63 subjects included in the analysis represented the top and bottom tertiles of the ED_{50} range of the α_2-AR agonist dexmedetomidine of a study population previously investigated in Study III in Turku. The GWAS did not yield statistically significant hits after correction for multiple testing, even though many markers of biologically plausible genes were associated with dexmedetomidine ED_{50} with small unadjusted *p* values. On the basis of the GWAS association data, 20 SNPs were selected for testing in the replication phase of the study. The replication phase included the top 5 loci from the entire GWAS dataset (smallest *p* values) and the top 15 loci (smallest *p* values) from a pre-defined candidate gene list of 256 α_2-AR-associated genes. The candidate gene list of the replication phase was based on an extensive literature search, although it still represents an incomplete state of knowledge of α_2-AR signalling pathways.

Before the GWAS era, the candidate gene approach was commonly applied in genetic studies of complex traits or diseases. This seemed a logical approach, since it was based on a particular biological hypothesis. This is exemplified by Study I, where *ADRA2B* was the investigated candidate gene. The candidate gene approach has been hampered by failures to confirm many of the initial reports of disease or phenotype associations. However, it can be theoretically speculated that candidate gene studies may have relatively greater statistical power than the GWAS approach, but nonetheless, the results must meet rigorous tests for both biological plausibility and statistical validity. Since no definitive results were obtained with GWAS, possibly as a result of the rather limited sample size, it was decided to extend the study with the candidate gene approach. The replication phase samples were genotyped at FIMM for 20 SNPs selected on the basis of the discovery phase results.

The design of Study IV does not represent the traditional form of pharmacogenetic research. The two independent study populations were investigated under similar controlled clinical pharmacological settings previously found to be appropriate for examination of DHV constriction responses. The subjects were healthy and in most respects similar and comparable methods were used. The main difference between the populations was their ethnic composition. Participants in the discovery phase of the study were Finns whereas participants in the replication phase were of both European and African descent. This limits the study's power to detect genetic variants unique to people of European descent. However, the alleles investigated by GWAS are common and likely to be shared with comparable frequencies across various populations (International HapMap

Consortium, 2005). The DHV responses of black and white Americans to infusions of dexmedetomidine have also been shown to be similar (Muszkat *et al.*, 2004).

6.2 General discussion

6.2.1 Study focus: cardiovascular effects of dexmedetomidine

The focus of the present series of studies was the role of α_2-ARs in dexmedetomidine-evoked blood vessel responses. This theme was approached by three clinical pharmacological studies and one pharmacogenetic investigation.

Before the present studies, knowledge on effects of α_2-AR activation on the human coronary vasculature was based on data from patients with chest pain undergoing diagnostic left-side catheterization. Two groups had reported that activation of α_2-ARs in the coronary vasculature by azepexole (BHT-933) could reduce MBF (Indolfi *et al.*, 1992, Baumgart *et al.*, 1999). Baumgart and colleagues reported that atherosclerosis augmented the microvascular vasoconstriction evoked by this clonidine analogue (Baumgart *et al.*, 1999). However, the impact of dexmedetomidine on cardiac function and perfusion had not been fully investigated in humans, even though dexmedetomidine had been thoroughly investigated in intensive care and anaesthesiological practice, and its systemic haemodynamic effects were well characterized (Ebert *et al.*, 2000).

Study I provided information on the effects of therapeutic (0.5 ng/ml) and high (5 ng/ml) circulating dexmedetomidine concentrations on systemic and pulmonary haemodynamics, MBF and cardiac function in healthy volunteers. The results revealed that therapeutic levels of dexmedetomidine reduced MBF in parallel with its effects on sympathetic nerve activity, myocardial work, HR and BP. Interestingly, the high dexmedetomidine concentration did not further reduce the mean MBF, but inter-individual variability was increased substantially. However, HR, SV, and ejection fraction showed further decreases, but systemic, pulmonary, and central venous BPs were increased above the baseline.

Therapeutic levels of dexmedetomidine thus evoked marked sympatholysis, which explains the observed decreases in systemic vascular resistance, myocardial work and MBF. High dexmedetomidine levels did not lead to any substantial further sympatholysis, but evoked clear systemic and pulmonary vasoconstriction, evidenced by increased vascular resistance and systemic, pulmonary and central venous BPs.

The vascular effects of dexmedetomidine result from vascular constriction by activation of α_2-ARs in vascular smooth muscle (Baumgart *et al.*, 1999, Talke *et al.*, 2003), vascular dilatation caused by activation of endothelial α_2-ARs leading to activation of NOS, which results in the release of NO (Coughlan *et al.*, 1992, Bruck *et al.*, 2001), and sympatholysis evoked by activation of α_2-ARs in the CNS and presynaptic α_2-ARs in the PNS (Aantaa *et al.*, 1990, Aantaa and Jalonen, 2006, Chrysostomou and Schmitt, 2008). *In vitro* evidence from studies on canine coronary arteries (Coughlan *et al.*, 1992) suggested that dexmedetomidine may induce additional vasoconstriction by activating vascular α_1-

ARs at high concentrations, which could affect both myocardial workload and perfusion. The indirect effects of dexmedetomidine on cardiac function include modulation of catecholamine release, influence on preload, afterload, and HR. These are tightly interconnected with the indirect effects of dexmedetomidine on MBF that include vascular myogenic responses, which correspond to alterations in perfusion pressure and perfusion-dependent dilatation, and local metabolic regulation (Konidala and Gutterman, 2004).

In Study I, high circulating dexmedetomidine concentrations caused individually variable effects on MBF, perhaps reflecting the well-established inter-individual variability in vascular responses to α_2-AR activation. Between the low therapeutic and the high dexmedetomidine concentrations, MBF was either significantly increased (three subjects) or decreased (five subjects) or remained practically unchanged (four subjects). However, regression analysis demonstrated that the changes in MBF between the two drug infusion phases were significantly associated with changes in the RPP ($r^2 = 0.45$; $p = 0.017$), reflecting the concept that MBF is usually balanced by the myocardial workload, in order to avoid significant mismatches between the oxygen demand and supply; indeed, myocardial hypoxia was not present as verified by ECG and TTE. This essential balance may be cautiously paralleled with the finding that dexmedetomidine concentrations of 0.6 – 1.2 ng/ml did not impair the CBF/CBR ratio in healthy volunteers (Drummond *et al.*, 2008). The TTE results indicated that already at therapeutic levels, dexmedetomidine causes myocardial depression that is similar to the effects of β-AR blockers. Flacke *et al.* (Flacke *et al.*, 1992) demonstrated that dexmedetomidine did not have direct effects on cardiac function and suggested that the observed myocardial depression was attributable to sympatholysis and decreased release of catecholamines, leading to reduced myocardial inotropy. Contractility (denoted by the pre-ejection period divided by the left ventricle ejection time; positive changes mean less contractility), contraction (denoted by the movement of the lateral annuli) and the ejection fraction were reduced during the high dexmedetomidine concentration compared with baseline measurements. Nonetheless, it is noteworthy that the differences between the low and the high dexmedetomidine concentration phases were relatively small and may be largely explained by the increased afterload during the high-dose phase.

The main findings of Study I were that low therapeutic concentrations of dexmedetomidine reduce MBF by sympatholysis and reduced myocardial work, and high concentrations do not further reduce MBF and do not cause clinically evident mismatches between cardiac oxygen demand and supply.

It is well established that the peripheral vascular responses to α_2-AR activation are complex. Therefore, one would speculate that activation of vascular α_2-ARs triggers regulatory processes that aim to match the oxygen demand in different organs, including the heart. Since activation of α_2-ARs in coronary arteries induces constriction (Indolfi *et al.*, 1992, Baumgart *et al.*, 1999), and impaired endothelial function augments coronary constriction in humans (Figueroa *et al.*, 2001) and dogs (Jones *et al.*, 1993), it is apparent that coronary arterial tone is a result of the cross-talk between vascular smooth muscle and endothelial cell function.

Based on the findings of Study I, Study II was designed to test whether inhibition of endothelial NOS activity would augment the arterial and venous constriction evoked by α_2-AR activation. Previously, activation of NOS by α_2-ARs has been established in animal models. It was also demonstrated that inhibition of NOS augmented dexmedetomidine-induced constriction of isolated canine coronary arteries *in vitro* (Coughlan *et al.*, 1992), but not in canine cerebral arteries *in vitro* or *in vivo* (Coughlan *et al.*, 1992, McPherson *et al.*, 1994). It was known that dexmedetomidine would cause significant constriction of human forearm (Masuki *et al.*, 2005) and digital arteries (Talke *et al.*, 2003), and DHVs (Muszkat *et al.*, 2004) *in vivo*. Previous evidence also indicated that L-NMMA augmented α_2-AR agonist-induced reductions in forearm blood flow (Lembo *et al.*, 1997). In addition, it had been reported earlier that in the human forearm microcirculation, endogenous NO attenuated noradrenergic constriction, and there was also indirect evidence to suggest that endothelial α_2-ARs were involved in the release of NO (Bruck *et al.*, 2001). Study II sought to investigate the effects of NOS inhibition on dexmedetomidine-induced constriction of human digital arteries and DHVs *in vivo*.

The main finding of Study II was that inhibition of NOS by L-NMMA substantially augmented dexmedetomidine-induced constriction of digital arteries, suggesting a notable role for endothelial NO release following α_2-AR activation opposing the constriction response. This effect was seen in all investigated subjects with minimal inter-individual variation. On the other hand, L-NMMA had rather variable effects on dexmedetomidine-induced constriction of DHVs. Among the 10 investigated subjects, L-NMMA clearly shifted the dose–response curve of dexmedetomidine for constriction to the left in three subjects (ED_{50} reduced by at least 10-fold), whereas in seven subjects, the effect of L-NMMA on dexmedetomidine ED_{50} was minor (less than five-fold difference in ED_{50} value) or even absent. These results suggest that activation of endothelial α_2-ARs in small arteries induces significant activation of NOS, and when endothelial NO release is blocked, vasoconstriction is intensified. In large veins, this mechanism was not uniformly observed. In murine small arteries *in vitro*, low concentrations of dexmedetomidine evoked endothelium-dependent dilatation, and both L-NMMA and rauwolscine abolished this effect (Wong *et al.*, 2010). It has also been reported that dexmedetomidine activates NOS and increases the release of NO from endothelial cells of human umbilical veins (Joshi *et al.*, 2007, Kim *et al.*, 2009). Joshi *et al.* also revealed that this action was inhibited by PTX, implying that this mechanism is mediated by G_i-proteins (Joshi *et al.*, 2007). As proposed before in this section, it can be assumed that α_2-AR-mediated endothelium-dependent dilatation opposing smooth muscle-dependent vasoconstriction is differently balanced in different vessels and organs, perhaps tuned to meet the specific perfusion requirements. It must be taken into account that the findings presented before were acquired with different methods in different settings and even in different animal species, and that these assumptions have not been fully evaluated in humans. Still, the results from studies with human vessels are rather consistent.

In the LTF sub-study of Study II, major inter-individual variability in the ED_{50} of dexmedetomidine-induced constriction was observed that is congruent with other studies, but the reason for this variability is unknown. The preceding findings formed the basis for Studies III and IV.

It was assumed that if a major share of the signalling cascade that is involved in vascular smooth muscle cell contraction after α_1-AR and α_2-AR activation is shared by both types of receptors, then the sensitivity of an individual's blood vessels to contraction induced by these mechanisms would be determined by factors that are shared by both receptor mechanisms. Furthermore, it was assumed that in the absence of major differences in receptor expression levels between individuals, the potencies of α_1- and α_2-AR agonists to induce vasoconstriction should be positively correlated within an individual. Study III was designed to investigate whether the potency of phenylephrine to induce vasoconstriction in one hand can predict the potency of dexmedetomidine to induce vasoconstriction in the opposite hand within an individual. In other words, if the sensitivity of the DHVs to respond to activation of α_1- and α_2-ARs would be determined by the same individual characteristics of a test subject, then the two ED_{50} values (sensitivity estimates) would be expected to be highly intercorrelated.

In Study III, simultaneously infusions of α_1- and α_2-AR agonists in gradually increasing concentrations into DHVs of the two hands were conducted and changes in vein diameters were assessed with the LVDT method. The estimates of the ED_{50} values for both drugs were calculated and the extent to which the dexmedetomidine ED_{50} was determined by the phenylephrine ED_{50} was analysed with and without clinical covariates. The main finding was that the phenylephrine ED_{50} value, BMI, age and hand temperature are significant determinants of DHV responses to dexmedetomidine, but only a relatively small fraction of the inter-individual variability in the sensitivity to dexmedetomidine could be explained by these factors. The findings suggested that α_1- and α_2-AR agonists provide independent information about α_1- and α_2-AR-mediated constriction responses and that constriction responses of DHVs to phenylephrine and dexmedetomidine are primarily dependent on distinct and separate molecular signalling mechanisms activated by α_1- and α_2-ARs. Interestingly, independently of this work and at the same time, another group published in parallel a similar study as our Study III, reporting no correlation between the DHV sensitivities (ED_{50}) for dexmedetomidine and phenylephrine in healthy human volunteers *in vivo* (Muszkat *et al.*, 2011).

Previous studies had reported that gender and age contribute to the inter-individual α-AR response variability (Alradi and Carruthers, 1985, Sofowora *et al.*, 2004, King *et al.*, 2005). The statistical models of Study III included these covariates, but in contrast to the previous studies, gender did not have a significant effect on the sensitivity to dexmedetomidine. One possible explanation for this difference is that the drug infusions were initiated only after the finger tip temperature exceeded 34 ºC. Female subjects needed more time than male subjects to warm their hands, suggesting this as a confounding factor for the gender differences in previous studies. Furthermore, a previous DHV study including both genders reported that women displayed biphasic constriction responses to the α_2-AR agonist azepexole, whereas this was not noted in men (King *et al.*, 2005). Female subjects were perceived to produce DHV dilatation in response to α_2-AR activation with low doses of azepexole (10 – 100 ng/min). King *et al.* suggested that the initial dilatation response might stem from the presynaptic effect of α_2-AR activation at low concentrations, thereby preferentially inhibiting neuronal NA release and consequently relaxing vascular smooth muscle cells. Based on our experience from the pilot sessions and excluded sessions of Study III, female subjects with insufficiently

warmed hands exhibited unpredictable and abnormal responses to dexmedetomidine, such as an initial dilatation that was later abolished with an increasing hand temperature. One plausible explanation is that dilatation is observed during the first infusion doses, because the studied hand is gaining temperature as a function of time and the initial drug concentrations are too small to oppose this temperature-dependent dilatation.

6.2.2 Inter-individual variability in the constriction responses to α-adrenoceptor agonist activation

Marked inter-individual differences in the vascular responses to α-AR activation have been reported recurrently. A range of over 1000-fold was observed between subjects in their responses to dexmedetomidine, measured as ED_{50} estimates (Muszkat *et al.*, 2004, Muszkat *et al.*, 2005a). In Study III, up to 6500-fold between-subject differences in dexmedetomidine ED_{50} and up to 520-fold differences in phenylephrine ED_{50} were observed. Other groups have reported smaller between-subject differences of 50- to 100-fold with NA (Alradi and Carruthers, 1985, Luthra *et al.*, 1991). Studies with monozygotic twin pairs have demonstrated that genes are strong determinants of the responses of DHVs to constrictors (Luthra *et al.*, 1991, Gupta and Carruthers, 1997). Despite this inter-individual variability, it has been previously demonstrated that there are no significant differences in DHV responses to dexmedetomidine between black and white Americans (Muszkat *et al.*, 2004, Kurnik *et al.*, 2008). Inter-individual variability has also been observed in *ADRA2B* 901_909del variant carriers (Muszkat *et al.*, 2005a) and other *ADRA2B* variant carriers (Muszkat *et al.*, 2005b), and these genetic variants themselves did not contribute substantially to this variability. It was recently reported that homozygous carriers of the SNP rs553668 (formerly known as the *Dra*I restriction fragment length polymorphism) of *ADRA2A* (the α_{2A}-AR gene) and the corresponding haplotype 4 contribute to the inter-individual variability in BP and HR responses to dexmedetomidine (Kurnik *et al.*, 2011), but the sample size of that study limits the generalizability of these results.

The *ADRA2B* 901_909del variant displayed no desensitization while insertion variant receptors showed rapid loss of sensitivity when incubated with an agonist *in vitro* (Small *et al.,* 2001). Nonetheless, the magnitude of desensitization of DHV responses to dexmedetomidine *in vivo* is minor (Muszkat *et al.*, 2010), which could be explained by activation of other α_2-AR subtypes, as dexmedetomidine is not subtype-selective. α_1-AR responses to phenylephrine did not show any desensitization in prolonged infusion (Dishy *et al.*, 2001), and therefore phenylephrine preconstriction is commonly used to study the effects of vasodilators in DHVs (Landau *et al.*, 2004). However, significant inter-individual variability in the potency of phenylephrine to induce vasoconstriction in our study as well in other studies (Sofowora *et al.*, 2004, Muszkat *et al.*, 2011) has been demonstrated, but the common Arg347Cys α_{1A}-AR polymorphism does not seem to contribute to this variability (Sofowora *et al.*, 2004).

Study IV sought to identify genetic factors contributing to the large inter-individual variability in α_2-AR-mediated vascular constriction induced by dexmedetomidine. The main finding of Study IV was the detection of an association of rs9922316 in the gene for PKC type beta, *PRKCB*, with inter-individual constriction variability, but the finding

provides only a partial explanation for the large inter-individual DHV response variation. The small sample size sets limitations for the interpretation of the result. Small effects of common variants may not have been detected in this sample, and at the same time, possible effects of rare allelic variants could not be distinguished at all. Theoretically, the larger the size of the sample the more likely it is that at least one gene variant related to the response variation can be uncovered. A key consideration in this GWAS context is statistical power that behaves directly as a function of effect size and sample size, when assuming that the typical trait- or disease-associated variant expected to be detected will be relatively common in the population (10 % – 30 % minor allele frequency) and conferring only a modest risk (relative risk = 1.1 – 1.4) (Hakonarson and Grant, 2011). However, the spectrum of inter-individual variability is broad and the prevalence of high- and low-responding phenotypes is equal; thus it can be speculated that the finding of allele A of rs9922316 being associated with a higher dexmedetomidine ED_{50} in both independent datasets can be considered to be relatively robust. However, we can only speculate on the impact of genetic variation of PKC beta and its heritability on other vessel types and diseases. In the genetics of agonist-promoted cellular signalling leading to sympathetically regulated vessel constriction, individual gene effects on vessel phenotypes may be expected to be smaller than those on intermediate phenotypes that are theoretically closer to the mechanisms of gene action.

The findings of Studies III and IV do not comprehensively explain the inter-individual variation in DHV sensitivity to dexmedetomidine, but may improve our understanding of the complexity of mechanisms of α_2-AR-mediated vasoconstriction. Prior to these present investigations, it was suggested that genetic factors would determine the inter-individual variation to a significant extent, but the current results imply that there are still unidentified factors affecting response variation. Even though the DHV responses have been highly reproducible within individuals over time and also between different measurement sites (right and left hand) (Luthra *et al.*, 1991, Schindler *et al.*, 2003), there may exist as yet unknown individual differences in ligand binding to polymorphic α_2-ARs, and positive and negative allosteric modulators may also affect the binding affinity and signaling of the receptors.

6.2.3 Clinical implications

α_2-ARs play central roles in the control of sympathetic tone and BP and are important targets for drugs. α_2-ARs are critical mediators of the actions of the catecholamines. The effects of α_2-AR activation on the CVS are mediated by α_2-ARs that reside in the CNS and PNS, and by postsynaptic α_2-ARs in target organs. A multitude of studies have revealed significant roles for α_2-ARs in vasoconstriction (Docherty, 1998, Muszkat *et al.*, 2011), vasodilatation (Figueroa *et al.*, 2001), and the effects of increasing plasma dexmedetomidine concentrations include hypotension followed by hypertension, increased systemic vascular resistance, decreased CO and bradycardia (Ebert *et al.*, 2000, Talke *et al.*, 2003). In Study I, vascular resistance and BP responded to low and high dexmedetomidine concentrations in a biphasic manner, reflecting initial α_2-AR activation in the CNS and PNS, which was later masked by the increasing postsynaptic effects in the CVS. Whereas α_1-AR antagonists are commonly used to treat hypertension, α_2-AR

antagonists such as yohimbine typically increase BP (Etzel *et al.*, 2005), which may be explained as a result of inhibition of CNS and peripheral presynaptic α_2-ARs resulting in increased sympathetic tone. This could offer a future opportunity for the development of a novel drug for treatment of hypotension as a stand-alone agent or to be used together with NA in the intensive care setting. Since studies with genetically engineered mice have suggested that α_{2B}-ARs are mainly responsible for the vascular constriction leading to the hypertensive effect (Link *et al.*, 1996), the discovery of a subtype-selective α_{2B}-AR antagonist could give rise to a novel type of antihypertensive agent.

It has been reported that G_i-dependent responses are impaired in atherosclerotic and degenerated endothelium (Vanhoutte, 2003). Since it has been demonstrated that impaired endothelial function augments coronary constriction in humans (Figueroa *et al.*, 2001) and dogs (Jones *et al.*, 1993), caution should be exercised when using high-dose dexmedetomidine treatment in patients with conditions that predispose to endothelial dysfunction, such coronary artery disease (Lerman and Zeiher, 2005, Toggweiler *et al.*, 2010), cigarette smoking (Toggweiler *et al.*, 2010), hypercholesterolaemia (John *et al.*, 1998) and diabetes (Petrofsky and Lee, 2005). In Study I, high dexmedetomidine concentrations in plasma (5 ng/ml) induced only minor additional sympatholytic effects compared to lower concentrations (0.5 ng/ml), but significantly increased peripheral vasoconstriction and elevated BP levels. The results of Study II showed that dexmedetomidine-induced α_2-AR activation had a significant component of vasodilatation *via* activation of endothelial NOS in parallel with the peripheral vasoconstriction. It is probable that increased peripheral resistance as a result of enhanced systemic vascular constriction resulting from endothelial dysfunction may induce myocardial ischaemia by increasing myocardial workload, leading to a mismatch between oxygen demand and supply during high-dose dexmedetomidine administration. Study I demonstrated that in healthy volunteers, low therapeutic dexmedetomidine concentrations reduce MBF and myocardial workload by sympatholysis, while high concentrations did not further reduce MBF and did not cause clinically detectable mismatches between myocardial oxygen demand and supply. Therefore, it was proposed that at least in the intensive care of patients that do not have the above-mentioned conditions, dexmedetomidine provides cardiac-safe sedation, analgesia, anxiolysis and maintenance of haemodynamic stability.

Extensive inter-individual variability in α_2-AR-mediated constriction responses was seen in all three clinical studies (I, II and III). It is important to try to shed light on the details of genetic variation behind the vascular phenomena, since they could assist in understanding of cardiovascular functions in health and disease. However, one can only speculate on the impact of genetic variation of PKC beta and its heritability on other vessel types and diseases. The responsiveness of DHVs to α_2-AR activation may be a local phenomenon and thus, the results of DHV studies (II and III) cannot be extrapolated to other types of veins or to arteries of any diameter. In a clinical perspective, it must be stressed that veins are not exposed to forces such as those exerted by the arterial BP on the arterial vasculature, and hence, inter-individual differences in venous responses cannot be directly linked with the causes or consequences of systemic hypertension. However, in Study I, the inter-individual variability in MBF was increased substantially at high dexmedetomidine concentrations, implying that inter-individual variability may have clinical consequences in terms of cardiac, renal and cerebral safety in clinical practice.

7. CONCLUSIONS

The following conclusions were drawn from the series of studies that formed the basis of this thesis:

1. Systemic infusions of dexmedetomidine had biphasic effects on haemodynamics, with initial decreases in BP and MBF at low concentrations, followed by increases in BP and CVR at high concentrations. Plasma concentrations of dexmedetomidine that significantly exceeded the recommended therapeutic level did not further reduce MBF below the level that is observed with the usual therapeutic concentrations, and did not induce any evident myocardial ischaemia in healthy subjects. This provides evidence in favour of the safety of high-dose dexmedetomidine infusions, but direct inferences should not be made to patients with cardiovascular diseases (I).

2. Dexmedetomidine exerted significant vasodilatory effects through activation of endothelial NOS, and when this endothelial component of the blood vessel response to dexmedetomidine was inhibited, peripheral vasoconstriction was augmented. This could indicate that the vasoconstrictive effects of dexmedetomidine could be accentuated in patients with impaired endothelial function (II).

3. The DHV constriction response to phenylephrine is a weak determinant of the DHV response to dexmedetomidine, suggesting that α_1-AR- and α_2-AR-mediated venous constrictions are independently regulated (III).

4. A polymorphism in the human *PRKCB* gene was found to be associated with the venous constriction response to dexmedetomidine, suggesting that PKC beta perhaps plays a significant role in the signalling pathways of dexmedetomidine to induce venous constriction. It remains to be investigated whether this genetic variation is associated with the systemic haemodynamic effects of dexmedetomidine (IV).

In addition to the above conclusions related to the roles of α_2-ARs in physiological and pharmacological regulation of cardiovascular functions, it may be stated that valuable new information was acquired in terms of several new technical approaches for the *in vivo* evaluation of cardiovascular drugs.

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