UNIVERSITY OF TURKU

FADOLMIDINE – AN ALPHA2-ADRENOCEPTOR AGONIST FOR SPINAL ANALGESIA

Analgesic effect and safety in preclinical *in vivo* models

Tiina Leino

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA - SER. D OSA - TOM. 1525 | MEDICA - ODONTOLOGICA | TURKU 2021

UNIVERSITY OF TURKU

FADOLMIDINE – AN ALPHA2-ADRENOCEPTOR AGONIST FOR SPINAL ANALGESIA

Analgesic effect and safety in preclinical *in vivo* models

Tiina Leino

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1525 | MEDICA – ODONTOLOGICA | TURKU 2021

University of Turku

Faculty of Medicine
Department of Biomedicine
Pharmacology, Drug Development and Therapeutics
Drug Research Doctoral Programme (DRDP)
Integrative Physiology and Pharmacology Research

Orion Corporation, Orion Pharma
Research and Development

Supervised by

Professor Ullamari Pesonen, PhD
Department of Biomedicine
Integrative Physiology and
Pharmacology Research
University of Turku
Turku, Finland

Professor, Vice President Antti
Haapalinna, PhD
Research and Development
Orion Corporation, Orion Pharma
Turku, Finland

Reviewed by

Professor Heikki Ruskoaho, MD, PhD
Faculty of Pharmacy
Pharmacology and Pharmacotherapy
University of Helsinki
Helsinki, Finland

Adjunct Professor Vesa Kontinen, MD, PhD
Faculty of Biomedicine
University of Helsinki
Helsinki, Finland

Opponent

Professor Eija Kalso, MD, PhD
Faculty of Medicine
Diagnostics and Therapeutics
University of Helsinki
Helsinki, Finland

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-8307-0 (PRINT)
ISBN 978-951-29-8308-7 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama Oy, Turku, Finland 2021

to my family

UNIVERSITY OF TURKU
Faculty of Medicine
Department of Biomedicine
Pharmacology, Drug Development and Therapeutics
TIINA LEINO: Fadolmidine – an alpha2-adrenoceptor agonist for spinal analgesia: analgesic effect and safety in preclinical *in vivo* models
Doctoral Dissertation, 171 pp.
Drug Research Doctoral Programme (DRDP)
Integrative Physiology and Pharmacology Research
January 2021

ABSTRACT

Pharmacological activation of α_2-adrenoceptors is known to induce a characteristic pattern of pharmacodynamic responses including sedation, bradycardia, initial hypertension followed by hypotension, hypothermia and analgesia. Thus, α_2-adrenoceptor agonists have several therapeutic applications e.g. as analgesics and sedatives. α_2-Adrenoceptor agonists, administered either by intrathecal (i.t.) or epidural injections, are potent analgesics in humans. However, at antinociceptive doses, some of these agents e.g. clonidine, the prototype α_2-adrenoceptor agonist with lipophilic properties, may produce pronounced systemic adverse effects such as hypotension, bradycardia and sedation. Fadolmidine is a polar α_2-adrenoceptor agonist especially developed for locally effective spinal analgesia.

This study is a part of the nonclinical development and characterization of fadolmidine for spinal analgesia. These *in vivo* studies revealed that fadolmidine is a potent analgesic after epidural and especially after i.t. administration in rats and dogs. At analgesic doses in rats/dogs, i.t. fadolmidine induced minor effects on blood pressure concomitantly with a decrease in heart rate. At high spinal doses, fadolmidine induced sedation, hypothermia and a mydriatic response in rats. In contrast, two established α_2-adrenoceptors agonists, dexmedetomidine and clonidine, exerted those adverse effects already at analgesic doses. After i.t. dosing, the concentration of fadolmidine in plasma was very low in rats. With i.t. infusion, fadolmidine achieved a good analgesic effect without evoking cardiovascular side effects, e.g. hypotension in dogs. Co-administration of i.t. fadolmidine with a local anaesthetic bupivacaine enhanced sensory-motor block in rats and dogs. This interaction of the analgesic effect was synergistic.

This study provides novel data about pharmacological properties of fadolmidine for further development for use in spinal analgesia.

KEYWORDS: α_2-Adrenoceptor agonist, fadolmidine, spinal analgesia

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen laitos
Farmakologia, lääkekehitys ja lääkehoito
TIINA LEINO: Fadolmidiini – alfa2- adrenoseptoriagonisti spinaali analgesiaan: analgeettinen vaikutus ja turvallisuus prekliinisissä *in vivo* malleissa
Väitöskirja, 171 s.
Lääketutkimuksen tohtoriohjelma (DRDP)
Integratiivinen fysiologia ja farmakologia
Tammikuu 2021

TIIVISTELMÄ

α_2-Adrenoseptoreiden kautta elimistössä välittyy useita fysiologisia vasteita. Niiden farmakologinen aktivaatio aiheuttaa esimerkiksi sedaatiota, sydämen sykkeen laskua, verenpaineen nousua ja laskua, hypotermiaa ja analgesiaa. α_2-Adrenoseptoriagonisteja käytetäänkin mm. kivunlievityksessä ja rauhoitteina. Selkäytimeen tai epiduraalitilaan annettuna α_2-adrenoseptoriagonistit ovat tehokkaita kipua lievittäviä lääkeaineita ihmisellä. Kuitenkin kipua lievittävillä annoksilla esim. laajasti käytetty rasvahakuinen α_2-adrenoseptoriagonisti, klonidiini, voi aiheuttaa haittavaikutuksia koko elimistön tasolla kuten verenpaineen ja sydämen sykkeen laskua sekä väsymystä. Fadolmidiini on polaarinen α_2-adrenoseptoriagonisti, joka on kehitetty erityisesti paikalliseen spinaalianalgesiaan.

Tämä tutkimus on osa fadolmidiinin nonkliinistä selvitystyötä ja tuotekehitystä spinaalianalgeettista käyttöä varten. *In vivo* -tutkimuksissa rotalla ja koiralla fadolmidiini osoittautui tehokkaaksi kipua lievittäväksi lääkeaineeksi epiduraalisesti ja erityisesti intratekaalisesti annettuna. Kipua lievittävillä annoksina fadolmidiinilla on vain vähän verenpainevaikutuksia sekä samanaikaista sydämen sykkeen laskua rotalla ja koiralla. Suurilla annoksilla fadolmidiini aiheuttaa sedaatiota, hypotermiaa ja mydriaasia rotalla. α_2-Adrenoseptoriagonistit deksmedetomidiini ja klonidiini aiheuttavat samanlaisia haittavaikutuksia jo kipua lievittävillä annoksilla. Fadolmidiinin plasmapitoisuus on rotalla alhainen intratekaalisen annostelun jälkeen. Koirilla intratekaali-infuusiona annettu fadolmidiinin kipua lievittävä teho on hyvä ilman merkittävää verenpaineen laskua. Fadolmidiini kombinoituna puudutusaine bupivakaiinin kanssa lisää sen sensorista ja motorista salpausvaikutusta rotalla ja koiralla. Tämä yhteisvaikutus on synergistinen.

Tämä tutkimus tarjoaa uutta tietoa fadolmidiinin farmakologisista ominaisuuksista kehitettäessä sitä spinaalianalgesiaan.

AVAINSANAT: α_2-Adrenoseptoriagonisti, fadolmidiini, spinaalianalgesia

Table of Contents

Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine (serotonin)
ANOVA	Analysis of variance
BP	Blood pressure
BT	Body temperature
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal fluid
DBH	Dopamine β-hydroxylase
DDC	DOPA decarboxylase
DHPG	3,4-dihydroxyphenylethylglycol
DOMA	3,4-dihydroxymandelic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DRG	Dorsal root ganglion
ED_{50}	Effective dose 50
GABA	Gamma-aminobutyric acid
GI	Gastrointestinal
HPLC	High-performance liquid chromatography
HR	Heart rate
HVA	Homovanillic acid
i.c.v.	Intracerebroventricular
i.p.	Intraperitoneal
i.t.	Intrathecal
i.v.	Intravenous
K^+	Potassium ion
LC	Locus coeruleus
L-DOPA	L-dihydoxyphenylalanine
MAP	Mean arterial pressure
MABP	Mean arterial blood pressure
MAO	Monoamine oxidase
MHPG	3-methoxy-4-hydroxyphenylglycol

MHPG-SO_4	3-methoxy-4-hydroxyphenylglycol sulphate
%MPE	Percent of maximum possible effect
MTA	3-methoxytyramine
NA	Noradrenaline
Na^+	Sodium ion
NM	Normetanephrine
NS	Nociceptive specific
P	Probability
PAG	periaqueductal gray
PNMT	phenylethanolamine-N-methyltransferase
RVM	rostral ventromedial medulla
s.c.	Subcutaneous
S.D.	Standard Deviation
S.E.M.	Standard error of the mean
TH	Tyrosine hydroxylase
WDR	Wide dynamic range

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals I-IV:

I **Leino T**, Viitamaa T, Haapalinna A, Lehtimäki J, Virtanen R. Pharmacological profile of intrathecal fadolmidine, a α_2-adrenoceptor agonist, in rodent models. *Naunyn Schmiedebergs Arch. Pharmacol.,* 2009; 380: 539–550.

II **Leino T***, Lehtimäki J*, Koivisto A, Haapalinna A, Pesonen U. *Equal contribution. Fadolmidine – Favourable adverse effects profile for spinal analgesia suggested by *in vitro* and *in vivo* models. *Eur. J. Pharmacol.*, 2020; 882: 173296.

III **Leino T**, Yaksh T, Horais K, Haapalinna A. Pharmacodynamics of intrathecal and epidural fadolmidine, an α_2-adrenoceptor agonist, after bolus and infusion in dogs – comparison with clonidine. *Naunyn Schmiedebergs Arch. Pharmacol.,* 2020; 393: 1459–1473.

IV **Leino T**, Viitamaa T, Salonen J S, Pesonen U, Haapalinna A. Effects of fadolmidine, an α_2-adrenoceptor agonist, as an adjuvant to spinal bupivacaine on antinociception and motor function in rats and dogs. *Submitted.*

1 Introduction

Pharmacological activation of α_2-adrenoceptors is known to induce a characteristic pattern of pharmacodynamic responses (Gyires et al. 2009; Nguyen et al. 2017; Michel et al. 2019) including sedation, analgesia (Yaksh 1985; Yaksh et al. 2017), bradycardia, initial hypertension (due to peripheral vasoconstriction) followed by hypotension (Eisenach & Tong 1991; Kroin et al. 1996) and hypothermia (Livingston et al. 1984; Sinclair 2003). The activation of presynaptic α_2-adrenoceptors reduces the release and turnover of noradrenaline or other transmitters from neurons (Langer 1997; Starke 2001; Knaus et al. 2007). Noradrenaline, as a key neurotransmitter released by sympathetic postganglionic nerve fibres, is involved in the autonomic regulation of various organs. Based on physiological effects, α_2-adrenoceptor agonists could have various beneficial effects that could be exploited in the clinic. These drugs are being used as premedication as well as for sedation, analgesia, and as adjuvants to general and regional anesthesia (Nguyen et al. 2017). Clonidine is an antihypertensive agent (Nguyen et al. 2017) and used also for the control of pain (Hassenbusch et al. 2002; Mastenbroek et al. 2017); detomidine and medetomidine are sedatives in veterinary use (Grimsrud et al. 2015; Sinclair 2003) and dexmedetomidine is utilized in critical care units for human patients (Keating 2015). In addition, α_2-adrenoceptor agonists are used in the treatment of glaucoma (Greenfield et al. 1997).

Pain is a subjective, complex, physiological and psychological phenomenon that can be acute or chronic and may be classified according to its cause (Doody & Bailey 2019). The International Association for the Study of Pain has defined pain as:''An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." Within the experience of pain, the concept of total pain describes the physical, psychological, social and spiritual factors that influence the experience of pain (Doody & Bailey 2019). Pain is a common symptom, reported by up to 84% of adult patients in hospital, with up to 36% of patients reporting severe pain (Gregory & McGrowan 2016). Furthermore, chronic pain has a weighted mean prevalence in adults of 20% (Geneen et al. 2017).

α_2-Adrenoceptor agonists have been used for the treatment of pain, preoperative anaesthesia (Michel et al. 2019) and acute opioid withdrawal (Michel et al. 2019;

Gowing et al. 2016). α_2-Adrenoceptor agonists such as clonidine (α_2- and α_1-adrenoceptor agonist, with an affinity of 200 : 1 for α_2- versus α_1-adrenoceptors, respectively) and dexmedetomidine (a highly selective α_2-adrenoceptor agonist) are extensively used in both anaesthesia and intensive care medicine (Giovannitti et al. 2015; Nguyen et al. 2017). Clonidine has been demonstrated to evoke analgesia in human neuropathic, postoperative and obstetric pain after epidural and/or i.t. administration (Chiari et al. 1999; Deer et al. 2017; Eisenach et al. 1996; Giovannoni et al. 2009; Mastenbroek et al. 2017; Zhang et al. 2016). Probably due to the reported shorter duration of its analgesic action by the i.t. route, the epidural administration of clonidine has been widely utilized (Castro & Eisenach 1989; Filos et al. 1994; Klimscha et al. 1995). Clonidine is well tolerated at antinociceptive doses, however it may produce pronounced side effects such as hypotension, bradycardia and sedation in spinal use (Carroll et al. 1993; Eiseanch et al. 1989; Engelman & Marsala 2013; Kumar et al. 2014). Clonidine, as a highly lipophilic drug (Aantaa & Scheinin 1993; Chan et al. 2010), has been shown to undergo rapid systemic absorption from the lumbar i.t. space (Castro & Eisenach 1989). Dexmedetomidine, another highly lipophilic drug (Aantaa & Scheinin 1993), is used as a sedative agent for critically ill patients requiring prolonged sedation and mechanical ventilator support in a critical care setting and as an adjunctive sedative agent for procedural sedation (Giovannitti et al. 2015).

α_2-Adrenoceptor agonists (such as clonidine and dexmedetomidine) are furthermore used as adjuvants with local anaesthetics to improve the quality and duration of spinal anaesthesia since their combination enhances the analgesic effect by prolonging the duration of sensory-motor block of the local anaesthetics (Bedder et al. 1986; Swain et al. 2017; Zhang et al. 2016). Local anaesthetics induce hypotension, and the addition of vasoconstrictors such as clonidine and dexmedetomidine with local anaesthetic drugs helps in the maintenance BP by decreasing their systemic absorption (Bajwa et al. 2012; Eisenach et al. 1996), and minimizing hemodynamic effects in the periphery (Staikou & Praskeva 2014; Strebel et al. 2004). However, in the clinic, the combination a local anaesthetic and clonidine (Niemi 1994; Pöpping et al. 2009; Staikou & Praskeva 2014) or dexmedetetomidine (Liu et al. 2020; Staikou & Praskeva, 2014) has evoked unwanted effects e.g. hypotension, bradycardia, sedation and urinary retention.

It has been suggested that agents with a lower lipid solubility might be advantageous for spinal use (Eisenach et al. 1994). Increasing lipophilicity is associated with more rapid and extensive absorption into the vasculature and redistribution in the body, which for α_2-adrenoceptor agonists could lead to a greater likelihood or intensity of sedative and haemodynamic unwanted effects. In addition, Yaksh et al. (2017) speculated that a more polar α_2-adrenergic agonist might have virtues in spinal drug delivery. Thus, a polar α_2-adrenoceptor agonist fadolmidine

with low lipophilicity is being developed for spinal analgesia. In addition, this kind of selective α_2-adrenoceptor agonist would be expected to achieve a good analgesic effect with less side effects such as hypotension, bradycardia, severe sedation and respiratory depression after i.t./epidural administration.

All four studies are preclinical pharmacodynamic studies, and part of the work aiming to evaluate the profile of adverse effects of fadolmidine when it is administered at spinal analgesic doses. Furthermore, novel findings show that fadolmidine delivered spinally especially i.t. can provide antinociception with less of the known use-limiting adverse effects as hypotension associated with other α_2-adrenoceptor agonists. In addition, when i.t. fadolmidine was tested as an adjuvant to a local anesthetic, it enhanced sensory-motor block and thus these two drugs could represent a suitable combination for spinal anaesthesia.

2 Review of the Literature

2.1 Biosynthesis and metabolism of catecholamines

In the synthesis of catecholamines, tyrosine (L-tyrosine) is taken up and concentrated in catecholaminergic neurons by an active transport mechanism, where it is first converted to L-dihydoxyphenylalanine (L-DOPA) by tyrosine hydroxylase; L-DOPA is converted to dopamine by DOPA decarboxylase (Cooper et al. 1991; Weiner & Molonoff 1994). The conversion of L-tyrosine to L-DOPA and further to dopamine occurs in the cytosol. Dopamine is taken up into the storage vehicles. In noradrenergic cells, dopamine is further converted to noradrenaline (NA) by dopamine β-hydroxylase. The neurotransmitter is stored in vesicles in nerve terminals where its concentration is high whereas the concentration of neurotransmitter in cytosol is low. Activation (depolarisation) of NA neurons releases the neurotransmitter from synaptic vesicles via an exocytotic mechanism to the synaptic cleft resulting in the activation of postjunctional receptors causing either depolarisation or hyperpolarisation of the target tissue. The presence of catecholamines at the synaptic cleft is terminated mainly by re-uptake neurotransmitter into the nerve terminals. Re-uptake of NA into the nerve terminals involves active transport by carrier proteins. Neurotransmitter in the synaptic cleft may be metabolized primarily by methylation of catechol-O-methyltransferase (COMT) and by oxidation of monoamine oxidase (MAO). COMT is distributed in different tissues e.g. in the glia cells where it is located both in cytosol and in cell membranes, whereas MAO is found in the mitochondria of the nerve terminals. Both COMT and MAO are widely distributed throughout the body. The main end metabolite of NA (in the periphery), adrenaline and dopamine is homovanillic acid (HVA). In the brain, the main metabolite of NA is 3-methoxy-4-hydroxyphenylglycol (MHPG) (Cooper et al. 1991; Weiner & Molonoff 1994). The biosynthesis and metabolism of catecholamines are illustrated in Figure 1.

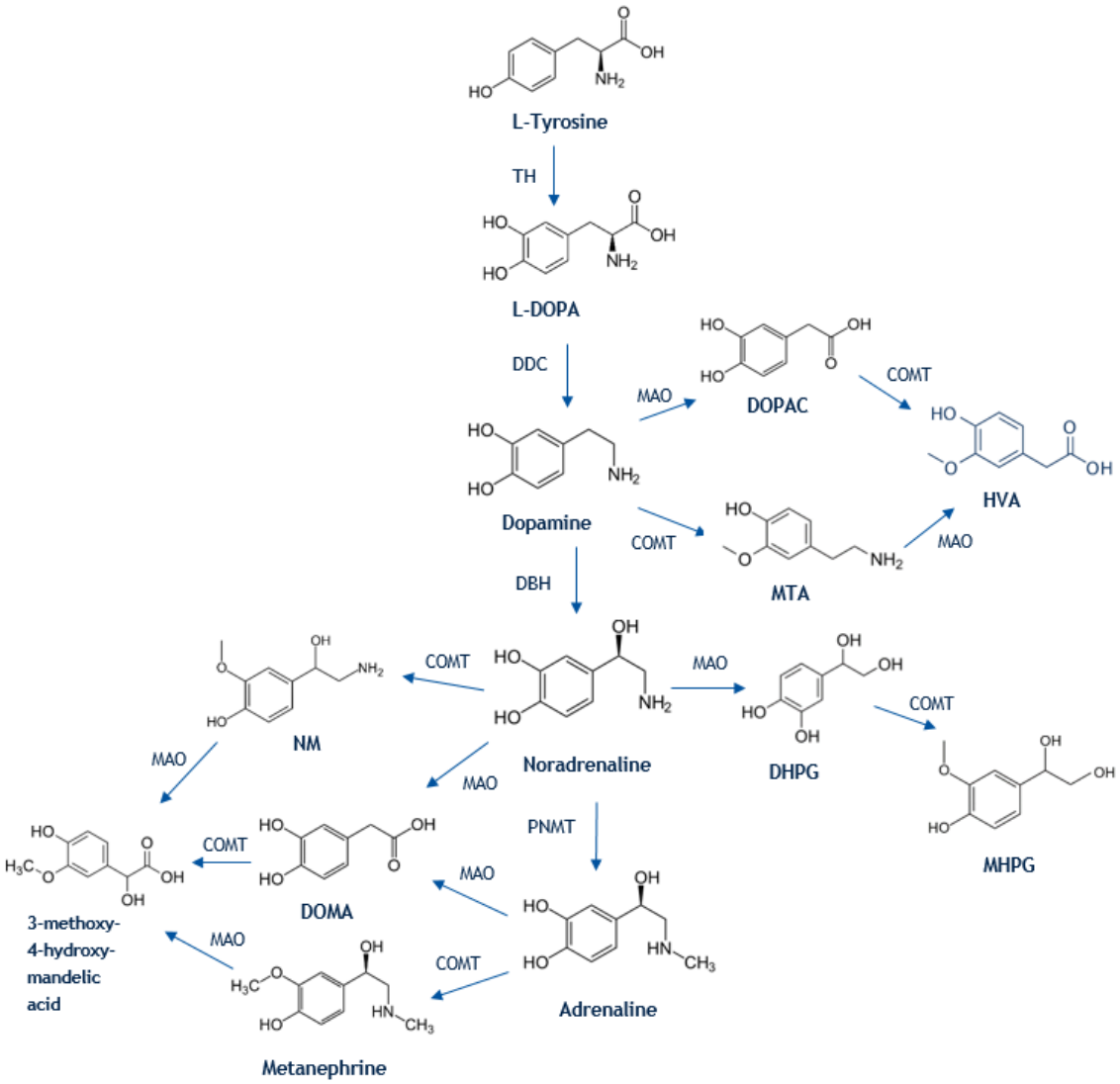

Figure 1. The biosynthesis pathway and metabolism for catecholamines (compiled and modified from Cooper et al. 1991; Weiner & Molonoff 1994). Abbreviations for compounds; L-DOPA = L-dihydroxyphenylalanine, DOMA = 3,4-dihydroxymandelic acid, DOPAC = 3,4-dihydroxyphenylacetic acid, DHPG = 3,4-dihydroxyphenylethylglycol, MHPG = 3-methoxy-4-hydroxyphenylglycol, HVA = homovanillic acid, NM = normetanephrine, MTA = 3-methoxytyramine. Abbreviations for enzymes; DBH = dopamine β-hydroxylase, DDC = DOPA decarboxylase, COMT = catechol-O-methyltransferase, MAO = monoamine oxidase, PNMT = phenylethanolamine-N-methyltransferase, TH = tyrosine hydroxylase.

2.2 α_2-Adrenergic receptors

Adrenergic receptors are classified into three guanine nucleotide-binding regulatory proteins (G-protein) coupled cell membrane receptors α_1-, α_2- and β-adrenoceptors (Bylund 2007) of which NA and adrenaline are endogenous ligands. A classification of adrenergic receptors is presented in Table 1. Based on the ligand binding and molecular cloning studies, α_2-adrenergic receptors (α_2-adrenoceptors) are further sub-classified into three subtypes α_{2A} /α_{2D} (orthologue of human α_{2A} in rodents), α_{2B} and α_{2C} (Bylund et al. 1992). α-Adrenoceptors are distributed throughout the body. α_{2A}-Adrenoceptors can be found in the brain, especially in Locus coeruleus (LC), a region which contains the cell bodies for the ascending and descending noradrenergic neurons (MacDonald & Scheinin 1995). In addition, the α_{2A}-adrenoceptor subtype is present in the brainstem, cerebral cortex, septum, hypothalamus, hippocampus and amygdala in the rat (Scheinin et al. 1994) and from spinal cord synaptosomes (Li & Eisenach 2001) and on central terminals of primary afferent nociceptors (presynaptic) and pain-relay neurons (postsynaptic) (Pertovaara 2006). In the central nervous system (CNS), the α_{2A}-adrenoceptor subtype influences neurotransmitter release, neuronal excitability and in the regulation of sympathetic outflow (Guyenet et al. 1994). The α_{2B}-adrenoceptor subtype can be found in peripheral tissues e.g. in the cardiovascular system i.e. in vascular smooth muscle as well as in the CNS. In the cardiovascular system, activation of the α_{2B}-adrenoceptor subtype induces a transient hypertensive response (Link et al. 1996). Furthermore, in the spinal cord, the α_{2B}-adrenoceptor subtype mediates the analgesic effect of nitrous oxide in descending NA neurons (Sawamura et al. 2000). α_{2C}-Adrenoceptors are found in the brain e.g. in hippocampus, cerebral cortex (MacDonald & Scheinin 1995) and LC as well as other brain regions containing noradrenergic, serotonergic and dopaminergic neurons and thus are suggested to be involved in the regulation of monoamine pathways of the brain (Rosin et al. 1996). Furthermore, α_{2C}-adrenoceptor subtypes are found in the spinal cord and adrenal chromaffin cells (Gyires et al. 2009; Knaus et al. 2007). In the CNS, activation of α_{2C}-adrenoceptors influences memory and behavioural functions (Scheinin et al. 2001). They may exert beneficial effects in attention deficient and post-traumatic stress disorders (Sallinen et al. 1998). Furthermore, the α_{2A}-adrenoceptor subtype is the main inhibitory presynaptic feedback receptor and along with the α_{2C}-adrenoceptor subtype, it participates in presynaptic regulation in CNS, whereas all three α_2-receptor subtypes serve as feedback regulators in peripheral sympathetic nerves (Knaus et al. 2007).

Endogenous agonists (i.e. NA and adrenaline) have similar affinity for all 3 subtypes (Giovannitti et al. 2015; Gyires et al. 2009). At the second messenger level, α_2-adrenoceptors function via G-proteins ($G_{i/0}$) to inhibit the activity of adenylyl cyclase and decrease cyclic adenosine monophosphate activity. In addition, in some cases, they cause hyperpolarization via opening of K^+-channels (Arima et al. 1998)

and inhibiting Ca^{2+}-influx (Soini et al. 1998; Timmons et al. 2004), leading to a suppression of neuronal firing and a further inhibition of transmitter release (Giovannitti et al. 2015).

Table 1. Classification of adrenergic receptors.

α_1-adrenoceptors	α_{1A}
	α_{1B}
	α_{1C}
α_2-adrenoceptors	α_{2A}
	α_{2B}
	α_{2C}
	α_{2D}*
β-adrenoceptors	β_1
	β_2
	β_3

* α_{2D} = orthologue of human α_{2A} in rodents. Modified from Bylund et al. 2007.

2.2.1 Pharmacology of α_2-adrenergic receptor agonists

α_2-Adrenoceptors are involved in various physiological functions, particularly in the cardiovascular system and the CNS (Gyires et al. 2009; Kamibayashi & Maze 2000). α_2-Adrenoceptors operate as presynaptic inhibitory feedback receptors to control the release of NA and adrenaline or other transmitters from neurons (Giovannitti et al. 2015). Some of the physiological/pharmacological effects mediated through α_2-adrenoceptors are summarised in Table 2.

Table 2. Selected physiological/pharmacological effects mediated by stimulation of α_2-adrenoreceptors.

Organo or tissue	α_2-Adrenoceptor subtype	Action
Brain	α_{2A}, α_{2C}	Inhibition of NA, dopamine, serotonin release
	α_{2A}	Sedative action
	α_{2A}, α_{2C}	Effects on memory and behavioural functions
	α_{2A}	Hypotensive action (vasodilatation in periphery)
	α_{2A}	Bradycardiac action
	$\alpha_{2A,}$ α_{2C}	Hypothermia
	α_{2A}	Mydriasis
Spinal cord	α_{2A}, α_{2B}, α_{2C}	Analgesia
Adrenergic neurons	α_{2A}, α_{2B}, α_{2C}	Presynaptic feedback inhibition of NA release
Adrenal medulla	α_{2C}	Feedback control of plasma adrenaline
Pancreatic β cells	$\alpha_{2A,}$ α_{2C}	Inhibition of insulin secretion
Pituitary	$\alpha_{2A,}$ α_{2C}	Stimulation of growth hormone release
Vascular smooth muscle	α_{2A}, α_{2B}	Arterial vasoconstriction
	α_{2C}	Venous vasoconstriction
Heart	$\alpha_{2A,}$ α_{2C}	Inhibition of NA release, bradycardia
Kidney	α_{2A}, α_{2B}, α_{2C}	Inhibition of renin secretion, increase glomerular filtration, increase secretion of sodium and water
GI tract	α_{2A}	Inhibition of GI motility
	α_{2A}	Ions transport and fluid secretion in the small intestine
	α_{2A}	Inhibition of gastric acid secretion
	α_{2B}	Gastric mucosal protection
Uterus	$\alpha_{2A,}$ α_{2C}	Decrease in the contractile response
Eye	α_{2A}	Decrease in intraocular pressure
Platelets	α_{2A}	Platelet aggregation

Derived from Fagerholm et al. 2011; Gáspár et al. 2007; Gyires et al. 2009; Intengan & Smyth 1996; Kamibayashi & Maze 2000; Knaus et al. 2007; Pertovaara 2006; Peterhoff et al. 2003; Scheinin et al. 2001; Trendelenburg et al. 1997; Vahabi & Kazemi 2011.

2.2.1.1 Sedative, hypothermic and mydriatic effects

In the CNS, α_2-adrenoceptors agonists inhibit NA, dopamine, serotonin and acetylcholine release and induce sedation, analgesia and hypothermia. Experimental data suggests that the sedative-hypnotic effects of α_2-adrenergic agonists are mediated by the activation of α_2-adrenoceptors in the brain (Gyires et al. 2009; Maze & Tranquili 1991; Nguyen et al. 2017; Pertovaara et al. 1994). The LC is an important target site for the sedation induced by α_2-adrenergic agonists such as clonidine (De Sarro et al. 1987; Sakamoto et al. 2013) and dexmedetomidine (Correa-Sales et al. 1992; Jorm & Stamford 1993). Presynaptic α_2-adrenergic receptor activation in these areas leads to decreased NA stimulation to the autonomic nervous system. In contrast, postsynaptic α_2-adrenoceptor stimulation causes hyperpolarization of neuronal membranes reducing the activity of the ascending noradrenergic pathways and inducing hypnosis and sedation in animals (Bloor et al. 1992; Drew et al. 1979; Gyires et al. 2009; Nguyen et al. 2017; Scheinin et al. 1989; Sinclair 2003). In addition, two α_2-adrenoceptor agonists guanfacine (Hayes et al. 1986; Minor et al. 1989) and tizanidine (Kawamata et al. 2003; Kroin et al. 2003) induce sedation in rats and dogs at a lower incidence than clonidine. Furthermore, in addition to their effects in animals, certain α_2-adrenoceptors agonists have induced sedation and sleep in humans e.g. dexmedetomidine (Aho et al. 1991; Belleville et al. 1992; Bloor et al. 1992; Keating 2015; Weatherall et al. 2017), clonidine (Cruickshank et al. 2016; Filos et al. 1994; Nguyen et al. 2017), tizanidine (Berry & Hutchinson 1988) and guanfacine (Rugino 2018).

Dexmedetomidine is a highly selective α_2-adrenoceptor agonist having sedative, anxiolytic, analgesic, and sympatholytic properties (Nguyen et al. 2017). Clonidine is a α_2-adrenoceptor agonist and used as an antihypertensive drug (Nguyen et al. 2017). Both have perioperative beneficial effects, which include a reduction of anaesthetic requirements, improving hemodynamic stability and providing analgesia (Nguyen et al. 2017).

In the hypothalamus, noradrenergic afferents in the medial preoptic area regulate body temperature (BT) (Alam & Mallick 1994; Kumar et al. 2007). α_2-Adrenergic agonists induce the hypothermic response by activating postsynaptic α_2-adrenoceptors in the preoptic area (Millan et al. 2000; Myers et al, 1987; Sinclair 2003). Clonidine (Drew et al. 1979; Livingston et al. 1984), medetomidine/ dexmedetomidine (Granholm et al. 2007; MacDonald et al. 1989; Scheinin et al. 1989) and guanfacine (Minor et al. 1989) were reported to produce a dose-dependent hypothermia in unanaestherized rats and dogs.

In addition, α_2-adrenergic agonists are known to induce mydriasis in rats, possibly mediated through central α_2-adrenoceptors by reducing the parasympathetic neural input to the constrictor muscle of the iris, an effect mediated by inhibitory α_2-adrenoceptors on the Edinger-Westphal nucleus in the midbrain (Heal et al. 1995).

Medetomidine (Scheinin et al. 1989), dexmedetomidine (Haapalinna et al. 2003; Lehtimäki et al. 2008; Sallinen et al. 2007) and clonidine (Yu & Koss 2004) evoked a clear mydriatic effect (i.e. pupil dilatation) after systemic and dexmedetomidine after i.t. administration (Horváth et al. 1994) as well in rats and tizanidine in mice after s.c. administration (Takayanagi et al. 1985).

2.2.1.2 Analgesic effect

When administered spinally, α_2-adrenergic agonists have evoked antinociception in various animal models (Eisenach et al. 1994; Yaksh 1985) and in humans (Eisenach et al. 1996; Giovannitti et al. 2015), and the effect is mediated primarily in the spinal cord (Bahari & Meftahi 2019; Eisenach et al. 1994; Pertovaara 2006; Yaksh 1985). The activation of spinal α_2-adrenoceptors suppresses the transmission of pain inputs to higher centres. In addition, adrenergic inhibitory pathways from the midbrain and brainstem to the spinal cord modulate pain processing in the dorsal horn (Bahari & Meftahi 2019, Pertovaara 2006). The activation of these descending inhibitory medullospinal pathways by α_2-adrenergic agonists induce postsynaptic cell hyperpolarisation in the dorsal horn and decrease pain transmission in spinal nociceptive neurons (D'Mello & Dickenson 2008; Pertovaara 2006). Systemic, spinal or LC administered α_2-adrenoceptor agonists e.g. dexmedetomidine (Asano et al. 2000; Guo et al. 1996; Kalso et al. 1991), clonidine (Asano et al. 2000; Post et al. 1987), tizanidine (Asano et al. 2000; McCarthy et al. 1990) and guanfacine (Hayes et al. 1986; Minor et al. 1989; Post et al. 1987) produced dose-dependent antinociceptive effects in rats. Furthermore, systemic or spinally administered detomidine (Pohl et al. 2012), dexmedetomidine (Pohl et al. 2012), clonidine (Kroin et al. 1996, 2003; Pohl et al. 2012), tizanidine (Kroin et al. 1996, 2003) and guanfacine (Hayes et al. 1986) have induced analgesia in dogs. In addition, clonidine (Detweiler et al. 1993; Eisenach et al. 1987; Smith et al. 1992), dexmedetomidine (Eisenach et al. 1994) and guanfacine (Smith et al. 1992) exerted analgesic effects in sheep/goats. In humans, administration of clonidine, dexmedetomidine (Eisenach et al. 1996; Pöpping et al. 2009; Xia et al. 2018; Zhang et al. 2016) and tizanidine (Berry & Hutchinson 1988) have been shown to induce analgesic effects.

2.2.1.3 Effects on cardiovacular system

The cardiovascular responses of α_2-adrenergic agonists are likely mediated through multiple sites of action (Eisenach and Tong 1991; Kroin et al. 1996; Nguyen et al. 2017; Sinclair 2003). α_2-Adrenergic agonists can activate α_2-adrenoceptors in the brain, reducing the sympathetic outflow (sympatholytic effect) from the CNS leading to peripheral vasodilatation. In addition, α_2-adrenergic agonists induce hypertension

by activating vascular α_2-adrenergic receptors and bradycardia by reducing the chronotrophic drive to the heart along with an increase in vagal outflow via parasympathetic stimulation and baroreflex activity (Eisenach et al. 1996; Kroin et al. 1996; Sinclair 2003). Centrally acting antihypertensive agents e.g. α-methyldopa, clonidine, guanabenz, guanfacine and moxonidine stimulate α_2-receptors on adrenergic neurons situated within the rostral ventrolateral medulla and consequently sympathetic outflow is reduced. Centrally acting agents also stimulate peripherally located presynaptic α_2-adrenergic receptors, but for the most part, this is of marginal clinical significance (Sica 2007).

α_2-Adrenergic agonists like clonidine (Ozawa et al. 1977), medetomidine (Savola 1989) and dexmedetomidine (Honkavaara et al. 2011) evoked a biphasic BP response, an initial increase and a delayed long-lasting decrease in BP and concomitantly an HR decrease in both phases after systemic administration. After spinal dosing, clonidine and dexmedetomidine induced also a biphasic effect on the BP; central α_2-adrenoceptors mediated a depressor effect at low to moderate doses and vascular α-adrenoceptors mediated a pressor response at higher doses in rats (Asano et al. 2000; Horvath et al. 2002; Solomon et al. 1989) and in dogs (Bloor et al. 1992; Pohl et al. 2012). However, in addition to the biphasic effects on BP also a rapid decrease in HR was reported in rats (Nagasaka & Yaksh 1990) and sheep (Eisenach et al. 1994) after intraspinal administration of dexmedetomidine. Furthermore, clear decreases in BP and HR were reported after epidural dosing of clonidine and guanfacine to goats (Smith et al. 1992). Dexmedetomidine and clonidine (Aantaa & Scheinin 1993) are lipophilic compounds which are able to cross the blood-brain barrier and are rapidly absorbed into the systemic circulation from the spinal space (Eisenach et al. 1996; Kawamata et al. 2003; Sabbe et al. 1994, Post et al. 1987; Solomon et al. 1989). In addition, two less selective α_2-adrenoceptor agonists, tizanidine (Kroin et al. 1996; Kroin et al. 2003) and guanfacine (Scholtysik 1986) also decreased BP and HR in rats and dogs. In humans, medetomidine (Scheinin et al. 1987), dexmedetomidine (Aho et al. 1991; Kallio et al. 1989; Uusitalo et al. 2018) and clonidine (Eisenach et al. 1996) dose-dependently decreased HR and BP after an initial hypertensive effect at larger doses following systemic administration. In the clinic, it has been suggested that guanfacine may be a useful alternative to clonidine in patients who are intolerant of clonidine because of excessive sedation (Cornish 1988). Because of their sedative and hypotensive effects, clonidine and dexmedetomidine, have long been used as adjuvants to other anesthetic agents by providing good hemodynamic stability with prolonged motor and sensory block and thus causing less side effects than the anesthetic compounds on their own (Bajwa et al. 2012; Mahendru et al. 2013).

2.2.1.4 Effects on kidney function and urinary

The α_2-adrenoceptor-mediated diuretic effect has been demonstrated in rats with various agonists including dexmedetomidine, clonidine and guanfacine (Harada & Constantinou 1993; Shockley et al. 1993). Furthermore, α_2-adrenergic agonists have been reported to induce a centrally mediated increase in urine output (Intengan & Smyth 1996) by lowering antidiuretic hormone and vasopressin secretion, as well as through a peripherally mediated increase in sodium excretion in the kidney (Cabral et al. 1998; Sinclair 2003). In humans, clonidine increases urine output and electrolyte excretion; this has been associated with a decrease in plama renin activity (Vahabi & Kazemi 2011). Furthermore, α_2-adenergic agonists have also been shown to interfere with the micturition reflex in conscious mice (Aro et al. 2015) and rats (Kontani et al. 2000) and in anaesthetised male rats (Streng et al. 2010). In dogs, the α_2-adrenoceptors in the kidney seem to inhibit neural NA release, which can have a role in controlling renal hemodynamics and thereby influence diuresis (Hisa et al. 1989). In the clinic, the combination a local anaesthetic and clonidine (Niemi 1994; Pöpping et al. 2009; Staikou & Praskeva 2014) or dexmedetetomidine (Liu et al. 2020; Staikou & Praskeva 2014) evoked adverse effects like hypotension, bradycardia, sedation and urinary retention.

2.2.1.5 Effects on GI system

The activation of presynaptic α_2-adenergic receptors is known to mediate several responses in the gastrointestinal (GI) tract i.e. it inhibits gastric acid secretion (Blandizzi et al. 1995; Müllner et al. 2001, 2002) gastric motility and GI transit (Fülöp et al. 2005; Gyires et al. 2009; Zádori et al. 2007) in rats. These responses are thought to be mediated through the activation of presynaptic α_2-adrenoceptors located on the vagus nerve leading to an inhibition of acetylcholine release. In accordance, clonidine (Asai et al. 1997; Pol et al. 1996) and dexmedetomidine (Asai et al. 1997) were reported to inhibit GI transit in the rat after their systemic administration, acting most probably via a peripheral parasympathetic effect, although part of the effect may be of central origin (Asai et al. 1997; Pol et al. 1996). In addition, tizanidine s.c. inhibited GI transit times in mice (Takayanagi et al. 1985). Furthermore also in humans, clonidine (Schiller et al. 1985; Malcolm et al. 2000) and dexmedetomidine (Iirola et al. 2011) inhibited GI transit although at low doses. However, at low doses no effects of clonidine on GI and colonic transit were also reported (Viramontes et al. 2001).

2.2.1.6 Effects on intraocular pressure

α_2-Adrenoceptors agonists e.g. clonidine (Harrison & Kaufmann 1977) and brimonidine (Burke & Potter 1986) decrease intraocular pressure (IOP) and in fact, brimodine is used in the treatment of glaucoma (Greenfield et al. 1997). After topical dosing with clonidine eye drops both systolic and diastolic blood pressure displayed a statistically significant reduction, although the magnitude of the decline was small (Harrison & Kaufmann 1977). Brimodine was shown to be safer than clonidine by evoking fewer systemic side effects based on its poor ability to cross the blood-brain barrier (Greenfield et al. 1997).

2.2.1.7 Endocrine effects

The activation of the α_{2A}-adrenoceptors on pancreatic β cells inhibits insulin secretion, and α_{2A}-adrenoceptors on sympathetic nerves and on adrenomedullary chromaffin cells limit the sympathoadrenal output and the activation of these receptors elevate blood glucose levels (Fagerholm et al. 2011; Ruohonen et al. 2012). Furthermore, a deletion of the α_{2C}-adrenoceptor subtype increased adrenaline secretion and evoked a potential increase in the blood glucose level via enhanced glycogenolysis (Ruohonen et al. 2012) thus α_{2C}-adrenoceptor activation may have a complementary role in this phenomenon (Peterhoff et al. 2003). Dexmedetomidine was found to reduce insulin release from the pancreas (Burton et al. 1997) and this increased plasma glucose concentrations in dogs (Restitutti et al. 2012). In humans, chronic treatment with oral clonidine gave rise to an elevation of the blood glucose level and decreased insulin secretion (Okada et al. 1986).

It has been reported that α_2-adrenoceptors agonists can stimulate the release of growth hormone (GH) releasing hormone at the hypothalamic level and thus increase GH secretion from the anterior pituitary (Balldin et al. 1993; Kiem et al. 1991). In healthy male volunteers, a single dose of a selective α_2-adrenoceptor agonist, medetomidine, was observed to increase GH levels (Kallio et al. 1989).

2.2.1.8 Other effects

The adrenergic system plays an important role in the control of uterine contractility. In non-pregnant animals, α_2-adrenoceptors are not involved in the control of myometrial contractions. In last-day-pregnant animals, the α_{2B}-adrenoceptors predominate and mediate contraction, while the α_{2A}- and α_{2C}-adrenoceptors decrease the contractile response to NA in vitro in rats (Gáspár et al. 2007). All three subtypes of α_2-adrenoceptors are coexpressed also in the human myometrium at term pregnancy; at that time, α_2-expression is dominated by the α_{2A}-subtype (Adolfsson et al. 1998). Dexmedetomidine increased uterine contractility at simulated clinical

plasma concentrations and clonidine only at much higher tissue bath concentrations on human myometrium *in vitro* (Sia et al. 2005).

Platelets express the imidazoline I_1 and I_2 -receptors as well as the α_2-adrenoceptors (Kawamoto et al. 2015). Adrenaline and NA produced a dose-dependent potentiation of adenosine diphosphate or collagen-induced platelet aggregation (Yokota et al. 2013). Dexmedetomidine and clonidine has both enhancing and suppressive effects on human platelet functions through their actions on the α_2-adrenoceptor and on the I-imidazoline receptor, respectively in vitro (Kawamoto et al. 2015; Pinthong et al. 2004).

2.3 Pain transmission

2.3.1 Pain pathways from periphery to the brain

High-intensity noxious stimuli impulses from the periphery are transmitted via primary afferent A delta (Aδ)- and C-fibres and low-intensity non-noxious stimuli impulses via A beta (Aβ)-fibres, through the dorsal root ganglion into the dorsal horn of the spinal cord (Pertovaara 2006, Yaksh et al. 2017). The dorsal horn of the spinal cord is critical to processing distinct modalities of noxious and innocuous sensation. Nociceptive small-diameter unmyelinated C-fibres responding to thermal, mechanical, and chemical stimuli (polymodal) and nociceptive medium-diameter thinly myelinated Aδ-fibers are predominately heat- and or mechanosensitive fibres. Non-nociceptive, large-diameter, highly myelinated Aβ-fibres respond only to touch (mechanoreceptors). In the dorsal horn, painful stimuli activate second-order neurons as nociceptive specific (NS) and wide dynamic range (WDR) neurons. NS neurons are mostly located superficially and which synapse with Aδ-, and C-fibres in lamina I and II (substantia gelatinosa), whereas most WDR neurons are located deeper in lamina V (Almeida et al. 2004; D'Mello & Dickenson 2008; Kibaly et al. 2016; Pertovaara 2006; Yaksh 1985). Non-nociceptive second-order neurons of dorsal horn respond to non-noxious stimulations from mechanical and thermal low-threshold Aβ and Aδ-fibres located within lamina I, II, III and IV (Almeida et al. 2004; Kibaly et al. 2016). There are excitatory, glutamatergic, and inhibitory, GABAergic or gycinergic interneurons and cholinergic interneurons within the spinal cord and these can increase or decrease the response of NS cells and WDRs and influence the output of the dorsal horn (D'Mello & Dickenson 2008). Projection neurons within lamina I and V constitute the major output from the dorsal horn to the brain. Lamina V neurons mainly project to the thalamus by the spinothalamic/spinoreticulothalamic tracts and from there to many cortical regions in the brain (Bahari & Meftahi 2019; D'Mello & Dickenson 2008; Pertovaara 2006). They carry primarily sensory information and so provide the sensory component of

the pain experience (Kibaly et al. 2016; Pertovaara 2006). In addition, there are projection neurones from the dorsal horn to limbic areas such as the parabrachial area and periaqueductal grey (PAG), which further transmit pain signals up to higher centres in the brain e.g. amygdala (Almeida et al. 2004; Kibaly et al. 2016). Lamina I projection neurons are very important as they contribute to the transmission of pain messages to the brain (Merighi 2018). These cells also project into brainstem areas such as the rostral ventromedial medulla (RVM) a region that has descending projections back to the dorsal horn modulating spinal processing (D'Mello & Dickenson 2008; Kibaly et al. 2016). There are also projection neurons from lamina III-VI (Aβ-fibres) which project predominantly to the thalamus (Almeida et al. 2004; D'Mello & Dickenson 2008; Kibaly et al. 2016). A schematic representation of pain processing pathways from the periphery to the brain are illustrated in Figure 2.

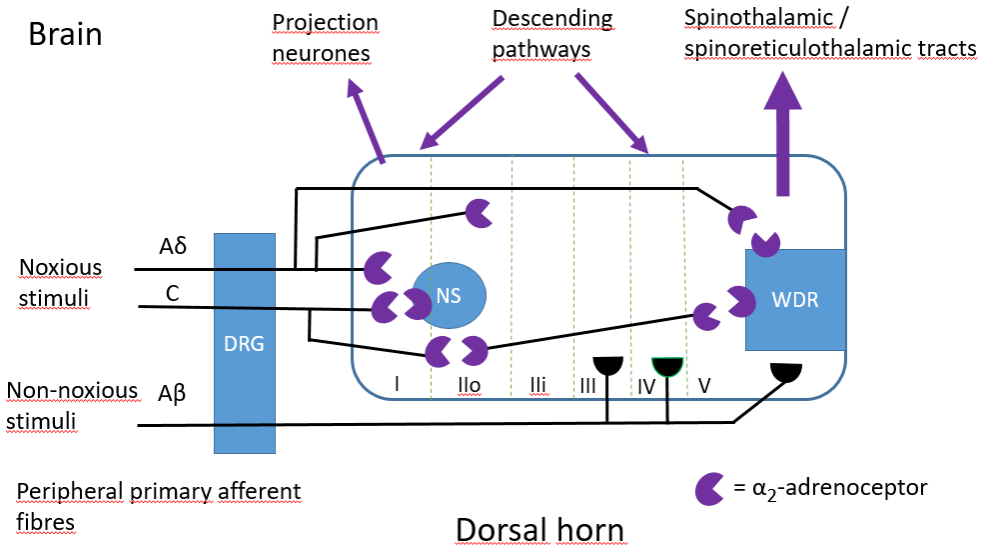

Figure 2. A schematic representation of the pain processing pathways from the periphery to the brain and α_2-adrenoceptors in the spinal dorsal horn (see paragraph 2.3.3). Aδ = A delta-fibre, C = C-fibre, Aβ = A beta-fibre, DRG = Dorsal root ganglion, NS = Nociceptive specific, WDR = Wide dynamic range, I-V = lamina I-V neurons. Lamina II can be divided into outer (IIo) and inner (IIi) parts, with the latter having a somewhat lower density of neurons. (Modified from Bahari & Meftahi 2019; D'Mello & Dickenson 2008).

Intact nociceptive primary afferent fibres are only little influenced by NA, sympathetic stimulation or sympathetic noradrenergic compounds (Pertovaara 2006, Sato & Perl 1991). However, peripheral nerve injury or inflammation or sensitization of the receptive field by heat or algogenic chemicals may lead to circumstances in which afferent myelinated Aδ and unmyelinated C nerve fibres become sensitive to

sympathetic stimulation and adrenergic compounds (D'Mello & Dickenson 2008; Pertovaara 2006).

The substantia gelatinosa (lamina II) of the spinal dorsal horn plays a critical role in nociceptive transmission (Almeida et al. 2004; D'Mello & Dickenson 2008; Yaksh 1985). The excitability and activity of lamina II neurons is mainly regulated by glutamate released from primary nociceptive afferents (Basbaum et al. 2009; Pertovaara 2006). Under pathological conditions, augmented glutamatergic transmission and resulting enhanced excitability of substantia gelatinosa neurons is expected to cause the hyperalgesia (increased sensitivity to painful stimuli) (Kuner 2010). Substantia gelatinosa structure consisting of neuronal cell bodies, neuropil, glia cells and synapses (Merighi 2018). Many pathological process within the CNS are mediated by complex interactions between neurons and resident glial cells. An activated spinal microglia plays an important role in the development of chronic pain (Tsuda et al. 2013). Furthermore, inhibition of microglial activation attenuates the development of neuropathic pain (Huang et al. 2014).

The sensitization of primary nociceptors or peripheral sensitization is one of the mechanisms involved in the development of neuropathic pain (Bahari & Meftahi 2019; Kibaly et al. 2016; Kuner 2010; Pertovaara 2006). When spinal neurons are subjected to repeat or high-intensity nociceptive impulses, they become progressively and increasingly excitable even after the stimulus is removed. This condition is known as central sensitization or wind-up phenomenon and leads to nonresponsive or chronic intractable pain (Herrero et al. 1999). Wind-up is a frequency-dependent increase in the excitability of spinal cord neurones, evoked by electrical stimulation of afferent unmyelinated C-fibres (Herrero et al. 1999). Repetitive noxious stimulation of C-fibres can result in prolonged discharge of dorsal horn cells. Repetitive episodes of wind-up may precipitate long-term potentiation (LTP), which involves a long-lasting increase in the efficacy of synaptic transmission. Both wind-up and LTP are believed to be part of the sensitization process involved in many chronic pain states (D'Mello & Dickenson 2008; Herrero et al. 1999; Kibaly et al. 2016).

2.3.2 Supraspinal noradrenergic pain modulation

In the brain, the noradrenergic system arises from cell groups in the brainstem, which are classified as A1-A7. Noradrenergic cell groups A7, A6 (LC) and A5 are located in the pons and provide a noradrenergic innervation that controls impulse transmission to the dorsal horn (desending inhibitory pathway) (Millan 2002; Pertovaara 2006). In addition, they provide ascending projections that target almost the entire forebrain (Bouret and Sara 2005; Pertovaara 2006, 2013). The pontospinal A7 noradrenergic nuclei has a high density of α_2-adrenoceptors (Scheinin et al. 1994)

and in the A6 nuclei, many of them are autoreceptors located on noradrenergic neurons (Aghajanian & Van der Maelen 1982; Pertovaara 2006). Increasing tonic discharge of LC neurons elevates the extracellular level of NA in various target areas e.g cortical areas, hippocampus, thalamus and amygdala. The prefrontal cortex is important in pain processing with other areas of the cerebral neocortex, hippocampus, PAG, thalamus, amygdala, and basal nuclei (Kibaly et al. 2016; Ong et al. 2019). Higher brain centers including thalamo-cortical regions are likely involved in the perception of the sensory and emotional qualities of pain (Groh et al. 2018). Descending modulatory pathways mediate the top-down regulation of nociceptive processing, transmitting cortical and limbic influences to the dorsal horn. These descending pathways from limbic regions are suggested to mediate the emotional, affective component of the pain experience. PAG contains a dense network of noradrenergic nerve fibres with the expression of α_2-adrenoceptors (Scheinin et al. 1994) and a low density of α_1-adrenoceptors (Day et al. 1997; Nalepa et al. 2005). It is postulated that a noradrenergic mechanism within PAG is not involved in spinal antinociception, but the PAG activates other noradrenergic nuclei in the brainstem, such as the A7 cell group (Bajic et al. 2001), that contribute to its desending antinociceptive action and this involves the α_2-adrenoceptors situated at the spinal cord level (Pertovaara 2006). Furthermore, central amygdala is the target of ascending fibers from the pain-responsive and stress-responsive nuclei in the brainstem. NA acting at presynaptic α_2-adrenoreceptors potently inhibits this synapse (Delaney et al. 2007). The amygdala plays a key role in the integration of noxious input with the endogenous analgesia and behavioural coping response (Li & Sheets 2018). The RVM modulates the afferent pain system (spinal or ascending nociception) from most structures of the forebrain (Kibaly et al 2016; Millan 2002; Pertovaara 2006). The stimulation of the RVM evokes the spinal release of NA (Hammond et al. 1985; Millan 2002) and antinociception (Yaksh 1985). The RVM directly projects to the dorsal horn of the spinal cord. The activation of the neurons in the descending pathway stimulates the release of serotonin and NA from their axons at the spinal level (Kibaly et al. 2016; Pertovaara 2006).

A schematic representation of the pain processing pathways and the modulatory systems of pain transmission in the spinal cord and brain is illustrated in Figure 3.

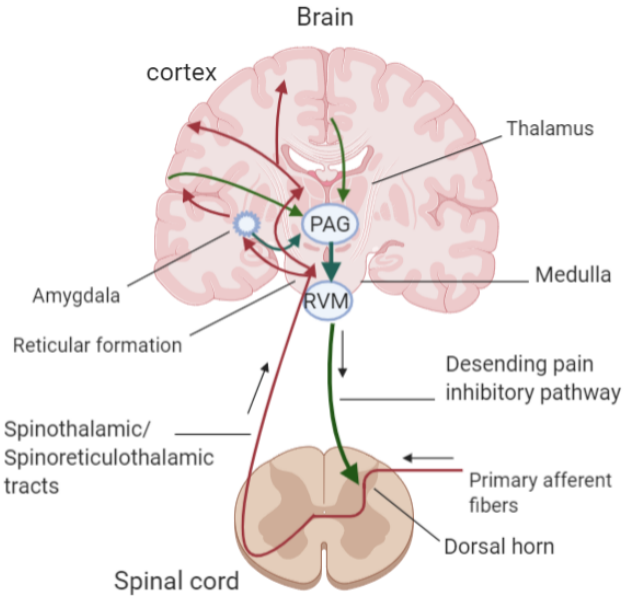

Figure 3. A schematic representation of the pain processing pathways (in red) and the modulatory systems of pain transmission (in green) in the spinal cord and brain (modified from Kibaly et al. 2016). PAG = periaqueductal gray matter, RVM = rostral ventromedial medulla.

Pontine noradrenergic cell groups are involved in descending modulation of pain through the action on spinal α_2-adrenoceptors (Bahari & Meftahi 2019; D'Mello & Dickenson 2008; Pertovaara 2006). For example, noxious stimuli activate brainstem adrenergic inhibitory pathways and increase the release of NA in the dorsal horn from descending terminals. These activate α_2-heteroreceptors on the presynaptic cell and suppress the release of glutamate and other stimulatory neurotransmitters. Both α_{2A}- and α_{2C}-adrenoceptor subtypes have been detected in the superficial layers of the dorsal horn of the spinal cord (Gyires et al. 2009; Pertovaara 2006; Stone et al. 1998). Pontospinal noradrenergic axons release NA and inhibit nociception by acting on α_{2A}-adrenoceptors on central terminals of nociceptive primary afferent nerve fibers. Besides α_{2A}-adrenoceptor α_{2C}-adrenoceptors, as hetero-receptors, were shown to inhibit the release of dopamine or serotonine in the CNS (Gyires et al. 2009). In addition, pontospinal noradrenergic axons are known to activate α_1-adrenoceptors on GABAergic and glycinergic inhibitory interneurons, which further hyperpolarize postsynaptic pain-relay neurons (Bahari & Meftahi 2019; Pertovaara 2006). Furthermore, pontospinal noradrenergic axons, by releasing NA, inhibit nociception by acting α_{2A}-adrenoceptors in the dorsal horn. In addition, the release of NA acting on α_{2C}-adrenoreceptors in excitatory interneurons is thought to inhibit nociception (Pertovaara 2006; Stone et al. 1998). α_{2A}-Adrenoceptor gene knockout mice

indicated that the α_{2A}-adrenoceptor has a major role in the pain suppressive effect induced by α_2-adrenergic compounds (Knaus et al. 2007; Pertovaara 2006).

Information from the environment and certain motivational states can activate the descending inhibitory pathway that is composed of neurons of which the cell bodies are locted in several regions of the midbrain as PAG and RVM and which the axons project into the spinal cord (Kibaly et al. 2016; Millan 2002). The neurons of PAG express opioid receptors which activation stimulate the serotonergic neurons in RVM which directly project to the dorsal horn of the spinal cord (Kibaly et al. 2016; Kwiat & Bashaum 1990). A noradrenergic pathway from the LC and subcoeruleus participated in the descending inhibitory system and projects to the neurons of the dorsal horn (lamina II, IV) (D'Mello & Dickenson 2008; Millan 2002; Pertovaara 2006). Activation of these neurons stimulate the release of serotonin and NA from their axons at the spinal level, which in turn induces secretion of enkephalins from the interneurons inhibiting presynaptic primary neurons in the dorsal horan as well as postsynaptic second-order neurons (Kibaly et al. 2016; Millan 2002). In the gate-control theory mechanism involves the balanced interplay between two different type of primary afferents fibres non-nociceptive Aβ and Aα (motor fibers) fibres and nociceptive C or Aδ fibres both coverge on a second-order WDR neuron in tht dorsal horn (Kibaly et al. 2016). In this spinal control pain model nonnociceptive stimuli (e.g. light touch) interfere with the transmission of painful stimuli and results in some pain relief. Non-nociceptive primary afferent fibres stimulate inhibitory interneurons in the dorsal horn whereas nociceptive fibres inhibit these inhibitory interneurons. An increase in the non-nociceptive input via the Aβ and Aα fibres reduces the firing rate of the dorsal horn second-order WDR neurons (Kibaly et al. 2016; Millan 2002).

2.3.3 Antinociceptive effects of α_2-adrenoceptor agonists

α_2-Adrenoceptors are located at various levels of the pain pathways from the periphery to the brain. The location of the α_2-adrenoceptors in the pain pathways in the spinal dorsal horn is illustrated in Figure 2. High densities of α_2-adrenoreceptors were found in the substantia gelatinosa (Yaksh 1986) and further, i.t. administration of an α-adrenergic agonist has elicited an increase in the nociceptive threshold in a variety of species (Fürst 1999). The analgesic properties of spinally administered α_2-adrenergic agonists like clonidine have been proposed to involve both central (in the dorsal horn of the spinal cord and brainstem) as well as peripheral sites of action (Chan et al. 2010; Eisenach et al. 1996; Nagappa et al. 2018; Wang et al. 2002). In the dorsal horn, treatment with an α_2-adrenergic agonist has been shown to activate presynaptic α_2-adrenergic receptors on small primary afferent C- and Aδ-fibres leading to an inhibition of the release of several neurotransmitters such as glutamate,

substance P, and calcitonin gene-related peptide from primary fibre terminals and postsynaptically hyperpolarizing dorsal horn WDR neurons (Bahari & Meftahi 2019; Pertovaara 2006; Yaksh 1985; Yaksh et al. 2017).

In the brain, α_2-adrenoceptor agonists decrease the firing rate of the LC and this is followed by reduced NA release in innervated brain regions (Correa-Sales et al. 1992). The LC is an important target site for the sedation, analgesia, and decrease in central sympathetic outflow induced by α_2-adrenergic agonists (De Sarro et al. 1987; Correa-Sales et al. 1992). The actions of an agonist at presynaptic α_2-adrenoceptors in these areas leads to decreased NA impulses to the autonomic nervous system. In contrast, postsynaptic α_2-adrenoceptor stimulation causes hyperpolarization of neuronal membranes reducing the activity of the ascending noradrenergic pathways. The pontospinal noradrenergic axons synapse with spinal pain-relay neurons in the spinal doral horn (Pertovaara 2006) and inhibit their activity by activating postsynaptic α_2-adrenoceptors (Sonohanata et al. 2004). In addition, α_2-adrenergic agonists can activate the descending inhibitory medullospinal pathways and inhibitory interneurons such as cholinergic interneurons and inhibitory interneurons. Activation of spinal muscarinic acetylcholine interneurons produces analgesia and inhibits dorsal horn neurons through potentiation of GABAergic/glycinergic tone and inhibition of glutamatergic input; this activation induces hyperpolarization of spinal postsynaptic nociceptive neurons cells (Bahari & Meftahi 2019; D'Mello & Dickenson 2008; Millan 2002; Pertovaara 2006). Furthermore, hyperpolarization of spinal postsynaptic nociceptive neurons decreases the release of nociceptive substances from substantia gelatinosa and prevents the transmission of pain inputs to higher centers (Bahari & Meftahi 2019; Eisenach et al. 1996; Kawasaki et al. 2003; Nguyen et al. 2017; Pertovaara 2006). Although there is some evidence for both a supraspinal and peripheral site of action, it is thought that it is the spinal mechanism which is responsible for most of the analgesic actions of α_2-adrenoceptor agonist drugs (Eisenach et al. 1996; Jaakkola et al. 1991; Pertovaara 1993; Yaksh 1985; Yaksh et al. 2017).

2.4 Drugs used for spinal analgesia

The spinal (i.e. epidural and i.t.) administration technique provides a good opportunity to achieve quick and efficient pain relief. Epidural analgesia is a good, and reliable method to provide intraoperative, postoperative (Nagappa et al. 2018) and labor analgesia (Sng & Sia 2017). Better drugs, techniques and delivery systems have enhanced the efficacy and safety of epidural analgesia (Sng & Sia 2017). In addition, i.t. drug delivery systems have proven efficacy for a wide variety of intractable pain conditions as well as evoking fewer adverse effects (Erdine & De Andrès 2006; Mercadante et al. 2012) than systemic therapy in patients with

advanced cancers (Lamer et al. 2016; Mercadante et al. 2012). The benefits of i.t. drug delivery include a potent analgesic response with a more stable therapeutic drug level, decreased latency, increased duration of action, and fewer pharmacological complications such as nausea, vomiting, sedation and constipation (Erdine & De Andrès 2006). In addition, i.t. infusion (Deer et al. 2017; Lamer et al. 2016; Prager et al. 2014) has been shown to be a suitable drug delivery route for chronic (Ghafoor et al. 2007) and acute postoperative (Peniche et al. 2018; Sen & Sen 2015) pain treatments. Lower dosages of the analgesics could be used and this might reduce the side effects of these compounds (Erdine & De Andrès 2006; Lamer et al. 2016). Table 3 lists the most commonly used Food and Drug Administration (FDA) approved drugs for i.t. drug delivery (Lamer et al. 2016).

Table 3. Analgetic drugs for i.t. drug delivery.

Drugs (FDA-approved medications)	Class
Morphine	Opioid
Ziconotide	N-type voltage dependent calcium channel blocker
Baclofen	Anticonvulsant (GABA-B receptor agonist)
Commonly used off-label medications	
Bupivacaine	Local anesthetic
Fentanyl	Opioid
Hydromorphine	Opioid
Clonidine	α_2-adrenoreceptor agonist

Modified from Lamer et al. 2016; Deer et al. 2019.

Opioids are commonly used in severe cancer and neuropathic pain relief but their potential serious adverse effects and tolerance have given rise to concerns about their use as a safe analgesics (Baldini et al. 2012). Spinal and epidural opioids are used to control pain following a wide variety of surgical procedures (Cohen et al. 2017; Lamer et al. 2016; Lanz et al. 1982; Reiz et al. 1981; Wang et al. 1979). However, the use of opioids introduces a risk for unwanted adverse effects like pruritus, nausea, urinary retention with the risk for the late respiratory depression being the most serious risk (Reiz et al. 1981; Swain et al. 2017). Opioids are the most frequently used local anesthetic adjuvants allowing a reduced amount of local anesthetics to be administered while achieving a longer duration of analgesic effect (Grangier et al. 2020; Swain et al. 2017). The combination has a synergistic analgesic effect (Penning & Yaksh 1992). However, opioids cause side effects and tolerance, limiting their usability (Grangier et al. 2020; Hayhurst & Durieux 2016; Sun et al. 2017; Swain et al. 2017).

Local anaesthetics are used for acute and chronic pain treatments. However, the duration of action and the dose dependent adverse effects such as sympathetic nerve

block (resulting in hypotension), prolonged motor block and toxicity problems (neurotoxicity, systemic toxicity) (Lirk et al. 2014; Russell 1982) limited their use. Adjuvants or additives are often used with local anaesthetitics to seek synergistic effects by prolonging the duration of sensory-motor block and limiting the cumulative dose requirements of local anaesthetics (Swain et al. 2017; Zhang et al. 2016).

α_2-Adrenoceptor agonists (e.g. dexmedetomidine and clonidine) are used in both anaesthesia and intensive care medicine. α_2-Adrenoceptor agonists are being evaluated as ways of obtaining analgesia by administering them spinally (Bahari & Meftahi 2019; Yaksh 1985; Yaksh et al. 2017). α_2-Adrenoceptor agonists administered either by i.t. or epidural injections were shown to be potent analgesics both in animal models and clinically (Giovannoni et al. 2009). However, in the clinic, hypotension is one of the main side effects e.g. this has been encountered after clonidine therapy (Eisenach et al. 1996; Bajawa et al. 2012). Thus, to minimize the adverse effects of α_2-adrenoceptor agonists they are only used as adjuvants for spinal analgesia (Ganesh & Krishnamurthy 2018). The combination of an α_2-adrenoceptor agonist with a local anaesthetic allows for a reduction of the doses of both drugs and furthermore, causes less side effects in perioperative anaesthesia (Bajwa et al. 2012; De Kock et al. 2001; Dobrydnjov et al. 2002; Strebel et al. 2004; Zhang et al. 2016). Furthermore, α_2-adrenoceptor agonists potentiate opiate efficacy in anaesthesia and in acute and chronic pain management and providing an opiate-sparing effect (Tonner 2017). Table 4 describes the adverse events of spinally administered opioids, local anaesthetics and α_2-adrenoceptor agonists.

Table 4. Adverse effects of spinal administered opioids, local anaesthetics and α_2-adrenoceptor agonists.

Opioids	Local anaesthetics	α_2-adrenoceptor agonists
respiratory depression	hypotension	hypotension
constipation	muscle relaxation	bradycardia
urinary retention	urinary retention	increased sedation
nausea	toxicity	dryness of mouth
pruritus		urinary retention
hypotension		inhibition of gastrointestinal motility
tolerance and dependence		

Derived from Bajawa et al. 2012; Eisenach et al. 1996; Grangier et al. 2020; Hayhurst & Durieux 2016; Lirk et al. 2014; Liu et al. 2020; Russell 1982; Staikou & Praskeva 2014; Sun et al. 2017; Swain et al. 2017.

The N-type voltage dependent calcium channel blocker, ziconotide is FDA approved for i.t. use for treating chronic, severe pain. However, a narrow therapeutic index and reported side effects such as dizziness, nausea and somnolence have limited its use (Yaksh et al. 2017). The clinically approved GABA-B receptor agonist baclofen is

used to regulate motor tone in the case of spasticity where it also exerts nociceptive effects, however producing a prominent dose-dependent motor weakness (Yaksh et al. 2017).

Due to the risk for harmful adverse-effects caused by the current treatments, the management of postoperative pain is often unsatisfactory. There is a clear need for a safe and effective analgesic drug for the treatment of severe postoperative pain. Furthermore, technical improvements are still necessary to help reduce the complications and side effects associated with the procedure (Erdine & De Andrès 2006; Lamer et al. 2016). Research of agents and techniques for prolonging the action of a local anaesthetic without having deleterious actions, either primary systemic effects or neurotoxicity has been ongoing (Swain et al. 2017). According to experts in this field, there is a need for novel opioids with a reduced risk of tolerance and dependence (Berger & Whistler 2010) or more polar α_2-adrenoceptor agonists which could be advantageous for increasing the duration of analgesic action while decreasing systemic exposure after spinal delivery (Yaksh et al. 2017).

2.5 Fadolmidine as a spinal analgesic

Fadolmidine 3-(1H-Imidazol-4-ylmethyl)-indan-5-ol, HCl (previous name MPV-2426 hydrochloride), is a potent full agonist of all (A, B and C) the human α_2-adrenoceptor subtypes with EC_{50} values (nM) of 0.4, 4.9 and 0.5, respectively (Lehtimäki et al. 2008); it is being developed as a spinal analgesic (Lehtimäki et al. 1999) with a local mode of action (Lehtimäki et al. 2008; Pertovaara & Wei 2000). Moreover, fadolmidine is a full agonist at human α_{1A}- and α_{1B} -adrenoceptors but at approximately ten times higher concentrations than at α_{2A} and α_{2C}-adrenoceptor subtypes (Lehtimäki et al. 2008). In addition, the binding profile of fadolmidine demonstrates a binding affinity for the human serotonin 5-HT_3 receptors (IC_{50} 6.3 nM). The chemical structures of fadolmidine and another α_2-adrenoceptor agonist, dexmedetomidine as a comparison, are illustrated in Figure 4.

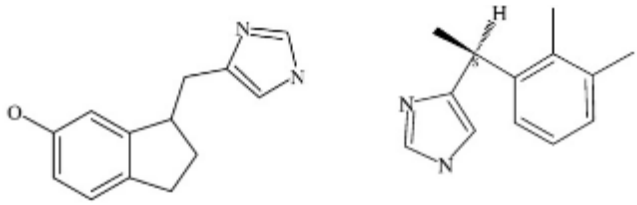

Figure 4. Chemical structures of fadolmidine and dexmedetomidine (derived from Lehtimäki et al. 2008).

Fadolmidine has been demonstrated to exert powerful spinal antinociceptive activity in several different animal models: in acute nociceptive tests (Lehtimäki et al. 1999; Onttonen et al. 2000; Talke et al. 2003; Xu et al. 2000a, 2000b) and in postoperative (Onttonen & Pertovaara 2000), neuropathic (Pertovaara & Wei 2000; Xu et al. 2000b) and inflammatory (Pertovaara & Kalmari 2003; Xu et al. 2000b) pain models in rats and in an acute nociceptive test in sheep (Eisenach et al. 1999). Fadolmidine has been reported to produce antinociception with greater potency when delivered i.t. than epidurally (Eisenach et al. 1999), whereas after i.v. (Eisenach et al. 1999; Wei et al. 2002), s.c. (Pertovaara & Wei 2000) and intraplantar (Ontonen & Pertovaara 2000; Wei et al. 2002) administration, fadolmidine had no or only weak antinociceptive properties. However, intraarticularly fadolmidine has been shown to produce a dose-dependent suppression of arthritic pain-associated vocalization in rats (Ansah & Pertovaara 2007).

The antinociception induced by i.t. fadolmidine is due to its action on spinal α_2-adrenoceptors (Onttonen et al. 2000; Pertovaara & Wei 2000; Xu et al. 2000a). Furthermore, fadolmidine at an antinociceptive i.t. dose, had no significant effects on the monosynaptic H-reflex which is a non-nociceptive motor response (Onttonen et al. 2000). I.t. fadolmidine attenuated ascending nociceptive signals (both neuropathic and non-neuropathic) to the RVM in neuropathic rats evoked both by thermal and mechanical stimuli (Pertovaara & Wei 2000). The role of the RVM in descending feedback control of spinal nociception is well-established (Basbaum & Fields 1984; Fields et al. 1991; Millan 2002). In addition, in the brain, the noradrenergic cell group A7, A6 (LC) and A5 in the pons with a high density of α_2-adrenoceptors (Scheinin et al. 1994) provide the noradrenergic innervation to the spinal cord (Clark & Proudfit 1993; Howorth et al. 2009). These cells groups are involved in descending modulation of pain through the action on spinal α_2-adrenoceptors (Pertovaara 2006). Fadolmidine has been reported to produce a tactile antihypersensitivity effect via noradrenergic cell group A7, but not in A6 (Wei & Pertovaara 2013).

Due to its pharmacokinetic properties, fadolmidine only poorly passed through the blood-brain-barrier after systemic (Eisenach et al. 1999; Lehtimäki et al. 1999) or i.t. administration (Onttonen et al. 2000; Pertovaara & Wei 2000), and thus did not gain access to the CNS (Xu et al. 2000a). Accordingly, the bioavailability of fadolmidine in a cerebrospinal fluid (CSF) was only 7% and 0.17% after epidual and i.v. administration, respectively, as measured in sheep (Eisenach et al. 1999). Furthermore, in sheep, i.t. fadolmidine, at an ED_{95} dose and even higher dose, produced a dose-independent reduction in thoracic and lumbar spinal cord blood flow (Eisenach et al. 1999).

For comparison, i.t. fadolmidine and dexmedetomidine, produced equipotent thermal antinociceptive (Onttonen et al. 2000; Xu et al. 2000b) and antihyperalgesic (Onttonen & Pertovaara 2000) effects. However, fadolmidine exerted a selective and

segmentally more restricted antinociceptive effect than dexmedetomidine following i.t. administration (Onttonen et al. 2000; Pertovaara & Wei 2000). According to the antinociceptive potency, the following rank order of α_2-adrenoceptor agonists was reported in gastrocnemius EMG model; dexmedetomidine > fadolmidine > tizanidine (Talke et al. 2003). In this model, however, higher analgesic doses were needed for antinociceptive effects than in a rat tail-flick test. In addition, at those antinociceptive doses, all of the compounds exerted almost equal sedative effects. Talke et al. (2003) speculated that in this model, the requirement dose for the antinociceptive effect may be increased significantly if there was the presence of discomfort in the animals. In addition, they considered that anxiety, pain, lack of interest in the environment, or motor dysfunction of animals could be attributed to the effect of sedation while the animals performed a locomotion test. In comparison to clonidine i.t., fadolmidine i.t. induced more potent analgesic effects in the rat tail-flick test (Lehtimäki et al. 1999). In a visceral pain model (with and without inflammation) fadolmidine and clonidine i.t. induced equipotent antinociceptive effects (Pertovaara & Kalmari 2003) whereas in a mechanical hyperalgesia model, the effects of clonidine were weaker (Onttonen & Pertovaara 2000).

Based on the different pharmacokinetic profiles of fadolmidine, dexmedetomidine and clonidine, after i.t. administration the sedative potency of fadolmidine has been shown to be weaker than similarly administered dexmedetomidine (Xu et al. 2000b) and clonidine (Lehtimäki et al. 1999). Furthermore, at antinociceptive i.t. doses, fadolmidine has been shown to be sedative only when injected directly into the LC in rats (Xu et al. 2000a). In addition, fadolmidine i.t. induced hypothermia only at clearly higher doses than clonidine i.t. in rats (Lehtimäki et al. 1999). Thus, in contrast to the highly lipophilic compound dexmedetomidine and to the lipophilic compound clonidine (Aantaa & Scheinin 1993) fadolmidine does not seem to redistribute so rapidly to the supraspinal space and spread into the CNS after i.t. and brainstem administration than dexmedetomidine (Xu et al. 2000a, 2000b) and clonidine (Lehtimäki et al. 1999). After systemic administration, fadolmidine displayed a considerably weaker CNS-mediated mydriatic response and sedative effect than dexmedetomidine (Lehtimäki et al. 2008).

Fadolmidine i.t. and epidurally at analgesic doses did not produce hemodynamic depression in sheep (Eisenach et al. 1999). Furthermore, Eisenach et al. (1999) predicted that the degree of hypotension from fadolmidine would be less than that from clonidine in humans. This suggests that as a species, sheep might be less sensitive to the hypotensive action of α_2-adrenergic agonists than humans (Eisenach & Dewan 1990). Furthermore, no (when administered i.t.) or small (when administered epidurally) decreases in HR were noted. Fadolmidine i.v. increased dose-dependently BP and inhibited electrically induced tachycardia in pithed rats

(Lehtimäki et al. 1999), as well as evoked increases in initial BP and decreases HR in anaesthetised rats (Lehtimäki et al. 2008).

In addition, various esters of fadolmidine with optimal lipophilicity properties were evaluated to decrease IOP after topical administration in rabbits. The pivaloyl ester of fadolmidine also decreased IOP in treated eyes of normotensive pigmented rabbits after unilateral ocular administration (Niemi et al. 2005).

The summary of pharmacological *in vivo* properties of fadolmidine and dexmedetomidine and clonidine as a comparison are presented in Table 5.

Table 5. The lowest dose (Dose) that produced a significant effect or potency (ED_{50}) of fadolmidine and the reference compounds dexmedetomidine (Dexmed) and clonidine on spinal (analgesic) and some supraspinal and peripheral *in vivo* pharmacological effects in mice, rats, rabbits and sheep.

PARAMETERS	FADOLMIDINE		DEXMED		CLONIDINE	
	Dose*)	ED_{50}	Dose*)	ED_{50}	Dose*)	ED_{50}
Noxious test: Thermal (rat, i.t.)						
- tail-flick	3[2], 2.5[3], 10(50)[4]	0.7[1]	3[2], 2.5[3], 12.5[4]	-	-	6.4[1]
- Heat / tail, withdrawal[5]	3	-	1	-	-	-
- Heat / paw withdrawal[5]	3	-	1	-	-	-
Thermal and mechanical (rat, s.c.)[8]						
- Tail-flick	>300	-	-	-	-	-
- paw pressure	>300	-	-	-	-	-
Mechanical / forelimb (sheep)[6]						
- i.t.	-	49	-	-	-	-
epidural	-	202	-	-	-	-
- i.v.	-	N/A	-	-	-	-
postoperative hyperalgesia, mechanical/hindpaw (rat)[7]						
- i.t.	3	-	3	-	>10	-
- intraplantar	N/A	-	-	-	-	-
Neuropathy (rat, i.t.)[8]						
- heat / tail, tail-flick	1	-	-	-	-	-
- heat / hindlimb, withdrawal	10	-	-	-	-	-
- noxious mechanical / paw pressure	1	-	-	-	-	-
- innocuous mechanical / paw pressure (tactile allodynia)	10	-	-	-	-	-
neuropathy (rat, i.v.)[9]						
- Chung model, tail-flick	300	-	30	-	-	-
arthritic pain (rat, intraarticular)[10]						
- vocalization score	30	-	-	-	100	-

PARAMETERS	FADOLMIDINE		DEXMED		CLONIDINE	
viskeral pain (rat, i.t.)[11)]						
- control	3	-	-	-	3	-
- a turpentine-induced inflammation	10	-	-	-	-	-
carrageenan / inflammation (rat, i.t.)[3)], paw flick	5	-	5	-	-	-
sedation (rat):						
- i.t.[1, 3, 4)]	5[3)], 10[4)]	30[1)]	2.5[3)],2.5[4)]	-	-	5[1)]
- Into the LC[**2)]	10	-	3	-	-	-
- s.c. (mice)[12)]	300	-	10	-	-	-
hypothermia (rat, i.t.)[1)]	10	-	-	-	4	-
mydriatic response (rat, i.v.)[12)]	100	-	3	-	-	-
BP[***)] and HR[****)]:						
sheep, i.t.						
- increase in BP	300	-	-	-	-	-
- bradycardia	N/A	-	-	-	-	-
sheep epidural						
- increase in BP	200	-	-	-	-	-
- bradycardia	1400	-	-	-	-	-
sheep i.v.						
- increase in BP	50	-	-	-	-	-
- bradycardia	300	-	-	-	-	-
pithed rats (i.v.)[1)]						
- increase in BP	0.23	-	0.84	-	-	-
- inhibition in electrically induced tachycardia	0.10	-	0.47	-	-	-
anaesthetized rats (i.v.)[1)]						
- increase in initial BP	3	-	N/A	-	-	-
- decrease in BP	10	-	3	-	-	-
- decrease in HR	1	-	1	-	-	-
intraocular pressure (rabbit, intraocular)[13)]	2.5	-		-	-	-

*) Dose: i.t., intraplantar, intraarticular and intraocular; µg/animal, and i.v. and s.c.; µg/kg, **) LC = Locus coeruleus, ***) BP = Blood pressure, ****) HR = Heart rate, N/A = not applicable. 1) Lehtimäki et al.1999, 2) Xu et al. 2000a, 3) Xu et al. 2000b, 4) Talke et al. 2003, 5) Onttonen et al. 2000, 6) Eisenach et al. 1999, 7) Onttonen & Pertovaara 2000, 8) Pertovaara & Wei 2000, 9) Wei et al. 2002, 10) Ansah & Pertovaara 2007, 11) Pertovaara & Kalmari 2003, 12) Lehtimäki et al. 2008, 13) Niemi et al. 2005.

3 Aims

Two α_2-adrenoceptor agonists i.e. clonidine and dexmedetomidine, are extensively used in both anaesthesia and intensive care medicine. However, while clonidine is reasonably well tolerated, it may produce pronounced side effects such as hypotension, bradycardia and sedation at analgesic doses, which may limit its usefulness under certain conditions.

Fadolmidine is a novel full α_2-adrenoceptor agonist with a local mode of action and it has been specially developed for use in spinal analgesia. Fadolmidine has been shown to possess analgesic properties as evaluated in different nonclinical animal pain models after its spinal administration. In contrast, the systemic effects of fadolmidine after spinal administration have not been studied previously thoroughly.

The studies in this thesis have been conducted as part of the nonclinical research program aiming to develop fadolmidine for spinal analgesia.

These specific aims were:

- To assess the analgesic effect of fadolmidine after i.t. administration in a rat tail-flick test and after i.t. and epidural administration in a dog skin twitch test.
- To evaluate the effects of fadolmidine on sedation, HR, BP and BT after i.t. administration in rats and i.t. and epidural administration in dogs.
- To examine the effects of fadolmidine on BP, HR, kidney function and urodynamics in rats.
- To study the effects of i.t. fadolmidine as an adjuvant to the local anesthetic bupivacaine on sensory-motor in rats and dogs, and to determine the distribution of ^{3}H-fadolmidine in plasma and spinal cord after i.v. and i.t. administration in rats.

4 Materials and Methods

4.1 General experimental design

In study I, the peripheral and central pharmacological effects of fadolmidine were compared to the specific α_2-adrenoceptor agonists, dexmedetomidine and clonidine all administered by i.t. injection. The profile of fadolmidine's adverse effects on cardiovascular variables, kidney function and urodynamic parameters was carried out in *in vitro* and *in vivo* in study II. In study III, the effects of fadolmidine on analgesia, HR, BP, BT were evaluated after i.t. and epidural administration in dogs and the effects were compared to the similar analgesic properties of a well-known α_2-adrenoceptor agonist i.e. clonidine. In study IV, the effects of i.t. fadolmidine as an adjuvant to a local anaesthetic, bupivacaine, on sensory-motor block were evaluated in rats and dogs.

4.2 Animals used in *ex vivo* and *in vivo* models

Animal experiments in the studies (I-II, IV) in Orion Corporation followed the European Communities Council Directive 86/609/EEC and were approved by the local Laboratory Animal Welfare Committee (610/712-86), Orion Corporation, Finland. Study III was performed in the University of California, San Diego (UCSD), California, USA, and it adhered to the rules of Association for Laboratory Animal Care (AALAC) and was approved by the Institutional Animal Care and Use Committee of the University of California, San Diego. The animals used are summarised in Table 6.

Table 6. Animals used in I-IV. All used animals were males.

STUDY	SPECIES	STRAIN	SUPPLIER
I	Rat	Sprague Dawley	B&K, Sweden
II	Rat Guinea pig	Sprague Dawley Dunklin-Hartley	B&K, Sweden B&K, Sweden
III	Dog	Beagle	Marshall Farms USA, Inc.
IV	Rat Dog	Sprague Dawley Beagle	B&K, Sweden Harlan-Winkelman Hundezucht, Germany

The rats were housed in groups of five per cage with aspen chips as housing material under controlled conditions of light (12 h light / 12 h dark), temperature (22 ± 2 °C) and humidity (50 ± 10%) (I, II, IV) in the animal facility of Orion Corporation, Orion Pharma. Standard rat food and tap water were available *ad libitum*.

The guinea pigs (II) were housed in groups of ten per cage with aspen chips as housing material under controlled conditions of light (12 h light / 12 h dark), temperature (22 ± 2 °C) and humidity (50 ± 10%) in the animal facility of Orion Corporation, Orion Pharma. Standard guinea pig food and tap water were available *ad libitum*.

The dogs were housed individually in runs with wood shavings, with automatic control of light (12 h light / 12 h dark) and temperature (between 18 to 27 ºC) and with *ad libitum* access to fresh food and water in the animal facility of the University of California, San Diego (III). In study IV, dogs were housed in groups of 2–3; after surgery they were housed individually with automatic control of light (12 h light / 12 h dark), temperature (18 ± 4 °C) and humidity (50 ± 20%) with a standard certified pelleted dog feed and tap water was available *ad libitum* in the animal facility of Orion Corporation, Orion Pharma.

4.3 5-HT_3 receptor activity measurements (II)

4.3.1 *In vitro* patch clamp measurments

Stably transfected 5-HT_{3A} and 5-HT_{3AB} cell lines were created in Molecular Biology of Orion Pharma in Viikki campus. The cells were incubated at 37 °C for 1–3 days in an 5% CO_2 and 95% O_2 atmosphere in Dulbeccos modified Eagle's medium with 10% fetal calf serum (heat inactivated), 10 I.U. penicillin and 10 µg streptomycin, 10 mM HEPES, 0.2 mg/ml geneticin. For the 5-HT_{3AB} cells medium

0.2 mg/ml hygromycin was added. The medium of cells was changed after 2–3 days. Approxmately once a week the cells were replaced by using 0.25% trypsin solution. The adhering cells were tested at a room temperature by using the tight-seal whole cell voltage-clamp configuration of the patch clamp technique (Hamill et al. 1981) with an Axopatch 200B amplifier (Axon Instruments, USA). The coverslip with cells was placed into an extracellular solution (in mM): 150 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 2.5 glucose and 10 HEPES (pH 7.4) with osmolarity of 305 mOsm (OM-6020 osmometer; DIC Kyoto Daiihi Kagagu Co. Ltd, Japan). The cells were perfused with the extracellular solution at 2 ml/min. The intracellular solution consisted of (in mM): 120 KCl, 5 BABTA (tetrapotassium salt), 0.5 $CaCl_2$, 1 $MgCl_2$, 2 adenosine triphosphate (disodium salt), 10 HEPES (pH 7.2) with osmolarity of 290 mOsm and calculated free Ca^{2+} concentration of 23 nM (with BAD4 program). The borosilicate glass pipettes (Clark Electromedical, England), a resistance between 1 and 1.5 MOhm, were pulled with a P-2000 puller (Sutter Instruments, Co. USA). The cellular capacitance was compensated by the circuitry in the amplifier. In all recordings, cell voltage was clamped to -70 mV except during voltage ramps and were digitized with Digidata 1200 interface (Axon Instruments, USA) at the sampling rate of 2 kHz. The recording was performed with Clampex 8.0 and analysed with Clampfit 8.0 software (Axon Instruments, USA). The dose response results were analysed and fitted with the free Hill equation in SigmaPlot 4.01.

All drugs were dissolved in extracellular solution and were given with RSC-200 rapid solution changer (Bio-Logic, France). The test items were perfused 20 s with increasing concentrations with 90–100 s washout in between the tests. The agonist effects of fadolmidine and a reference compound 3 μM serotonin (for 5-HT_{3A} cells) or 6 μM serotonin (for 5-HT_{3AB} cells) was first applied for 20 s and after a washout of 90-100 s increasing concentrations of fadolmidine were applied.

4.3.2 *Ex vivo* guinea pig ileum preparation

Guinea pigs (Dunklin-Hartley) were used for the experiments. Segments of approximately 20 cm of ileum valve were divided into 8–12 preparations (2 cm long each) in a physiological solution (mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$ and 6 glucose for electrical and chemical stimulation, respectively. Four ileal segments were studied in parallel by using a Schuler organ bath with tissue chamber volume of 10 ml (Hugo Sachs Elektronik, Germany) at 37 °C temperature (Hetofrig, Heto, Danmark). Isometric contractions were measured by Grass force-displacement transducer (model FT03; Grass Instruments, Quincy, USA) with Grass D.C. Low-Level Pre-Amplifiers and driver amplifiers (type 7DAG). The amplified signals were plotted by using a Grass ink writer oscillograph

type 7WU 16F. Preparations were allowed to stabilize under the resting tension of 1.0 g for 30 min and were washed three times by overflow.

Two boluses of 1 μM acetylcholine were given at six minute intervals to determine the individual maximal contractile capability of the segments. The test substances were given with 15 min intervals using half-logarithmic increments and two minute contact times. Between dosing intervals the segments were washed. Before agonist given atipamezole and prazosin (16–17 min before) and in some experiments ondansetron (15 min before) were added into the incubation medium to prevent possible effects on presynaptic α_2-adrenergic receptors and postsynaptic α_1-adrenergic receptors, and effects on 5-HT_3, respectively. Agonist mediated contractions were evaluated by tetrodotoxin, a sodium channel blocker. Isometric contractions of the ileal segments were measured as a response to 5-HT_3 agonists.

4.4 *In vivo* models

4.4.1 I.t. catheterization (I–IV)

4.4.1.1 Rats (I, II, IV)

I.t. catheters were implanted under midazolam (5 mg/kg, Dormicum® 5 mg/ml) and fentanyl (0.25 mg/kg)–fluanisone (8 mg/kg) (Hypnorm®) s.c. combination anaesthesia according to the method of Yaksh and Rudy (1976) with minor modifications. The catheter (PE10, Intramedic®) was introduced into the lumbar enlargement of the spinal cavity via the atlanto-occipital membrane below the skull. The location of the catheter tip was confirmed by lidocaine 0.5 mg (Lidocain pond® 50 mg/ml) i.t. approximately on the third day after catheterization. Transient paralysis of both hind limbs was the indication of successful catheterization. After at least a one-week recovery period, animals with visually observed normal neurological function were selected for the experiment.

4.4.1.2 Dogs (III, IV)

I.t. catheterization was performed according to the method of Atchison et al. (1986) with minor modifications. In study III, the dogs received atropine (0.04 mg/kg, intramuscular (i.m.), atropine® 0.4 mg/ml). Anaesthesia was induced with xylazine (1.5 mg/kg, i.m., Rompun® 20 mg/ml) and maintained under spontaneous ventilation with 1.0 - 3.0% isoflurane and 60% N_2O/40% O_2 (approximate values). In study IV, anaesthesia was induced with medetomidine (40 μg/kg, i.m., Domitor® 1 mg/ml) and maintained with a propofol i.v. bolus injection (6.5 mg/kg, Diprivan® 10 mg/ml) and

infusion (0.9 ml/kg/h, Diprivan® 10 mg/ml). The surgical areas in the head and neck were shaved and surgically prepared. Using a sterile technique, the cisterna magna between the skull base and C1 was exposed and an incision was made into the dura. The PE10 (Intramedic®) i.t. catheter (the dead space volume of 0.2–0.3 ml) (III) or a clear nylon epidural 19G catheter (Portex®, Portex limited) with the connector (the dead space volume of approx. 0.4 ml) (IV) was inserted and passed caudally to the $L_{2\text{-}3}$ level. The catheter's placement and patency were verified post-operatively by the appearance of clear flowing CSF from the catheter. The animals were allowed to recover with appropriate postoperative care.

The location of the catheter tip was confirmed by the distribution of dye delivered before exposure (III) or by administering 6 mg lidocaine (Lidocain® 20 mg/ml) i.t. approximately on the third day after catheterization (IV). A transient paralysis of both hind limbs was the indication of successful catheterization. All animals showed an uneventful postoperative recovery with no sensory or motor deficits or evidence of discomfort.

For infusion (III), a nylon vest with an infusion pump was placed on the habituated animal for 48 h prior to surgery. Following surgical recovery, all animals having been implanted with PE10 i.t. catheter started to receive a chronic infusion of approximately 100 µl/h of physiological saline for maintaining catheter patency. The infusion was stopped at least 30 min prior to dosing with the test compound. In study IV, the catheter was flushed once a week with physiological saline (0.5 ml/dog) to maintain catheter patency.

4.4.2 I.c.v. catheterization (I)

For i.c.v. catheterization, rats were anaesthesised as in the i.t. catheterization (see 4.4.1.1). An internal cannula 28G (Plastics one® Inc Roanoke, VA 24022) was inserted stereotactically into the right lateral ventricle of the brain (coordinates: bregma A: 0.8, L: 1.4, V: 3.7) by using the method of Kehne et al. (1981). After the surgery, the animals were allowed to recover for at least 5 weeks. The location of the cannulae tip was verified by i.c.v. injection of angiotensin II (200–300 ng per 2–3 µl; Angiotensin II TFA salt, RBI) 2 days before the hemodynamic measurements. Only animals exhibiting a drinking response and normal CNS function in rotarod and cage track tests were used for the experiment.

4.4.3 Epidural catheterization (III)

The surgical preparation of the dogs for epidural catheterization was performed according to the method described originally by Durant and Yaksh (1986). The dogs were anaesthetised as in the i.t. catheterization (see 4.4.1.2) in study III. The lower

back of the dog was surgically prepared. A small skin incision over the L_7/S_1 interspace was made and a PE50 (Intramedic®) catheter was passed into the epidural space at approximately the L_{1-2} level via an 18G Tuohy needle. The catheter was tunneled s.c. and connected with a s.c. installed injection port (vascular-access-port model SLA, Access Technologies) with a dead space volume of 0.1 ml. The animals were allowed to recover with appropriate postoperative care. All animals showed an uneventful postoperative recovery with no sensory or motor deficits or evidence of discomfort.

4.4.4 Implantation of radio-telemetry transmitter (II, IV)

4.4.4.1 Rats (II)

The rats were anaesthetised as in the i.t. catheterization (see 4.4.1.1). The rats were operated and the functioning of the telemetry system was confirmed according to instructions provided Data Sciences (Data Sciences, St. Paul, MN). The surgical area was shaved and surgically prepared. A midline abdominal incision was made and the descending aorta was carefully isolated. The catheter tip of the telemetry transmitter (model TL11M2-C50-PTX) was inserted upwards just above the iliac bifurcation of the descending aorta and fixed with cyanoacrylate adhesive glue and cellulose fibre patch (5 x 5 mm). The body of the telemetry transmitter was inserted in the peritoneal cavity, sutured to the inside of the muscle wall and the incision was closed. The rats were housed individually (in polypropylene cages), and allowed to recover for at least one week before i.t. catheterization. After recovery, animals with visually observed normal neurological function were used in the experiments.

4.4.4.2 Dogs (IV)

I.t. catheterization (see 4.4.1.2) and implantation of radio-telemetry transmitter were operated simultaneously under sterile conditions. The implantation of the radio-telemetry transmitter was undertaken and the functioning of the telemetry system was confirmed according to instructions provided by Data Sciences (Data Sciences, St. Paul, MN). The surgical areas were shaved and surgically prepared. The device body of the sterile telemetry transmitter (model TL10M2-D70-PCT) was tunnelled into the pouch under the skin. An incision in the inguinal area over the femoral artery (deep femoral or muscular branch) was made and the tip of the catheter was passed cranially into the artery. The vessel was ligated and at the end, the incisions were closed. The animals were allowed to recover with appropriate postoperative care. After recovery for at least one week, those animals with visually observed normal neurological function were used in the experiments.

4.4.4.3 I.t. and epidural dosing (I–IV)

I.t. administration in rats: the test drugs were administered i.t. by a Hamilton syringe in a volume of 10 µl. The injection of each drug was followed by an additional physiological saline injection of 10 µl or 15 µl (in cardiovascular experiments (I)) or sterile water injection of 15 µl in cardiovascular experiments (II) to flush the drug remaining in the catheter lumen (I, II, IV).

I.t. administration in dogs: the test drugs were administered as a bolus i.t. injection by a syringe in a volume of 1.0 ml (III) or 0.5 ml (IV). The injection of each drug was followed by an additional physiological saline injection approximately of 0.5 ml (III, IV).

I.t. infusion in dogs: the test drugs were administered as a bolus injection by a syringe in a volume of 1.0 ml followed by a 24-h continuous i.t. infusion with an infusion pump (Panomat C-10, Distronic Medical Syatems) in a volume of 100 µl/h (III).

I.c.v. administration in rats: the test drugs were administered i.c.v. by Hamilton syringe in a volume of 2 µl (I).

Epidural administration in dogs: the test drugs were administered as a bolus epidural injection by a syringe in a volume of 1.0 ml. The injection of each drug was followed by an additional physiological saline injection of 0.1 ml to flush the drug remaining in the catheter lumen (III).

4.5 Nociceptive methods

4.5.1 The rat tail-flick test (I, IV)

The rat tail-flick test (Asano et al. 2000) was performed with an analgesia meter (Ugo Basile, model DS-20, Italy) consisting of an infrared heating spot and an automatic tail-flick detector. The rats were habituated to the plastic immobilization chamber three times on one day (I) or twice a day (2 x 2 min) (IV) before the start of the experiment. The infrared beam (a noxious heat stimuli) was focussed on the tail of an immobilized rat. After a sensation of the pain, the rat withdrew the tail from the heat beam and the time (s) to respond was recorded. Failure to respond in 5 s was the maximum (cut-off time) to prevent tissue damage. The predrug latency was recorded and a bolus injection of test compound was administered i.t. (10 µl). Latencies were recorded at 30 min after the test compound administration (I) or at the observation time points (IV). Both predrug and postdrug latencies were determined three times to diminish the effects of a possible unspecific reaction, and the mean value was used (Table 7).

4.5.2 The dog skin twitch test (III, IV)

The thermally evoked skin twitch response (Yaksh et al. 1994) was measured using a probe with approximately 1 cm^2 surface area maintained at approximately 62.5 ± 0.5 °C. The probe was applied sequentially to the shaven thoracolumbar areas of the animal's back. When a brisk contraction of the local musculature within 1–3 s of probe placement was detected, the probe was removed and the latency recorded. After a failure to respond within 6 s (cut-off time, to prevent tissue damage), the probe was removed and a value was assigned as the latency. The nociceptive response was presented as the mean of the two latencies. The predrug latency was recorded and a bolus injection of test compound was administered i.t. (1.0 ml (III) or 0.5 ml (IV)) or epidurally (1.0 ml (III)) or during 24-h continuous i.t. infusion (1.0 ml followed by 100 µl/h (III)) and the latencies were recorded at the observation time points. During the measurement, the dogs were standing on the operation table (Table 7).

4.5.3 Motor block (IV)

Simultaneously with the analgesia measurement, the onset and the duration of motor block were evaluated after administration of a local anaesthetic, bupivacaine, by defining the time between completion of the i.t. injection and the time when the dog's hind limbs were unable to support its weight and when the animal was again able to support its own weight, respectively (Table 7).

Table 7. Nociceptive and motor block observations in the studies I, III and IV.

STUDY	SPECIES (STRAIN, WEIGHT)	MODEL, DOSING ROUTE AND N*	TEST COMPOUNDS AND DOSES	OBSERVATION TIME POINT
I	Rat (Sprague Dawley, 210–480 g)	Tail-flick test, i.t., N=8–13, cross-over manner within drugs	Saline 10 µl per rat Fadolmidine 0.1, 0.3, 1, 3 and 10 µg per rat Dexmedetomidine 0.1, 0.3, 1, 3 and 10 µg per rat Clonidine 0.1, 0.3, 1, 3, 10 and 30 µg per rat	The dose-response effects at 30 min after drug injection
IV	Rat (Sprague Dawley, 293–443 g)	Tail-flick test, i.t., N=7–16	Bupivacaine 0 (saline 10 µl), 1, 3, 10, 30, 50, 100 and 300 µg per rat	The time points: 0, 10, 20, 30, 45, 60, 90 and 120 min after dosing
			Fadolmidine 0 (saline 10 µl), 0.3, 1, 3 and 10 µg per rat Bupivacaine 10 µg and fadolmidine 0 (saline 10 µl), 0.3, 1, 3 and 10 µg per rat	The time points: 0, 0.5, 1, 2, 4 and 6 h after dosing
			Fadolmidine (µg) + bupivacaine (µg): 2 x ED_{50} (2.4 + 175), 1 x ED_{50} (1.2 + 87), ½ x ED_{50} (0.6 + 45), ¼ x ED_{50} (0.3 + 22), 1/6 x ED_{50} (0.2 + 15), 1/8 x ED_{50} (0.15 + 11), 1/10 x ED_{50} (0.12 + 9), 1/12 x ED_{50} (0.1 + 7), 1/16 x ED_{50} (0.08 + 5.4)	The time points for isobolographic analysis: 0, 10, 20, 30, 45, 60, 120, 180, 240, 300, 360 and 420 min after dosing
III	Dog (Marshall farms, 12–14 kg)	Skin twitch test, i.t., N=5, cross-over manner and epidurally, N=5, cross-over manner	Fadolmidine 0 (saline 1.0 ml), 30, 100 and 300 µg per dog Clonidine 0 (saline 1.0 ml), 30, 100 and 300 µg per dog	The time points: 0, 0.33, 1, 2, 4 and 8 h post injection
		Skin twitch test, 24-h i.t. infusion, N=3–5, cross-over manner	Saline 500 µl + 100 µl/h per dog Fadolmidine 100 µg + 50 µg/h per dog Clonidine 100 µg + 100 µg/h per dog	The time points: 0, 1, 2, 4, 8 and 24 h after initiation of infusion
IV	Dog (Harlan-Winkelman Hundezucht, 8–11 kg)	Skin twitch test and motor block, i.t., N=5, cross-over manner	Bupivacaine 3 mg per dog Fadolmidine 60 µg per dog Bupivacaine 3 mg and fadolmidine 60 µg per dog	Skin twitch test. The time points: 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h after dosing Motor block: the onset and the duration of motor block

*N= number of animals per dose, i.t. = intrathecal.

4.6 Sedation, BT and respiratory rate (I–IV)

4.6.1 Motor coordination (I, IV)

The motor coordination (measuring moderate sedation) was evaluated on a Rota-rod treadmill (Ugo Basile, Italy) consisting of four drums (diameter of 70 mm, 4 r/min, separated by five flanges) in trained and habituated rats. After training, only the rats that were able to stay for at least 2 min on the rotating rod were selected for the test. The rats were administered test compound i.t. (10 µl) and placed on the rotarod to measure their ability to remain on the rotating rod (measuring time 120 s).

In study I, 15 min after test compound injection, the rotarod performance and then the motor activity test (for 10 min) and BT measurement were carried out at the observation time points. In study IV, after test compound injection, the motor score observation and then the rotarod performance and BT measurement were carried out at the observation time points (Table 8).

4.6.2 Motor activity (I)

A spontaneous locomotor activity test is mainly an objective measure of (slight) sedation. The motor activity of a single rat was recorded in a polypropylene animal cage (38 x 22 x 15 cm) surrounded by photobeam frames by Photobeam Activity System (PAS, Cage Rack®, San Diego instruments, USA). After test compound administration, the animal was placed into the cage for measuring its spontaneous locomotor activity for 10 min. Each breaking of the photobeams of the frame was counted as a signal of motor activity (Table 8).

4.6.3 Open field (II)

Exploratory activity (measuring mild sedation) was investigated in trained (4 x 3 min/one training session) rats in the open field test by using a non-reflecting black plastic open arena (70 x 70 cm) surrounded by walls (38 cm high) in a calm and noiseless room. Animals were allowed to habituate to the surroundings for at least 1 h before the start of the experiment. Ambulation of an animal was monitored by a video camera (mounted 220 cm above the arena) linked to a computer through an image analyser (Poly-Track video tracking system, San Diego Instruments, USA). The arena was divided into nine equal squares (23 x 23 cm) by the computer software (Chromotrack, Prototype Systems Ltd., USA) and the amount of ambulation was counted manually as the number of squares visited. Rats were habituated for handling (immobilisator) for two days before testing and also to the open field system (1 x 3 min) twice daily. At the beginning of the measurement, the rat was

placed gently in the centre of the open-field and allowed to explore freely. On the testing day, a compound was administered i.t. (10 µl) and spontaneous motor activity (2 x 3 min) was measured in the open field setup along with the MAP, HR (see 4.7.4.1) and BT (see 4.6.7) measurements at the observation time points. After the measurements, the rat was returned to its home cage (Table 8).

4.6.4 Motor score (IV)

The motor score was evaluated by a slightly modified method of Penning and Yaksh (1992) in rats. The following grading bilaterally was used; 1) sedation (scored 0–2), 2) the placing/stepping reflex of the left (scored 0–2) and right (scored 0–2) hind legs, 3) the muscle tone of the right (scored 0–2) and left (scored 0-2) hind legs by stretching the legs, and 4) the righting reflex (scored 0–2). The scores were 0 = absent, 1 = impaired and 2 = normal, the normal baseline score being thus 12. The muscle tone of the fore-limbs (right (scored 0-2) and left (scored 0–2)) was also measured. The rats with pretest scores of 16 were included in the study. Each test compound was given i.t. (10 µl). The motor scores were carried out at the observation time points. The measurements were performed in a blinded manner. After the motor score observation, the rotarod performance and BT measurement were carried out (Table 8).

4.6.5 Behavioural index (III)

The specific behavioural indices assessed were state of arousal (postural/behavioural indication of alertness), muscle tone (the state of muscle vigor or tension) and motor coordination (complimentary / balance muscle activity) in dogs. Scores of 0-3 were assigned for each parameter, with 0 being normal function and 3 being severely abnormal, as described in detail elsewhere (Yaksh et al. 1997). After test compound dosing (i.t. or epidural) behavioural index was assessed with the nociceptive measurement (see 4.5.2) and with the other physiological observations (see, 4.6.7, 4.6.8 and 4.7.3) at the predetermined time points (Table 8).

4.6.6 Sedation score (IV)

Sedation was scored (0 - 4) according to the following criteria in dogs: 0 = normal alertness and responsiveness to the investigators, 1 = quiet response, eyes closed, but readily alerted and retaining head tone continuously, 2 = quiet, drowsing, eyes transiently closed, minimal neck tone, but arousable, 3 = significant depression, eyes remain shut, loss of neck tone, difficult to arouse, 4 = not arousable, total loss of neck tone, no overall response to strong stimuli applied to paws. After test compound

dosing, the sedation score was assessed with the nociceptive measurement (see 4.5.2) and with the other physiological observation (see 4.5.3, 4.6.4, 4.6.7, 4.6.8 and 4.7.4.2.) at time points. During the measurement, the dogs were standing on the operation table (Table 8).

4.6.7 Body temperature (I–IV)

BT from rats were measured from the rectum by a thermometer inserted to a depth of 2.5 cm (I), or by a digital thermometer (Ellab, Denmark) to the depth of 2 cm (IV), or by the telemetry system (Data Science, St. Paul, MN) from the peritoneal cavity (II). In dogs, BT was measured from the rectum by a thermometer inserted to a depth of 3-4 cm (III, IV). During measurements, the dogs were standing on the operation table (III, IV). A baseline value of BT was measured and a test compound was given i.t. (in rats) or i.t. and epidurally (in dogs) and BT was measured at the observation time points. BT was measured after motor coordination (Rota-rod treadmill) and motor activity (Cage Rack®) measurements in study I, and during home cage and open field measurements in study II, and with the other physiological observations in studies III and IV (Table 8).

4.6.8 Respiratory rate (III, IV)

The respiratory rates were measured by observation of chest expansion and contraction in dogs. During measurements, the dogs were standing on the operation table. After test compound dosing (i.t. and epidural), the respiratory rate was measured simultaneously with the nociceptive measurement (see 4.5.2) and with the other physiological observations (see 4.6.5, 4.6.7 and 4.7.3 (III), and 4.5.3, 4.6.4, 4.6.6, 4.6.7 and 4.7.4.2 (IV)) at time points (Table 8).

Table 8. The effects on sedation, BT and respiratory rate in studies I–IV.

STUDY	SPECIES (STRAIN AND WEIGHT)	MODEL, DOSING ROUTE AND N*	TEST COMPOUNDS AND DOSES	OBSERVATION TIME POINT
I	Rat (Sprague Dawley, 390–400 g)	Rota-rod treadmill, Cage Rack® and rectal BT, i.t., N=8/group, cross-over manner within drugs	Fadolmidine 0 (water 10 µl), 1, 3, 10 and 30 µg per rat Dexmedetomidine 0 (water 10 µl), 0.3, 1, 3 and 10 µg per rat Clonidine 0 (water 10 µl), 0.3, 1, 3 and 10 µg per rat	Rota-rod: the dose-response effects measured for 2 min 15 min after drug dosing Cage Rack: the dose-response effects measured for 10 min approx. 17 min after drug dosing Rectal BT: The dose-response effects measured at 27 min after drug dosing
IV	Rat (Sprague Dawley, 193–407 g)	Motor score, Rota-rod treadmill and rectal BT, i.t., N=8-16/dose	Bupivacaine 0 (saline 10 µl), 1, 3, 10, 30, 50, 100 and 300 µg per rat	The time points: 10, 20, 30, 45 and 60 min after dosing
			Fadolmidine 0 (saline 10 µl), 0.3, 1, 3 and 10 µg per rat	The time points: 0.5, 1, 2, 4 and 7 h after dosing
			Bupivacaine 300 µg and fadolmidine 0 (saline 10 µl), 0.3, 1, 3 and 10 µg per rat	The time points: 0.5, 1, 2, 4 and 7 h after dosing
II	Rat (Sprague Dawley, 300–500 g)	Open field and core BT, i.t., N=4/group	Fadolmidine 0 (water 10 µl), 1, 3, 10 and 30 µg per rat Clonidine 0 (water 10 µl), 10, 30 and 100 µg per rat	Open field 1 and 2 measured (for 2 x 3 min) at 19 and 59 min, respectively after dosing BT: Home cage 1, 2 and 3 at 15, 55 and 95 min, respectively after dosing
III	Dog (the same animals as in the nociceptive measurements in Table 7)	Behavioural indices, rectal BT and respiratory rate, i.t. and epidural bolus injection (see Table 7)	See Table 7: Fadolmidine bolus i.t. and epidural injection Clonidine bolus i.t. and epidural injection	The time points: 0, 0.33, 1, 2, 4 and 8 h after dosing
		Behavioural indices, rectal BT and respiratory rate, 24-h i.t. infusion (see Table 7)	See Table 7: Saline 24-h continous i.t. infusion Fadolmidine 24-h continous i.t. infusion Clonidine 24-h continous i.t. infusion	The time points: 0, 1, 2, 4, 8 and 24 h after initiation of infusion
IV	Dog (the same animals as in the nociceptive measurements in Table 7)	Sedation score, BT and respiratory rate, i.t. bolus injection (see Table 7)	See Table 7: Bupivacaine i.t. Fadolmidine i.t. Bupivacaine and fadolmidine i.t.	Sedation score and respiratory rate. The time points: 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h after dosing BT. The time points: 0, 1, 2, 3, 4 and 6 h after dosing

*N = number of animals, i.t. = intrathecal, BT = body temperature.

4.7 Cardiovascular measurements (I–IV)

4.7.1 Anaesthetised rats (I)

The rats were anaesthetised with sodium pentobarbital (75 mg/kg i.p., Mebunat® 60 mg/ml). The trachea of rat was cannulated to allow the animal to breathe spontaneously. The left femoral artery was cannulated (PE60, Intramedic®) for BP and HR continuous measurement via Micro MP-15 (Hugo Sachs Electronik KG, Germany) transducer connected to Grass Model 7D Polygrap (Grass Instrument Co., USA). HR was obtained from the BP pulse signal. The rectal BT was kept constant at 37 ± 0.5 °C by a lamp. After a stabilization period of 10–20 min, only rats with the MAP of 85 mmHg (in i.c.v. study) or 90 mmHg (in i.t. study) or higher were used for the test. One bolus i.t. (10 µl) or i.c.v. (2 µl) injection of physiological saline or test compound was given and the effects were measured for 60 min (Table 9).

4.7.2 Bezold-Jarisch reflex in rats (II)

The rats were anaesthetised with sodium pentobarbital (1.25 mg/kg, i.v., Mebunat® 60 mg/ml) and BT was kept constant at 37˚C by water-filled heating block. The left femoral artery was cannulated with a polyethylene tube and connected to a MP-15 pressure transducer (Micron Instruments, USA) for a MAP measurement. The transducer was connected to two channel bridge amplifier (type 301, Hugo Sachs Electronik, Germany) and voltage signals were further delivered through an A/D-converter to the computer by using based data acquisition system MP100WS (Biopac Systems Inc., USA). Right femoral vein was cannulated for the i.v. dosing. Three boluses of 0.2 ml were given at 5 minutes intervals. At first, saline injection was given to determine volume effects on HR and/or MAP and then ondansentron (5-HT_3 receptor antagonist), prazosin (α_1-adrenoceptor antagonist), atipamezole (α_2-adrenoceptor antagonist) or both prazosin and atipamezole. In the end, a bolus injection of fadolmidine, 2-methylserotonin (5-HT_3 agonist) or dexmedetomidine was given. MAP and HR were continuously monitored and registered (Table 9).

4.7.3 Non-invasive method in dogs (III)

MABP and HR measurements were made using a tail cuff manometer (Dinamap 8100, Criticon, USA) in conscious dogs. Baseline values of MABP and HR were measured and test compounds or physiological saline were given i.t. or epidurally. MABP and HR values were measured simultaneously with the nociceptive measurement (see 4.5.2) and with the other physiological observations (see 4.6.5, 4.6.7 and 4.6.8) at various time points. (Table 9).

4.7.4 Hemodynamic and body temperature measurements with the telemetry system (II, IV)

4.7.4.1 In rats (II)

MAP and HR from the BP pulse signal and BT were recorded and analyzed by a Dataquest IV system (Data Sciences, St. Paul, MN) in conscious freely moving rats. The telemetry system consisted of the transmitters (model TL11M2-C50-PTX) receivers (model RLA2000), consolidation matrix (BCM100) and the Dataquest LabPRO™ software. Baseline values of MAP and HR, and BT were recorded at the sampling rates of 500 Hz and 250 Hz, respectively. A test compound was given i.t. and the 10 s signals were recorded every 5 min during the whole study (120 min), both in the home cage and synchronously with the motor activity measurements in the open field setup (see 4.6.3) at time points. After the open field measurements, the rat was returned to the home cage. Telemetry measurements at time points 20 and 25 min and 60 and 65 min after the injection were taken as the values for open field 1 and open field 2, respectively. The telemetry values at 15, 55 and 95 min after the injection were taken as the value for the home cage 1, 2 and 3, respectively (Table 9).

4.7.4.2 In dogs (IV)

MAP and HR from the BP pulse signals were recorded and analysed using the Dataquest IV system (Data Sciences, St. Paul, MN) in conscious dogs. The telemetry system consisted of the transmitters (model TL10M2-D70-PCT), receivers (model RLA2000), a consolidation matrix (model BCM100) and software Dataquest LabPRO (version 3.11). BP recordings (20 s) were taken every 5 min at the sampling rate of 500 Hz during the experiment starting 20 min before drug administration, and continuing 10 min before each measurement point. During the measurement, the dogs were standing on the operation table. After test compound dosing, MAP and HR measurements were made simultaneously with the nociceptive measurements (see 4.5.2) and with the other physiological observations (see 4.5.3, 4.6.6, 4.6.7 and 4.6.8) at predetermined time points (Table 9).

Table 9. Cardiovascular measurements in studies I–IV.

STUDY	SPECIES (STRAIN, WEIGHT)	MODEL, DOSING ROUTE AND N*	TEST COMPOUNDS AND DOSES	OBSERVATION TIME POINT
I	Rat (the same animals as in the Table 6, 270–500 g)	MAP and HR in anaesthetised rats, i.t., N=5-10/dose	Saline (10 µl) per rat Fadolmidine 0.3, 1, 3, 10 and 30 µg per rat Dexmedetomidine 0.1, 0.3, 1, 3 and 10 µg per rat Clonidine 0.3, 1, 3, 10 and 30 µg per rat	The time points: 0, 2, 5, 10, 20, 30, 40, 50 and 60 min after dosing
I	Rat (Sprague Dawley, 340–550 g)	MAP and HR in anaesthetised rats, i.c.v., N=2-4/dose	Saline (2 µl) per rat Fadolmidine 0.3, 1 and 3 µg per rat Dexmedetomidine 0.3, 1 and 3 µg per rat	The time points: 0, 2, 5, 10, 20, 30, 40, 50 and 60 min after dosing
II	Rat (the same animals as in the Table 8)	MAP and HR in telemetered rats (see Table 8)	See Table 8: Fadolmidine i.t. Clonidine i.t.	Home cage 1, 2 and 3. Time points:15, 55 and 95 min after dosing, respectively Open field 1 and 2. Time points: 20 and 25 min, and 60 and 65 min, respectively after dosing
II	Rat (Sprague Dawley, 225–378 g)	Benzol-Jarisch reflex; MAP and HR in anaesthetised rats, i.v., N=72	Fadolmidine 3 µg per rat, i.v. Dexmedetomidine 3 µg per rat, i.v. + Atipamezole (600 µg per rat i.v.) / prazosin (30 µg per rat, i.v.) / ondansetron (30 µg per rat, i.v.)	The dose-response effects
III	Dog (the same animals as in the Table 7)	MABP and HR by non-invasive method (see Table 7)	See Table 7: Fadolmidine bolus i.t. and epidural injection Clonidine bolus i.t. and epidural injection	The time points: 0, 0.33, 1, 2, 4 and 8 h after dosing
		MABP and HR by non-invasive method (see Table 7)	See Table 7: Saline 24-h continuous i.t. infusion Fadolmidine 24-h continuous i.t. infusion Clonidine 24-h continuous i.t. infusion	The time points: 0, 1, 2, 4, 8 and 24 h after initiation of infusion
IV	Dog (the same animals as in the Table 7)	MAP and HR in telemetered dogs (see Table 7)	See Table 7: Bupivacaine i.t. Fadolmidine i.t. Bupivacaine and fadolmidine i.t.	The time course at 0, 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.5, 2, 3, 4 and 6 h after dosing

*N= number of animals, i.c.v. = intracerebroventricular, i.t. = intrathecal, i.v. = intravenous, HR = heart rate, MABP = mean arterial blood pressure, MAP = mean arterial pressure.

4.8 Supplementary *in vivo* studies

4.8.1 Mydriatic response (I)

The rats were anaesthetised by i.p. injecting sodium pentobarbital (45 mg/kg, Mebunat® 60 mg/ml). The baseline value of the pupil diameter (mm) was measured under a stereomicroscope and one bolus i.t. (10 µl) of test compounds was given. The mydriatic response was observed every fifth minute for one hour after dosing (Table 10).

4.8.2 GI motility (I)

The rats were fasted overnight (approximately 15 h) tap water given ad libitum. On the study day, rats were given the test compound i.t. (10 µl) and half an hour later, charcoal suspension (10% charcoal (Merck) in 0.25% carboxymethylcellulose (Orion corporation)) was administered intragastrically by gavage in a volume of 1.0 ml per rat. Thirty min after charcoal dosing, the rat was sacrificed by CO_2. The small intestine was dissected and the distance (cm) travelled by the charcoal marker was measured (Table 10).

4.8.3 Kidney function measurements (II)

Rats were weighed and randomised into groups (for i.v. and i.t. dosing) a day before the experiment. The rats were fasted for approximately 15 h. Tap water was given *ad libitum*. On the study day, the rats received tap water (30 ml/kg) perorally and were treated immediately either i.v. (1 ml/kg b.wt.) or i.t. (10 µl/rat) with fadolmidine or saline. The positive control substance furosemide (2.0 mg/kg, Furesis 10 mg/ml) s.c. was given. For urine collection, rats were placed in metabolic cages (Tecniplast, Cod.170022) either two animals (i.v. dosing) or one animal (i.t. dosing) per cage and food and water was deprived. After 6 h, the urine output was measured and the rats were sacrificed. The urine was centrifuged (Jouan Plasma 1000) at 600 g for 7 min and osmolarity of urine was determined by using Osmostat OM-6020 osmometer (Kyoto, Japan). The urine samples were stored in the freezer at -20 °C prior to analysis of Na^+ and K^+ concentrations (the Yhtyneet laboratoriot Oy, Finland) (Table 10).

4.8.4 Urodynamic measurements (II)

Rats were anaesthetised with urethane 1.0–1.4 g/kg i.p. and the rectal BT was kept constant at 37 °C by a water-filled heating block. The abdomen was opened and the urinary bladder was exposed. A i.v. cannula (22G) was introduced into the bladder

dome and via the cannula a physiological saline was infused at a speed of 0.1 ml/min by a perfusion pump (Perfusor ED 2, B. Braun melsungen AG, Germany) to the bladder dome. For the measurement of intravesical pressure a MP-15 pressure transducer (Micron Instruments, USA) was connected to the cannula. To measure voiding volume, a plastic tube (length 110 mm, inner diameter 1.0 mm) was connected to the penile urethra to direct the urine to a beaker connected to a force transducer (type K30, Hugo Sachs Elektronik, Germany). Changes in the intravesical pressure and the voiding volume of the volume-evoked micturition were monitored. Intravesical pressure and voiding volume signals were amplified by a two channel bridge amplifier (type 301, Hugo Sachs Elektronik, Germany). The signals were delivered through an A/D-converter to a computer based data acquisition system MP100WS (Biopac Systems Inc., USA). After stabilising of the volume evoked micturition cycles (when at least three reproducible cycles were detected), the test compound (10 µl) was given i.t. Only rats capable of performing reproducible voiding cycles were used. The test compounds were tested in separate experiments. Both fadolmidine and saline groups were included in all studies for comparisons (Table 10).

Table 10. Supplementary *in vivo* studies I–II.

STUDY	PARAMETERS	SPECIES (STRAIN AND WEIGHT), DOSING ROUTE AND N*	TEST COMPOUNDS AND DOSES	OBSERVATION TIME POINT
I	Mydriatic response	Rat (Sprague Dawley, 210–310 g), i.t., N=4–5/dose	Fadolmidine 1, 3, 10 and 30 µg per rat Dexmedetomidine 0.3, 1, 3 and 10 µg per rat Clonidine 1, 3, 10 and 30 µg per rat	The time points: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min after dosing
I	Gi motility	Rat (Sprague Dawley, 180–380 g), i.t., N=6–10/dose	Fadolmidine 0 (saline 10 µl), 1, 3, 10 and 30 µg per rat Dexmedetomidine 0 (saline 10 µl), 1, 3, 10 and 30 µg per rat Clonidine 0 (saline 10 µl), 1, 3, 10 and 30 µg per rat	The dose-response effects: the distance travelled by the migrating charcoal marker at 30 min after dosing
II	Kidney function	Rat (Sprague Dawley, 209–274 g), i.v., N=5–6/dose and (250-300 g), i.t., N=8/dose	Saline 1 ml per kg, i.v. or 10 µl per rat, i.t. Fadolmidine 1, 3, 10 and 30 µg per rat, i.v. or i.t. Furosemide 20 mg per kg, s.c.	Urine output, osmolarity, and Na+ and K+ concentrations and excretion
II	Urodynamic	Rat (Sprague Dawley, 198–349 g), i.t. N=3–19/dose	Saline 10 µl per rat Fadolmidine 1, 3 and 10 µg per rat Dexmedetomidine 1, 3 and 10 µg per rat Clonidine 3,10 and 30 µg per rat Morphine 0.1, 0.3 and 1 µg per rat	Category (0–5) of voiding cycles

*N= number of animals, i.t. = intrathecal, i.v. = intravenous, s.c. = subcutaneous.

4.9 Rat brain neurochemistry (I)

The rats were given one bolus i.t injection (10 µl) of test compound 1 and 3 h before sacrifice. The rats were sacrified by decapitation, and the brains (cerebrum and cerebellum) were removed, frozen, and stored at -70 °C for not more than one week before analysis. NA and its metabolite MHPG-SO_4 and 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were analysed from the homogenate of whole brain tissue. NA, 5-HT, 5-HIAA concentrations were determined by electrochemical detection after separation by HPLC on a reverse phase C18 column, and NA metabolite, MHPG-SO_4 concentration was determined fluorometrically as described earlier (Haapalinna et al. 1997; MacDonald et al. 1988) (Table 11).

4.10 Pharmacokinetics of fadolmidine in rats (IV)

In the pharmacokinetics study, an unlabelled stock solution of the test substance was prepared by dissolving 10.0 mg of fadolmidine in 50 ml of 0.050 M hydrochloric acid to produce a mass concentration of 200 µg/ml of the drug with storage at 0–5 °C until being used within ten days of preparation.

^{3}H-labeled fadolmidine (radiochemical purity of 98.36%, determined by TLC), ref. no. TRQ8005 (Amersham), specific radioactivity ca. 1850 GBq/mmol (50 Ci/mmol), was custom synthesized by Amersham International plc. (Amersham, UK.), and stored in the freezer at -20 °C for the pharmacokinetic study.

The preparation of a test formulation for i.t. administration: a measured amount of ^{3}H-labeled fadolmidine in methanol was evaporated to dryness under a gentle flow of nitrogen at 30 °C. The residue was dissolved in an aliquot of the unlabeled fadolmidine stock solution described above. Then the pH of the solution was adjusted to 6.0 with 0.1 M NaOH and finally its volume was brought to 1.5 ml by adding purified water. The target concentration of the test compound in the solution was 0.100 mg/ml and radioactivity 111 MBq/ml (3 mCi/ml). Preparation of a test formulation for i.v. administration: a measured amount of ^{3}H-labeled fadolmidine in methanol was evaporated to dryness and dissolved in a dilution of the above stock solution. Then the pH of the solution was adjusted to 6.0 with 1 M NaOH and finally its volume was brought to 40.0 ml by adding purified water. The target concentration of the test compound in the solution was 3.0 µg/ml and radioactivity 3.7 MBq/ml (0.1 µCi/ml). The solutions for i.t. and i.v. administration were stored at 4 °C and were used for dosing within three days from preparation. Specific radioactivity (counted in a Wallac 1214 RackBeta liquid scintillation counter) was calculated taking into account the dilution factors and sample volumes.

Prior to the drug treatment, the rats were fasted overnight. Food was available to those rats remaining three hours after dosing. Tap water was available *ad libitum* except during dosing and sampling. On the dosing day of drug, a single i.t. (10 µl)

or i.v. (300 µl) bolus dose of ^{3}H-fadolmidine formulation was given via the i.t. catheter or via the tail vein to rats (Table 11).

Table 11. Brain neurochemistry and pharmacokinetics of fadolmidine (I, IV).

STUDY	PARAMETERS	SPECIES (STRAIN AND WEIGHT), DOSING ROUTE AND N*	TEST COMPOUNDS AND DOSES	SAMPLING TIME POINT
I	Brain neurochemistry	Rat (Sprague Dawley, 250–330 g), i.t., N=10/drug	Saline 10 µl per rat Fadolmidine 3 µg per rat Dexmedetomidine 3 µg per rat Clonidine 6 µg per rat	The time points: 1 and 3 h after dosing
IV	Pharmacokinetics	Rat (Sprague Dawley, 285–404 g), i.v., N=6/time point (258–311 g), i.t., N=6/time point	Fadolmidine 3 µg/kg	Plasma, the time points: 0.083, 0.17, 0.33, 0.5, 1, 2, 3, 5, 7, 12, 24 and 72 h Spinal cord, the time points: 0.17, 1, 5, 24 and 72 h

*N= number of animals, i.t. = intrathecal, i.v. = intravenous.

4.11 Chemicals (I–IV)

Fadolmidine, dexmedetomidine and atipamezole were synthetised by Orion Corporation. Clonidine was purchased from Sigma and RBI and morphine from Leiras. Bupivacaine (Bicain spinal® 5 mg/ml) and furosemide (Furesis® 10 mg/ml) were purchased from Orion Corporation. 2-Methylserotonin, phenylephrine and tetrodotoxin were purchased from Research Biochemicals International, ondansetron (Zofran® 2 mg/ml i.v. inject) from Glaxo UK Ltd., prazosin from Pfizer and acetylcholine from Sigma.

4.12 Statistical analysis (I–IV)

In study I, nonparametric Friedman's paired analysis of variance (ANOVA) tests were made for the overall analysis of the effects on motor activity and motor coordination and Wilcoxon signed rank test (two-tailed) for the comparison of the effect between control treatment and drug doses. The overall analysis of the effects on BT was performed by paired ANOVA with paired two-tailed *t* test as post hoc test and the mydriatic effect of drugs was performed by ANOVA for repeated measures with LSD (least significant difference) post hoc test. The effects on GI motility was performed by overall analysis of ANOVA with two-tailed Dunnett's *t* test as the post hoc test. In the i.t. study, homogeneity between doses of baseline

values of the MAP and HR was tested by ANOVA and the effects of drugs was analysed using one-factor ANOVA for repeated measures with two-tailed Dunnett's *t* test as the post hoc test. One-way ANOVA with Dunnett's *t* test as the post hoc test were used for the neurochemistry data. In the i.c.v. study, homogeneity between groups of baseline values of MAP and HR were tested by factorial ANOVA.

In study II, the effects of drugs on Bezold-Jarisch reflex were analysed by one-way ANOVA followed by *t* test. In kidney function measurements, statistical analyses were done by using the one-way ANOVA, followed by the Bonferroni corrected (by the number of comparisons made) *t* test as a post hoc test. The Bonferroni *P*-value <0.01 (five comparisons) was considered statistically significant. The open field data (HR, MAP and BT) was analysed by repeated measures ANOVA for multiple comparisons, followed by Tukey's post hoc test.

In study III, statistical analyses of MABP, HR, respiratory rate and BT values after i.t. and epidural bolus injection were performed separately for each compound and administration route by analysis of covariance model for repeated measurements and for baseline between doses and groups with one-way ANOVA. Contrasts (pair-wise comparisons) were made to characterize the differences in more detail. The 24-h i.t. infusion data were analysed in the same way as bolus injection. Maximum effects of doses were assessed using analysis of covariance model, with one within-factor dose, for repeated measurements with the contrasts.

In study IV, the magnitude of motor blocking effect and the duration of action were analysed by using dose levels with observed positive motor score effects. The Kruskal-Wallis test for ranks was used to evaluate overall treatment differences (studies 1 and 2) and followed by using Dwass, Steel, Critchlow-Fligner pairwise comparisons (study 1). The overall statistical significance of treatments on BT in rats was tested with two-way repeated measures ANOVA (Greenhouse-Geisser method), followed by a post hoc two-tailed Dunnett's *t* test. Statistical analyses of MAP and HR during the first 1.5 hours (during the analgesic effect of bupivacaine), and analgesia (skin twitch), respiratory rates and BT during the measuring time (6 hours, for the nature of the responses) following i.t. injection were performed by analysis of covariance for repeated measurements and for baseline between groups with one-way ANOVA with two within-dog factors i.e. dose (d) and time (t). The time points before dose administration being used as a covariate, and contrasts (pair-wise comparisons) were made to characterize the differences in more detail.

The results were expressed as the mean ± S.D. or mean ± S.E.M. *P*-values **P*<0.05, ***P*<0.01 and ****P*<0.001 were considered statistically significant.

5 Results

5.1 Characterization the effect of fadolmidine on 5-HT_3 receptors (II)

Functional effects of fadolmidine were studied in *in vitro* cell assays in human embryonic kidney cells transfected with human 5-HT_3 receptors (expressing recombinant either 5-HT_{3A} or 5-HT_{3AB} receptors) by the patch clamp technique and *ex vivo* guinea pig (male Dunklin-Hartley) ileum preparation by measuring isometric contractions (Figure 5). In transfected cells and in the guinea pig ileum preparation, fadolmidine demonstrated partial agonism at 5-HT_3 receptor.

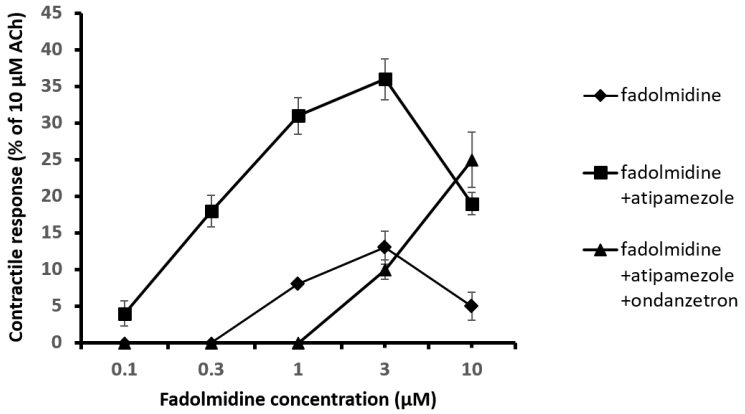

Figure 5. Contractile dose response of fadolmidine in guinea pig ileum *ex vivo* preparations without and with atipamazole or atipamazole + ondansetron. α_2-Adrenoceptor antagonist atipamazole (1 µM, n=7) potentiated and 5-HT_3 antagonist ondansetron (1 µM, n=5) inhibited fadolmidine (n=4) induced contractions being indicative of the 5-HT_3 agonistc property of fadolmidine. Data are presented as mean ± S.E.M. (II: Fig 2).

5.2 Pharmacological properties of fadolmidine *in vivo*

5.2.1 Effects on analgesia after spinal dosing (I, III, IV)

The analgesic effects (%MPE) of fadolmidine were evaluated after i.t. administration in the rat tail-flick test and after i.t. and epidural bolus injections and during a 24-h

continuous i.t. infusion in dog skin twitch test. In addition, the analgesic effects of i.t. fadolmidine as an adjuvant with a local anaesthetic bupivacaine were tested in rats and dogs. Furthermore, the interaction of the analgesic effect of bupivacaine and fadolmidine was evaluated by using an isobolographic analysis.

The analgesic effects of fadolmidine were compared to the effects of dexmedetomidine in rats and clonidine in rats and dogs. I.t. (i.e. into the lumbar spinal cord) administered fadolmidine (0.1 (only in study I), 0.3, 1, 3 and 10 µg) (I, IV), dexmedetomidine (0.1, 0.3, 1, 3 and 10 µg) (I), and clonidine (0.1, 0.3, 1, 3, 10 and 30 µg) (I) produced dose-dependent antinociception in a rat tail-flick test. The rank order of potency of the three α_2-adrenoceptort agonists based on their ED_{50} values was as follows; fadolmidine > dexmedetomidine > clonidine. Furthermore, fadolmidine and clonidine induced dose-dependent antinociception after i.t. (30, 100 and 300 µg) and epidural (30, 100 and 300 µg) bolus injections in a dog skin twitch test (III) (Figure 6).

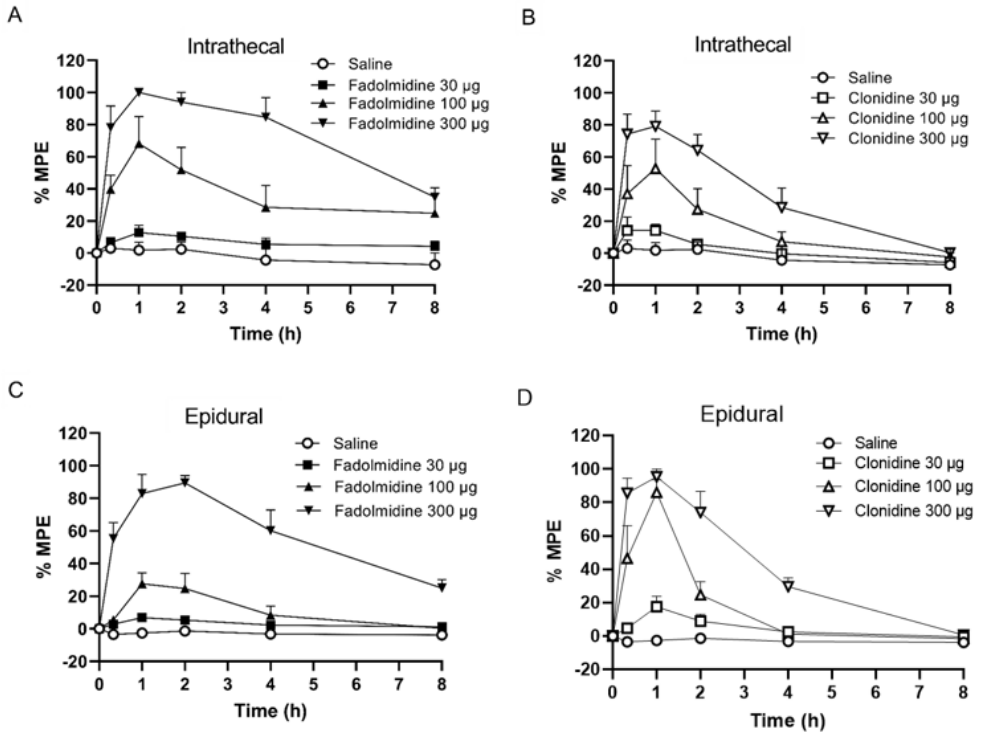

Figure 6. Time course of analgesic effects (%MPE; percent of the maximum possible effects) after (A) fadolmidine i.t. bolus injection, (B) clonidine i.t. bolus injection, (C) fadolmidine epidural bolus injection and (D) clonidine epidural bolus injection in the skin twitch model in dog, n = 5 dogs at each time point. The washout period between treatments was at least 48 h. Values are mean ± S.E.M. (III: Fig.1).

The antinociceptive potency of fadolmidine was weaker than when administered epidurally than when given i.t. and weaker than that of epidural clonidine. The antinociceptive potencies (ED_{50} values) of fadolmidine, dexmedetomidine and clonidine are presented in Table 12.

Table 12. The antinociceptive potency (ED_{50} values) of fadolmidine, dexmedetomidine and clonidine after i.t. or epidural administration in rats tail-flick and dogs skin twitch models (I, III).

Nociceptive model	Animal	Compound, dosing route	ED_{50} (µg/animal) (95% confidence interval)
Tail-flick	Rat	Fadolmidine, i.t.	0.73 (0.24–2.2)
		Dexmedetomidine, i.t.	2.2. (1.1–4.3)
		Clonidine, i.t.	6.4 (4.6–9.0)
Skin twitch	Dog	Fadolmidine, i.t.	67 (48–94)
		Clonidine, i.t.	78 (47–126)
		Fadolmidine, epidural	128 (104–158)
		Clonidine, epidural	51 (42–62)

During a 24-h continuous i.t. infusion, fadolmidine (at the dose of 100 µg bolus injection and 50 µg/h) achieved a good analgesic effect. After the initiation of infusion with either fadolmidine (100 µg + 50 µg/h) or clonidine (100 µg + 100 µg/h), the mean %MPE values were approximately 60 % and 50 % within the first 4 hours and then approximately 36 % and 39 % for the following 20 hours, respectively (III: Fig 2).

Co-administration of fadolmidine (1, 3, 10 µg, i.t.) and a low dose of a local anaesthetic, bupivacaine (10 µg, i.t.), was observed to produce an increase in the magnitude and duration of the analgesic effect as compared to either compound alone in the rat tail-flick test (IV). In the isobolographic analysis, the ED_{50} dose of bupivacaine of 87 µg/rat and 1.2 µg/rat for fadolmidine (data in file, Orion Corporation) was used. The isobolograph for the interaction of the analgesic effect of bupivacaine and fadolmidine was synergistic in its nature (IV: Fig 4). The calculated ED_{50} values for fadolmidine and bupivacaine were 0.26 µg (confidence limits; CL 0.21–0.34 µg) and 19.4 µg (CL 15.3–24.5 µg), respectively. The isobolographic analysis of the analgesia interaction was performed graphically by the methods of Tallarida et al. (1989) and Tallarida (1992). The isobolograph is presented in Figure 7.

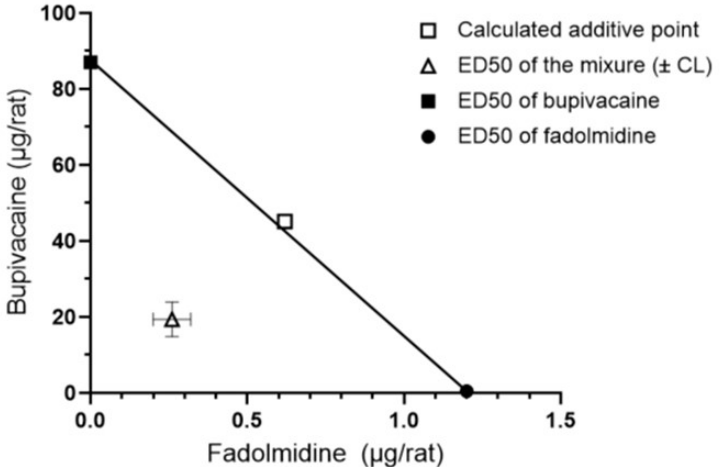

Figure 7. Isobolograph for the i.t. interaction of bupivacaine and fadolmidine in the rat tail-flick test given 30 min before testing. The X and Y axes show the dose (µg/rat) of fadolmidine and bupivacaine, respectively. The calculated ED_{50} values (n=7/dose group) for fadolmidine (solid circle) and bupivacaine (solid square) were 0.26 and 19.4 µg, respectively. The the theoretical additive line is the line between the X-axis and Y-axis. The point (open square) in the middle of the line is the theoretical additive point calculated from the separate ED_{50} values. The experimental point (open triangle) is below the additive line. The experimental point under the additive line indicating synergism of the combination (IV: Fig 4).

Furthermore, in dogs, the combination bupivacaine (3 mg, i.t.) and fadolmidine (60 µg, i.t.) increased the magnitude and duration of analgesic effects and prolonged motor block (IV). Added morphine 1 mg/kg, i.v. 30 min after i.t. dosing of the combination bupivacaine and fadolmidine increased the analgesic effects of the combination without effects on the duration of motor block (Figure 8).

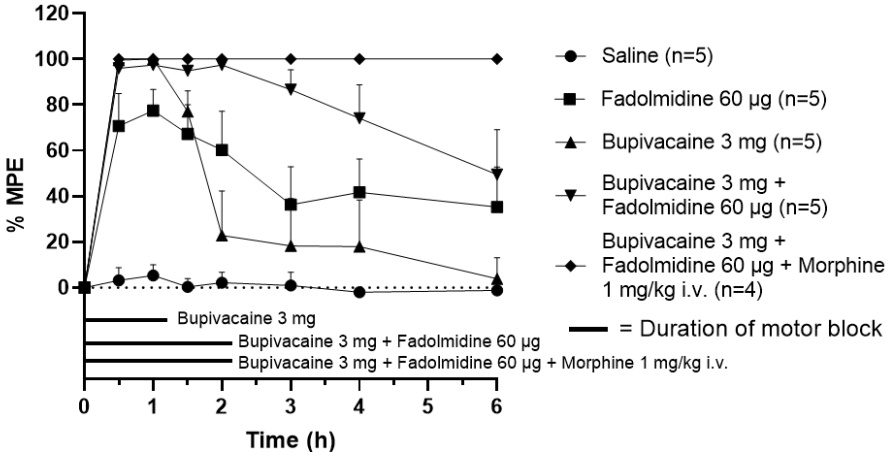

Figure 8. The time course of the analgesic effect (%MPE; percent maximum possible effect) and the duration of motor block (the values are mean) of i.t. fadolmidine 60 µg and saline (0.5 ml) alone and combined with bupivacaine 3 mg, and the combination of bupivacaine 3 mg + fadolmidine 60 µg + morphine 1 mg/kg i.v. in the skin twitch test in dogs. Each %MPE point represent mean ± S.E.M. of 5 dogs except in the combination bupivacaine 3 mg + fadolmidine 60 µg + morphine 1 mg/kg i.v. group n=4. (Modified from IV: Fig 6).

5.2.2 Effects on sedation, BT and mydriatic response (I–IV)

Sedation and hypothermia are characteristic pharmacodynamic responses for α_2-adrenoceptor activation in the brain. Fadolmidine (1–30 µg, i.t.) induced considerably less sedation than dexmedetomidine (0.3–10 µg, i.t.) and clonidine (0.3–10 µg, i.t) at the analgesic i.t. doses measured in motor activity and motor coordination tests in rats (I: Table 2). In moving animals (in open field conditions), no significant sedation (a decrease in a locomotor activity) was observed at the analgesic dose ranges (1–3 µg) of fadolmidine (II: Table 2). Furthermore, fadolmidine alone at the doses of 0.3, 1 and 3 µg had no effects on motor scores and rotarod performance but when it was combined with the high dose (300 µg) of bupivacaine, the motor score values and rotarod performance times were shortened and the duration of effects were prolonged dose-dependently in rats (IV: Table 3). In dogs, fadolmidine and clonidine 30 µg i.t. and epidurally had no effect on muscle tone (sedation) whereas after doses of 100 and 300 µg i.t. and epidurally, mild to moderate signs of hind limb muscle weaknesses were observed (III: Table 1 and 2) as during a 24-h continuous i.t. infusion (III: Table 3). Furthermore, no major effects on scored (0–4) sedation were observed after i.t. fadolmidine (60 µg) alone and in the combination with bupivacaine (3 mg) (IV).

Fadolmidine decreased BT (estimated -1 °C in BT) in rats at the dose of 10 µg i.t. or above, i.e. at over ten times higher doses than the ED_{50} dose in the analgesic tail-flick test (I: Table 2). In dogs, the decreases in BT (statistically significantly)

were seen at the dose of 100 µg i.t. and epidurally or above, i.e. at higher doses than the ED_{50} dose in the analgesic skin twitch test or at the analgesic dose, respectively (III: Table 1 and 2). During a 24-h continuous i.t. infusion no effects of fadolmidine on BT were observed (III: Table 3). In comparison, both dexmedetomidine (0.3–10 µg, i.t.) and clonidine (0.3–10 µg, i.t.) decreased BT at doses lower than the analgesic ED_{50} dose in rats (I: Table 2). In dogs, clonidine (30–300 µg) decreased BT at the analgesic dose after i.t. and epidural (at the dose of 100 µg) administration and during a 24-h continuous i.t. infusion (100 µg + 100 µg/h) (III: Tables 1, 2 and 3). No significant difference was obtained for BT in either fadolmidine (60 µg, i.t.) alone or in its combination with bupivacaine (3 mg, i.t.) as compared to control (IV).

As clinical signs, sporadic emesis/vomiting and urination were observed during the treatment with fadolmidine i.t. (III).

The agonist activity of fadolmidine (1–30 µg, i.t.) on central α_2-adrenergic receptors was assessed by measuring the mydriatic response, and the effect was compared to dexmedetomidine (0.3–10 µg, i.t.) and clonidine (1–30 µg, i.t.) elicited effects (I). Fadolmidine elicited a dose-related ($P = 0.004$) increase in pupil diameter and the maximum increase was achieved only at the highest tested dose (30 µg) of fadolmidine (1 vs 30 µg: $P<0.001$) with the lower doses being inactive. Dexmedetomidine ($P<0.001$) and clonidine ($P<0.001$) elicited a dose-related mydriatic effect which began to display statistical significance at the dose of 1 µg (0.3 µg vs 1 µg: $P<0.001$) and 10 (1 vs 10 µg: $P<0.001$), respectively.

5.2.3 Effects on BP and HR (I–IV)

In anaesthetised rats, fadolmidine (0.3–30 µg) after an i.t. bolus injection at the dose of 1 µg decreased BP and at the doses above 1 µg, there was a brief initial increase and then a long-lasting decrease in MAP (I: Fig 5). HR decreased during both phases of the pressure changes. Dexmedetomidine (0.1–10 µg, i.t.) and clonidine (0.3–30 µg, i.t.) decreased MAP and HR at all tested doses. The lowest dose for a statistically significant hypotension effect, when compared with the control was 3 µg of fadolmidine ($P<0.05$), 0.1 µg of dexmedetomidine ($P<0.01$) and 0.3 µg of clonidine ($P<0.05$). After i.c.v. administration, fadolmidine (0.3, 1 and 3 µg) decreased MAP and HR at the two highest doses and dexmedetomidine (0.3, 1 and 3 µg) at all doses (I). In conscious rats, in resting conditions (at home cage), fadolmidine (1, 3, 10 and 30 µg, i.t.) decreased HR dose-dependently and increased initial MAP, whereas in actively moving rats (in open field conditions), there were no effects at analgesic doses (II: Fig 7).

In conscious dogs, fadolmidine at the doses of 30, 100 and 300 µg had biphasic effects on BP after i.t. and epidural bolus injection (III: Table 1 and 2). The maximum effect of fadolmidine on BP (dose (d): $P=0.002$) at lower i.t. doses was hypertension and at the highest i.t. dose hypotension. After epidural dosing (d: $P=0.22$), the effects

were reversed. In contrast, clonidine evoked hypotensive effects both after i.t. and epidural administration at the doses of 30, 100 and 300 µg (III: Table 1 and 2). The maximum effect of clonidine on BP after i.t. (d: P=0.23) and epidural (d: P=0.10) dosing was mild hypotension. Concomitant with the BP changes, HR decreased in a dose-dependent manner after fadolmidine and clonidine treatments (d*time (t): $P \leq 0.005$). When fadolmidine (60 µg, i.t.) was combined with bupivacaine (3 mg, i.t.), an increase in MAP was evident. Bupivacaine and fadolmidine alone decreased and increased MAP, respectively (IV: Table 4). No significant effects on HR were seen after fadolmidine, bupivacaine, nor with the combination of bupivacaine and fadolmidine.

The 5-HT_3 agonistic effects of fadolmidine (3 µg/rat, i.v.) were further studied in Bezold-Jarisch reflex model in anaesthetised rats by measuring 5-HT_3 mediated effects in MAP an HR (II: Fig 3). Fadolmidine (3 µg/rat, i.v.) induced a transient increase in MAP followed by reflectory bradycardia. These effects were partly inhibited by atipamezole (600 µg/rat i.v.) and prazosin (30 µg/rat i.v.), and almost abolished when both blockers were co-administered. The selective 5-HT_3 receptor antagonist ondansetron (30 µg/rat, i.v.) was devoid of any effects on MAP or HR and it did not change the effects of fadolmidine on MAP or HR.

The potency of the analgesic effect (ED_{50} values) and some of the above tested supraspinal and peripheral (e.g. sedation (motor activity and muscle tone), hypothermia, hypotension and hypertension) *in vivo* pharmacological effects (at the dose) of i.t. fadolmidine in rats and i.t. and epidural fadolmidine in dogs are summarized in Table 13.

Table 13. Potency (dose) of spinal (analgesic) and some supraspinal and peripheral *in vivo* pharmacological effects of i.t. fadolmidine in rats (I) and i.t. and epidural fadolmidine in dogs (III).

PARAMETER	RAT, I.T.	DOG, I.T.	DOG, EPIDURAL
Analgesia[a]	0.73	67	128
Sedation[b]	19	100	100
Hypothermia[c]	10	300	100
Hypotension[d]	1.0	100	30
Hypertension[e]	3.0	100	100
RATIO			
Sedation / analgesia	26	1.5	0.8
Hypothermia / analgesia	14	4.5	0.8
Hypotension / analgesia	1.4	1.5	0.2
Hypertension / analgesia	4.1	1.5	0.8

[A] Tail-flick in rats and skin twitch in dogs, ED_{50} (µg/animal), [B] Decrease in motor activity in rats [ED_{50} (µg/animal)], and muscle tone in dogs, [C] Estimated -1°C in BT in rats and dogs, [D] No statistical significant dose in hypotension in rats and dogs, [E] Initial increase in BP (hypertension) in rats and dogs.

5.2.4 Supplementary *in vivo* studies (I, II)

The effects of fadolmidine, dexmedetomidine and clonidine (1–30 µg, i.t.) on GI motility were studied by using the charcoal propulsion test in rats (I). The effects of fadolmidine (1–30 µg, i.v. or i.t.) on urine output and electrolyte excretion were measured in conscious rats to study the potential effects on kidney function (II). After i.v. doses, fadolmidine increased only the sodium ion concentration and excretion but statistically insignificantly. The effects of fadolmidine (1–10 µg, i.t.), dexmedetomidine (1–10 µg, i.t.), clonidine (3–30 µg, i.t.) and morphine (0.1–1 µg, i.t.) on urinary function were evaluated in anaesthetised rats (II). The results are summarised in Table 14.

Table 14. The effects of GI motility, urine output, sodium excretion and voiding function after i.t. administration in rats (I, II).

PARAMETER	FADOLMIDINE	DEXMEDETOMIDINE	CLONIDINE	MORPHINE
Inhibition of GI motility[a]	18	1.3	4.9	-
Urinary output[b]	30	-	-	-
sodium concentration[C]	3	-	-	-
Voiding cycle[D]	1	1	30	0.3

[A] Inhibition of GI transit, ED_{50} (µg/rat), [B] Statistically significant increase in urine output, µg/rat, [C] Statistically significant increase in sodium concentration, µg/rat, [D] Interruption in volume-evoked voiding cycles catecory ≥4, µg/rat.

5.2.5 Rat brain neurochemistry (I)

The effects of fadolmidine (3 µg, i.t.), dexmedetomidine (3 µg, i.t.) clonidine (6 µg, i.t.) and control on rat brain neurochemistry were evaluated. NA and its metabolite MHPG-SO_4, and 5-HT and its metabolite 5-HIAA were measured from the homogenate of brain tissue at 1 and 3 h after compounds dosing (I).

In the control group the NA levels were 2.01 ± 0.05 nmol/g and 1.87 ± 0.02 nmol/g, and the 5-HT levels were 2.80 ± 0.15 nmol/g and 2.47 ± 0.18 nmol/g at 1 and 3 h, respectively. There were no drug associated differences in the NA, 5-HT and 5-HIAA levels between drugs at 1 (P=0.064, P=0.05 and P=0.07, respectively) and 3h (P=0.31, P=0.09 and P=0.85, respectively). The MHPG-SO_4 levels in the control animals were 0.50 ± 0.02 nmol/g and 0.56 ± 0.04 nmol/g at 1 and 3 h, respectively. Drug-associated differences in the MHPG-SO_4 levels were measured between drugs at 1 (P=0.03) and 3 h (P=0.0053). Compared to control

dexmedetomidine and clonidine exhibited a tendency to decrease the amount of MHPG-SO_4 at 1h, and the decrease was statistical significant after clonidine administration at 3h ($P<0.05$). The effects of drugs on the turnover ratios of metabolite to parent amines at 1 and 3 h are presented in Table 15.

Table 15. Comparison of the effect of fadolmidine with dexmedetomidine and clonidine on ratios of metabolite to parent amines in the brain at 1 and 3 h after their i.t. administration (I: Table 3).

DRUG AND DOSE (µG/RAT)	MHPG-SO_4/NA		5-HIAA/5-HT	
	1h	3h	1h	3h
Control	0.25 ± 0.003	0.30 ± 0.02	1.05 ± 0.05	1.23 ± 0.07
Clonidine 6	0.20 ± 0.01*	0.22 ± 0.03*	0.92 ± 0.05	0.99 ± 0.10
Dexmedetomidine 3	0.19 ± 0.01**	0.23 ± 0.02	0.83 ± 0.06*	0.98 ± 0.08
Fadolmidine 3	0.25 ± 0.01	0.28 ± 0.01	1.03 ± 0.06	1.00 ± 0.06
ANOVA; *P* values for treatment	*P*=0.0003	*P*=0.026	*P*=0.035	*P*=0.098

Values are mean ± S.E.M., n=5 in each group. The neurochemistry data were analysed by one-way ANOVA followed by Dunnett's *t* test i.e. drug versus control. NA noradrenaline, MHPG-SO_4 3-methoxy-4-hydroxyphenylglycol sulphate, 5-HT 5-hydroxytryptamine, 5-HIAA 5-hydroxyindoleacetic acid. *P<0.05; **P<0.01 (when compared with the corresponding control response).

5.2.6 Pharmacokinetics of fadolmidine in rats (IV)

Total and dose-corrected (after i.t. administration in plasma) radioactivity in plasma and the corresponding concentration in mass equivalent of ^{3}H-fadolmidine (free base) in spinal cord after i.t. and i.v. administration at the dose of about 3 µg/kg to rats are presented in Figure 9 (IV: Fig 7). Mass equivalents of the i.t. dosing were corrected (dose-corrected) according to the radioactive dose ratio in order to allow for comparison with the i.v. dosing. The conversion to mass equivalents was based on the specific activity of the formulations. The radioactive doses were 4.198 MBq/kg (1.194 MBq/rat) after i.t. and 2.918 MBq/kg (1.004 MBq/rat) after i.v. administration. The corresponding doses were 3.564 µg/kg (1.013 µg/rat) after i.t. and 2.538 µg/kg (0.873 µg/rat) after i.v. administration. The tested dose of fadolmidine 1 µg/rat, i.t. was approximately the ED_{50} dose of the analgesic i.t. dose. The results show that the circulating level of ^{3}H-fadolmidine-related radioactivity in rats rapidly reached its maximum. After i.t. administration, the concentration of ^{3}H-fadolmidine in plasma was very low. The systemic elimination of fadolmidine was faster than its elimination from the i.t. space.

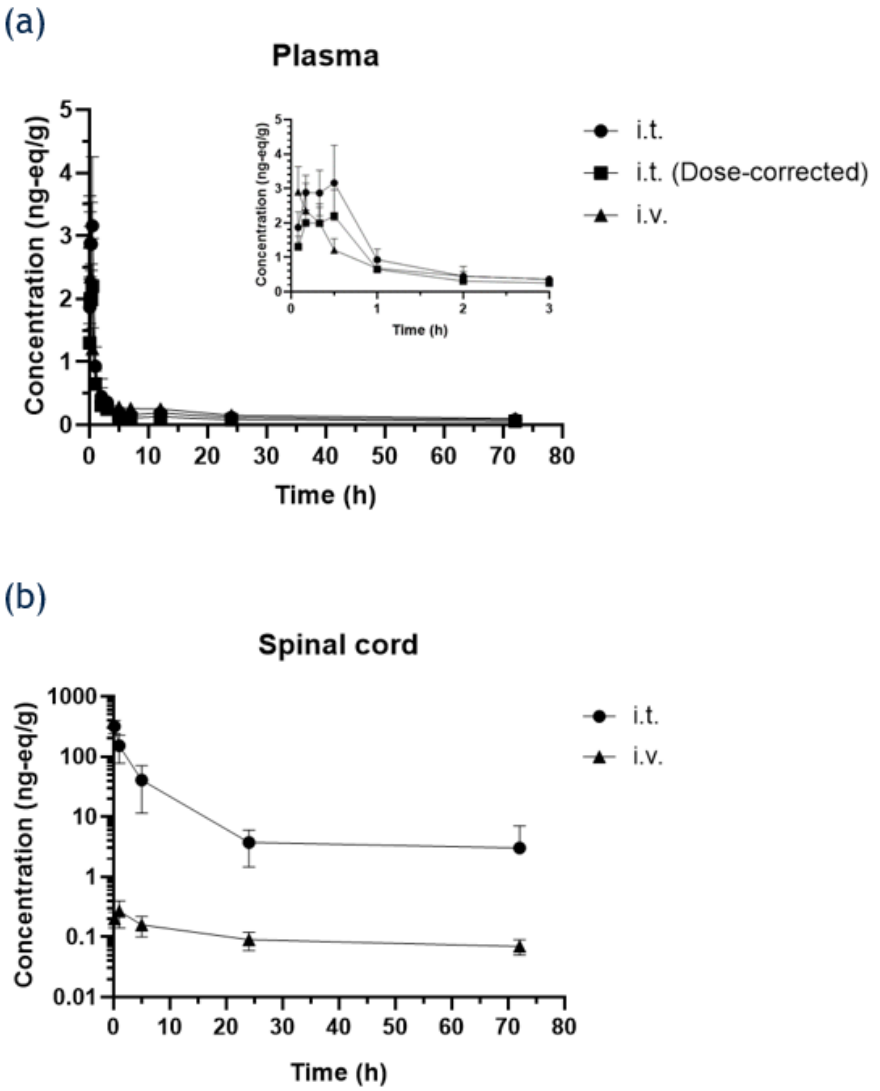

Figure 9. Drug-related total and dose-corrected (after i.t. administration in plasma) radioactivity in rat plasma (a) and spinal cord (b) converted to mass equivalents of ^{3}H-fadolmidine base after an i.t. or i.v. dose of about 3 µg/kg of the compound (hydrochloride). For the dose-correction, the radioactive doses were 4.198 MBq/kg and 2.918 MBq/kg after i.t. and i.v. administration, respectively. Values are presented as mean ± S.D. (n=6 per time point) (IV: Fig 7).

6 Discussion

α_2-Adrenoceptor agonists induce a characteristic pattern of pharmacodynamic responses including sedation as well as variable changes on BP and bradycardia and furthermore, they are potent analgesic agents after i.t. or epidural injection. However, while some of these compounds e.g. clonidine, are well tolerated, at antinociceptive doses, they may produce pronounced hemodynamic side effects such as hypotension and bradycardia as well as sedation. Fadolmidine (MPV-2426) is a full α_2-adrenoceptor agonist (Lehtimäki et al. 1999) with a local mode of action developed as a spinal analgesic by Orion Corporation, Orion Pharma. This thesis work has been a part of the biological evaluation of compound, in which the pharmacological profile *in vitro* and *in vivo* was studied in different models in order to support the development work of fadolmidine.

6.1 Concentration of fadolmidine in plasma after i.t. administration

After i.t. administration, ^{3}H-fadolmidine-radioactivity was rapidly distributed in the spinal cord, close to the administration site in rats. In contrast, the concentration of ^{3}H-fadolmidine in plasma was very low. In addition, after i.v. administration ^{3}H-fadolmidine radioactivity in the spinal cord was low. It has been proposed that fadolmidine, as a polar and a less lipophilic α_2-adrenoceptor agonist than clonidine (Aantaa & Scheinin 1993; Remko et al. 2006) or dexmedetomidine (Aantaa & Scheinin 1993), induces a local, dose-dependent antinociceptive action, possessing a weak ability to redistribute from the injection site to the supraspinal space and further to the CNS (Xu et al. 2000a). Furthermore, in the present study the duration of the analgesic effects of fadolmidine was longer than observed with clonidine after epidural and especially after i.t. bolus injection at high, equal analgesic doses in dogs. In addition, i.t. fadolmidine has been reported to reduce spinal cord blood flow in sheep (Eisenach et al. 1999). Furthermore, when the cardiovascular effect were examined, at the analgesic i.t. doses fadolmidine injection induced only minor effects (an increase or decrease) on MAP concomitantly with a decrease in HR, while after epidural dosing the effects were more evident. The small cardiovascular effects are in accordance with the determined concentration of ^{3}H-fadolmidine in plasma after

i.t. administration. In sheep, CSF distribution ($t_{½\alpha}$) and elimination half-life ($t_{½\beta}$) times of fadolmidine after i.t. and epidural injection were 0.33 and 15 h, and 0.23 and 17 h, respectively (Eisenach et al. 1999). However, here during 24-h continuous i.t. infusion of fadolmidine at a steady state, the elimination half-life time of fadolmidine in dog's plasma was approximately 0.27 h. Thus, the overall results can be summarized as follows: the present pharmacokinetic, efficacy and safety results and earlier findings (Pertovaara 2004) indicate that after i.t. and epidural administration either most of the fadolmidine remains at the site of application or it is rapidly eliminated if it reaches the systemic circulation. The systemic elimination of fadolmidine seems to be faster than its elimination from the i.t. space.

6.2 Analgesic effect following spinal administration

In our studies, fadolmidine evoked a dose-dependent, α_2-adrenoceptor activation mediated analgesia (an increase in thermal response latency) after i.t. administration in a rat tail-flick test and after i.t. and epidural bolus injection as well as during 24-h continuous i.t. infusion in a skin twitch test in dogs. Furthermore, i.t. fadolmidine as an adjuvant to a local anaesthetic, bupivacaine, enhanced the sensory-motor block. The present results are in line with the previous reports of work done in rats (Onttonen et al. 2000; Pertovaara & Wei 2000).

The analgesic effect of spinally administered α_2-adrenergic agonist is mediated through α_2-adrenergic receptors located on small primary afferents C and A-delta fibres on WDR neurons (Gabriel & Gordin 2001; Kawasaki et al. 2003; Yaksh 1985) and in the descending inhibitory medullospinal pathways in the dorsal horn of the spinal cord (Bahari & Meftahi 2019; Millan 2002).

In a comparison with two recognized α_2-adrenoceptor agonists, dexmedetomidine and clonidine, the rank order of the maximal antinociceptive potency in acute pain model following i.t. administration was as follows; fadolmidine > dexmedetomidine > clonidine in the rat tail-flick test and comparable between i.t. fadolmidine and clonidine in the dog skin twitch test. The antinociceptive potency values of fadolmidine (Lehtimäki et al. 1999), dexmedetomidine (Horváth et al. 2001; Joó et al. 2000) and clonidine (Lehtimäki et al. 1999; Ouyang et al. 2012) are in line with the earlier reported results of these compounds in a rat tail-flick test. The analgesic potency of fadolmidine was greater than that of dexmedetomidine and clonidine. which is in accordance with the rank order potency of these compounds as α_2-adrenoceptor agonists at the receptor level (Lehtimäki et al. 1999, 2008; Pohjanoksa et al. 1997). Fadolmidine and dexmedetomidine are full α_2-adrenoceptor agonists (Lehtimäki et al. 2008) whereas clonidine is a partial α_2-adrenoceptor agonist, being approximately ten times weaker than fadolmidine (Lehtimäki et al. 1999) and dexmedetomidine (Lehtimäki et al.

2008; Pohjanoksa et al. 1997). However, in contrast to our result, also equal antinociceptive potencies of i.t. fadolmidine and dexmedetomidine were reported in the rat tail-flick test (Xu et al. 2000a, 2000b).

After epidural injection in dogs, the antinociceptive potency of fadolmidine in skin twitch test was approximately two times greater than with the i.t. route, while after clonidine administration this ratio was opposite in dogs. The differences in the antinociceptive potency after i.t. and epidural administration of fadolmidine are in line with the results reported in sheep in a mechanically induced pain model (Eisenach et al. 1999). Furthermore, in dogs when the duration of the analgesic effects of fadolmidine and clonidine were followed up to 8 h, the durations of analgesic effects following epidural dosing of fadolmidine (Eisenach et al. 1999) and clonidine (Eisenach et al. 1987) were comparable with the values obtained earlier in sheep. However, after i.t. dosing, the duration of analgesia with fadolmidine was approximately double that observed with clonidine. In addition to the differences in efficacy between these compounds at α_2-adrenoreceptors, some of the difference at least partly, can be explained by their physicochemical properties i.e. differences in their polarity and lipophilicity and pharmacokinetics (Eisenach et al. 1999; Onttonen et al. 2000; Onttonen & Pertovaara 2000; Pertovaara & Wei 2000; Xu et al. 2000a, 2000b).

The effects of fadolmidine and clonidine on analgesia have been compared during a 24-h continuous i.t. infusion in dogs. I.t. infusion (Deer et al. 2017; Prager et al. 2014) has been reported to be a suitable drug delivery route for chronic pain (Abdolmohammadi et al. 2015; Ghafoor et al. 2007) and acute postoperative pain (Peniche et al. 2018; Sen & Sen 2015) treatments. In dogs, fadolmidine and clonidine induced appreciable antinociceptive effects during 24-h continuous i.t. infusion, evoking both equipotent maximal analgesic effects when the i.t. infusion dose of clonidine was approximately two higher that that of fadolmidine. Physicochemical and pharmacokinetics properties of fadolmidine and early findings with clonidine (Castro & Eisenach 1989; Pettinger 1980) support the proposal that the differences between the effects of fadolmidine and clonidine may be based, at least partly, on the physicochemical properties and elimination half-lives of compounds. Thus, in contrast to fadolmidine, clonidine infusion could lead to the drug's accumulation in the body during a 24-hour continuous i.t. infusion. However, a progressive loss in the antinociceptive actions was observed, which may indicative of the development of tolerance to the analgesic effect of these types of compounds, as reported earlier during 28 days' epidural infusion of clonidine in dogs (Yaksh et al. 1994) and in chronic spinal infusion in rats (Takano & Yaksh 1993), but not documented in humans (Eisenach et al. 1989). Thus, further studies will be needed to clarify this phenomenon during long-term fadolmidine infusions.

A local anaesthetic, bupivacaine, can produce adequate pain relief and is commonly used in spinal anaesthesia. Bupivacaine inhibits the nociceptive response and induces motor block by blocking voltage sensitive sodium channels in efferent autonomic and motor nerves. However, the short duration of action and dose-dependent cardiovascular adverse effects, like hypotension may limit the use of bupivacaine (Patro et al. 2016; Russell 1982). In the clinic, supplemental vasoconstrictors are required for BP maintenance and subsequently to decrease the systemic absorption of local anaesthetics (Bajwa et al. 2012; Eisenach et al. 1996; Staikou & Praskeva 2014). Furthermore, the combination allows a reduction of the doses of both drugs and furthermore, causes less side effects in perioperative anaesthesia (Bajwa et al. 2012; De Kock et al. 2001; Strebel et al. 2004). Co-administration of i.t fadolmidine with bupivacaine produced an increase in the magnitude and duration of the antinociceptive response and prolonged the duration of bupivacaine induced motor block but did not affect the time of onset of the motor block produced by bupivacaine. The isobolographic analysis of the data revealed that the interaction of nociceptive response of fadolmidine and bupivacaine was synergistic in its nature. Furthermore, the duration of sensor block was much longer than the duration of motor block. Added morphine i.v. increased the magnitude and the duration of analgesic effect further. It is known that the combination of i.t. α_2-adrenergic agonist (e.g. clonidine, tizanidine) and a local anaesthetic produced a synergistic antinociceptive action in rats (Kawamata et al. 1997; Nishiyama & Hanaoka 2004) and dogs (Bedder et al. 1986; Mensink et al. 1987) and it also prolonged the motor block more than that of either compound alone. The mechanism behind the prolongation of a nerve block duration of a local anaesthetic with an α_2-adrenoceptor agonist, clonidine (Kroin et al. 2004) and dexmedetomidine (Brummett et al. 2011) seems to be mediated by an inhibition of the hyperpolarization-activated cation current and not via an α_2-adrenoreceptor mediated mechanism. It has been demonstrated that α_2-adrenergic agonists can hyperpolarize motor neurons and this property may exert a modest effect upon motor tone (Davis et al. 1989; Tanabe et al. 1990). However, in the rat tail-flick and formalin tests, the duration of analgesic effects were not prolonged by the combination, suggesting that the duration of the effect of bupivacaine was long enough to be influenced by clonidine (Nishiyama & Hanaoka 2004). In contrast in the present studies, the duration of analgesic effects of the combination bupivacaine and fadolmidine were clearly increased as compared to the either compound on its own. Furthermore, in humans, clonidine as an adjuvant to local anaesthetics for peripheral nerve and plexus blocks has been reported to prolong the duration of analgesia and sensory block by about 2 h (Pöpping et al. 2009).

6.3 Limitations and advantages of methods and protocols used to measure analgesia

The effects of fadolmidine on analgesia were evaluated following spinal administration in an acute rat tail-flick test and in a dog skin twitch test. However, there are a few limitations or issues which should be taken into account in the methods and protocols of these studies, e.g. they were carried out only in male animals. α_2-Adrenoceptor-induced antinociception has been reported to be sex-specific and attenuated by estrogen in female rats and requires the presence of testosterone in male rats (Ansonoff & Etgen 2001; Nag & Mokha 2016) thus our findings might not be generalised to female subjects.

This is first time that the analgesic effects of fadolmidine have been characterized in dogs. Fadolmidine displayed high affinity and full agonist efficacy at all three human α_2-adrenoceptors suptypes (A, B and C) (Lehtimäki et al. 2008). Although the selectivity of fadolmidine has not been specifically studied at canine α_2-adrenoceptors: in dogs, fadolmidine administration dose-dependently induces all of the physiological effects known to be mediated via α_{2A}-adrenoceptors, including anxiolysis/sedation, antinociception, decrease in BP and decrease in HR as well as the α_{2B}-adrenoceptor mediated initial hypertensive phase in BP (Gyires et al. 2009). In addition, several studies have shown the similarity of the canine α_{2A}-adrenoceptor to the human α_{2A}-adrenoceptor with both mediating the recognized anxiolytic/sedative effects (Schwartz et al. 1999) and in the dog, it is the same α_2-adrenoreceptor (α_{2B} subtype) which mediates contraction of dog saphenous vein as encountered in humans (MacLennan et al. 1997). Furthermore, another advantage for evaluating the analgesic effect in dogs compared to rats is that the spinal space (subarachinoid) of a dog is larger and closer to that in humans. In addition, there is the relatively smaller size of the catheter with respect to the spinal cord and i.t. space in the dogs compared to the situation in rats. The duration of subarachnoid conduction motor blockade in dogs has also been shown to be qualitatively similar to values for spinal anaesthesia reported in humans (Feldman & Covino 1981). In addition to the analgesic effects, safety parameters i.e. sedation, MAP, HR, respiratory rate and BT were evaluated in dogs. A conscious dog is the recommended and commonly-used species to examine potential effects of drugs on BP and HR (ICH S7A guidance, US FDA, S7A 2001) due the sensitivity of dogs to those effects. Thus, the results reveal that the dog seems to be a suitable species for the evaluation of both the analgesic and the cardiovascular effects of α_2-adrenergic compounds.

The analgesic effects were evaluated following i.t. (in rats and dogs) and epidural (in dogs) administration of the drugs in the chronic i.t. or epidural catheterized animals. In i.t. catheterized rats, the catheters tips were in the lumbar enlargement and in dogs at the level of L_{2-3}: in epidural catheterized dogs at the level of L_{1-2}. The location of the catheters tips was confirmed by administering lidocaine i.t. in rats and

dogs or by the distribution of dye delivered before exposure in dogs. Transient paralysis of both hind limbs after administration of an anaesthetic via the cannula was the indication of successful catheterization. In addition, only animals with visually observed normal neurological function were selected for the experiments. However, no histopathology samples (e.g. for evaluation the effects of the chronic catheterization and the local fadolmidine dosing to the spinal cord), from the spinal cord and/or spinal and epidural spaces were taken at the end of the studies.

Both the rat tail-flick and dog skin twitch tests are acute heat-induced (a noxious heat stimuli) pain models without changes in spontaneous or evoked behavioral responses of animals as is evident in persistent pain models where there is a peripheral injury or in inflammation pain models (Bannon & Malmberg 2007). In the heat-induced pain models, an increase in thermal tail flick or skin twitch response latencies was measured. The tail flick is a spinal reflex, but it is subject to supraspinal influences that can affect this reflex (Millan 2002). Potential problems with the rat tail-flick test are maintaining the animal in a correct posture without inducing unwanted stress and the role of the tail in the thermoregulation of the rodent (Barrot 2012). In the present study, in an attempt to reduce unwanted stress, the animals were habituated to the handling and immobilization chamber before the start of the experiment. Furthermore, the noxious stimulus (the beam) was directed to different locations on the tail during a testing session to prevent tissue damage. The skin twitch test is also a reflex test that is applicable for large laboratory animals such a dogs or sheep but there may be individual variations between testing sessions (Allen & Yaksh 2004). In our study, however, there were no major individual variations in baseline values in the testing sessions or between individuals. The dogs were habituated to the handling, environment and situation of measurement before the start of the experiment. In addition, the measuring order of the parameters during the testing session is essential, starting from the sensitive and less stressful observations to more unpleasant measurements e.g. behavioural/sedation ≥ BP/HR > respiratory rate > analgesia > measurements of rectal body temperature.

Barrot (2012) suggested that preclinical pain tests can make rapid progress in assessing the fundamental aspects of physiological pain, but efforts are still needed to achieve the translation to patient treatment e.g. exploiting chronic pain models (Taneja et al. 2012). Furthermore, in the studies, the relevance of the protocol e.g. model, test, procedures and duration of treatment are all critical issues that should be assessed. Nonetheless, Taneja et al. (2012) have stated that there is a common consensus that experimental models replicate symptoms. They added that an assessment of the concentration–effect relationship is necessary for translational purposes. Thus, the drug discovery process needs robust, reproducible, and high-throughput assays, including large animal models, that can be used to screen and to prioritize high numbers of compounds and focus on those with the best chance of

successful development (Whiteside et al. 2016). Thus, in the present study, the potential limitations in study designs were that no pharmacokinetics samples (i.e. blood and CSF samples) were taken in the dog experiments in order to avoid any discomfort to the animals during pharmacodynamics studies. Multiple sampling timepoints would be necessary to determine the pharmacokinetic distribution of fadolmidine and its concentrations in different physiological spaces. However, the ^{3}H-fadolmidine concentrations in spinal cord and plasma were determined after i.t. and i.v. administration in rats. Another limitation is that a rather low number of dogs were tested in the i.t. infusion part of the study. Furthermore, in the combination of i.t. bupivacaine and fadolmidine with morphine i.v. experiment, it would have been interesting to test the effect of morphine i.v. alone as this could had verified the ability of morphine to further potentiate the analgesic effects of the combination of fadolmidine and bupivacaine.

6.4 Fadolmidine induces sedation, hypothermia and mydriasis

α_2-Adrenergic agonists induce central mediated effects such as sedation, hypothermia (Giovanniitti et al. 2015; Michel et al. 2019; Millan et al. 2000; Nguyen et al. 2017; Sinclair 2003) and mydriasis (Heal et al. 1995). Sedation was evoked by fadolmidine (Xu et al. 2000a), clonidine (De Sarro et al. 1987; Sakamoto et al. 2013) and dexmedetomidine (Correa-Sales et al. 1992); this is mediated by their actions at the LC, with inhibition of the regulation of sleep and wakefulness. The effects of i.t. fadolmidine on sedation were evaluated in the exploratory and motor activity tests, which measure slight and moderate sedation, respectively. The effects of i.t. fadolmidine on motor activity and in motor coordination were evident only at doses which were over 20 and 40 times higher than the analgesic ED_{50} dose. In moving animals (in open field), where the animals showed high motor activity by exploring their surroundings, no significant sedation was observed at the analgesic dose ranges (1–3 µg) of i.t. fadolmidine. Furthermore, fadolmidine had no effects on the motor score. In contrast, dexmedetomidine and clonidine caused an inhibition in the motor activity and dexmedetomidine further decreased motor coordination at clearly lower doses than at its analgesic ED_{50} dose. The results are in line with the findings of Xu et al. (2000b) that i.t. injection of fadolmidine caused less sedation than dexmedetomidine in a dark field spontaneous locomotor activity model. In addition, after systemic (s.c.) injection, fadolmidine evoked sedation only at very high doses ($\geq$ 300 µg/kg, s.c.) in comparison to the sedation evoked by dexmedetomidine ($\geq$ 10 µg/kg, s.c.) (Lehtimäki et al. 2008; Wei et al. 2002) which further support the finding that fadolmidine does not pass well across the blood-brain barrier and further distribute into the CNS (Xu et al. 2000a). In dogs, the specific behavioural indices

and sedation scores were used for evaluating the sedation level. Spinally adminstered fadolmidine caused a depression of the muscle tone only at doses higher than that needed for analgesia (ED_{50} value) and this effect was comparable with clonidine. In summary, when considering the analgesic effects of spinal fadolmidine, sedation was only evident at higher doses in the used models and species.

α_2-Adrenergic agonists induce hypothermia by activating noradrenergic afferents in the medial preoptic area in the hypothalamus (Kumar et al. 2007). I.t. fadolmidine caused a decrease in BT doses at doses higher than the analgesic dose (ED_{50} values) in rats and dogs. After epidural administration, a decrease in BT was observed at the analgesic dose (ED_{50} value) in dogs. However, dexmedetomidine and clonidine i.t. also decreased BT at analgesic doses in rats as did clonidine i.t. and epidurally in dogs.

The mydriatic effect, the assessment of the pupillary response, is a sensitive method for the estimation of the supraspinal effects of an α_2-adrenoceptor agonist (Heal et al. 1995; Yu & Koss 2005). Fadolmidine induced mydriasis only at high doses after systemic (i.v. and s.c.) (Lehtimäki et al. 2008) and i.t. ($\geq$ 14 times higher dose than the analgesic ED_{50} dose) administration. Instead, dexmedetomidine (Horváth et al 1994, Lehtimäki et al. 2008) and clonidine (Heal et al. 1995) induced dose-dependently mydriasis after both systemic and i.t. administration in rats.

Activation of α_2-adrenoceptors results in widespread alterations in the release rates of monoamine neurotansmitters. The spread of fadolmidine, dexmedetomidine and clonidine from the lumbar spinal space to brain was assessed by measuring the turnover of brain NA and 5-HT after i.t. administration at the selected analgesic dose. Both dexmedetomidine (3 µg) and clonidine (6 µg) decreased NA and/or 5-HT turnover significantly in brain whereas fadolmidine (3 µg) caused no effects in rats. These results with fadolmidine on central monoamine turnover are highly in line with the findings of the behavioural results e.g. very little evidence of sedation, hypothermia and mydrasis, supporting the suggestion that fadolmidine (Xu et al. 2000a) has a very limited ability to penetrate into the CNS as compared to dexmedetomidine (MacDonald et al. 1988, 1989) and clonidine (Antkiewicz-Michaluk et al. 2017). However, the actual concentrations of the compounds in the brain were not determined, thus further distribution and blood-brain barrier penetration studies of fadolmidine will be needed for evaluating its ability to penetrate into the CNS after i.t. administration.

The mechanism of α_2-adrenoceptor agonists on the depressive effect of respiratory rate is not clear, but it likely reflects calmness and mild sedation (Sinclair 2003). The effect on the respiratory rate was assessed by observation of chest expansion and contraction in dogs. Fadolmidine i.t. had a slight while clonidine i.t. and epidurally exerted a more prominent depressive effect on the respiratory rate at doses higher than the analgesic ED_{50} dose. In humans, however, clonidine has not

caused or potentiated respiratory depression to any appreciable extent (Eisenach et al. 1996; Nguyen et al. 2017).

As clinical signs, sporadic emesis/vomiting and urination were observed during the treatment of fadolmidine i.t. in dogs. However, dogs are known to be a very sensitive species to the emesis induced by α_2-adrenoceptor agonists (Sinclair 2003). Both dexmedetomidine (Jin et al. 2017) and clonidine (Samieirad et al. 2018), have been described to prevent nausea and vomiting during general anaesthesia in humans.

6.5 Effects of spinal fadolmidine on BP and HR

Spinally administered fadolmidine resulted in variable changes on BP and a dose-dependent decrease in HR. In anaesthetised rats, fadolmidine i.t. at lower doses decreased MAP whereas at higher doses, fadolmidine increased initial MAP both in anaesthetized and in conscious animals. After i.c.v. administration, a decrease (but not a dose-dependent decrease) in MAP was noted.

In addition to rats, the cardiovascular effects were evaluated in dogs since this animal species has been shown be highly sensitive to the cardiovascular effects of α_2-adrenergic compounds (Eisenach & Grice 1988; Kroin et al. 2003). Additionally, Beagle dogs are used widely to characterize developmental compounds as potential drugs for human therapy. Furthermore, a conscious dog is a recommended and a commonly used species to examine the potential effects of drugs on BP and HR (ICH S7A guidance, US FDA, S7A 2001). In dogs, the trends in BP were towards an initial hypertension after i.t. injection but hypotension after epidural administration. The cardiovascular responses of α_2-adrenoceptor agonists are likely mediated through multiple sites of action (Eisenach and Tong 1991; Michel et al. 2019; Nguyen et al. 2017; Sinclair 2003). α_2-Adrenoceptor agonists can activate α_2-adrenoceptors in the brain, reducing the sympathetic outflow from the CNS leading to peripheral vasodilatation. In addition, α_2-adrenoceptor agonists induce hypertension by activating vascular α_2-adrenoceptors and reduce chronotrophic drive to the heart along with an increase in vagal outflow and baroreflex activity.

Correspondingly, the two recognized α_2-adrenoceptor agonists, clonidine and dexmedetomidine, decreased MAP and HR at all tested i.t. doses in anaesthetised rats. After dosing with fadolmidine, during the initial hypertension phase, HR decreased more slowly than after dexmedetomidine and clonidine administration probably due to its less prominent central sympatholytic mechanism immediately after i.t. dosing. These results are in line with earlier findings with dexmedetomidine, where a rapid decrease in BP and HR was noted following i.t. dosing (Nagasaka & Yaksh 1990). Clonidine evoked the well known biphasic effect on MAP, a central α_2-adrenoceptors mediated a depressor effect at low to moderate doses whereas

vascular α-adrenoceptors mediated a pressor response at higher doses (Horváth et al. 2002; Solomon et al. 1989). Dexmedetomidine and clonidine (Aantaa & Scheinin 1993) as lipophilic compounds can cross the blood-brain barrier and are rapidly absorbed systemically from the spinal space (Eisenach et al. 1996; Kawamata et al. 2003; Sabbe et al. 1994, Post et al. 1987; Solomon et al. 1989).

In dogs, as in rats, the trends of fadolmidine on BP were towards an initial hypertension with i.t. injection and hypotension following epidural injection, whereas with clonidine, the tendencies were towards hypotension with both dosing routes. The differences seen in the BP values likely reflect the different abilities of fadolmidine and clonidine to redistribute to the supraspinal space and hence to the periphery (Eisenach et al. 1999; Onttonen et al. 2000; Pertovaara and wei; Xu et al. 2000a, 2000b) reflecting at least partly their physicochemical properties as well as their efficacy at α_2-adrenoceptors (Aantaa & Scheinin 1993, Lehtimäki et al. 2008). The results are in line with the *in vitro* results of fadolmidine and clonidine. Clonidine is a lipophilic molecule with logP (partition coefficients (P), octanol/buffer pH 7.4 at 20 °C, UV spectroscopy) value of 1.6, molecular weight (MW) of 230.55, pKa values of 8.2, and polar surface area (PSA) of 36.4 (Remko et al. 2006). In comparison, fadolmidine is slightly less lipophilic than clonidine. The corresponding *in vitro* values of fadolmidine at pH 7.4 are: a logP value of 1.7, MW of 214.27, pKa of 6.8, and PSA of 48.9 (data on file, Orion Corporation). In the light of the existing documentation, fadolmidine would be expected to penetrate slightly less well through cell membranes and in addition, it would be predicted to be associated with reduced clearance and thus cause less systemic adverse effects than clonidine after spinal administration. The pharmacokinetic results of fadolmidine, a rapid clearance of the drug after reaching the systemic circulation, support those findings. However, fadolmidine does redistribute to some extent to the periphery and at higher doses, it may redistribute further to induce hypotension possibly by reducing spinal and/or supraspinal sympathetic activity. Nonetheless, the results are not in accordance with the earlier reported findings in sheep that no hemodynamic depression was observed after either the i.t. or epidural injection of fadolmidine (Eisenach et al. 1999) or clonidine (Eisenach et al. 1987). This suggests that as a species, sheep might be less sensitive to the hypotensive action of α_2-adrenergic agonists than humans (Eisenach & Dewan 1990; Eisenach et al. 1999).

The effects of fadolmidine and clonidine on safety paramaters e.g. BP and HR have been compared during a 24-h continuous i.t. infusion in dogs. At comparative analgesic doses, fadolmidine exerted no hypotensive effects whereas clonidine induced hypotension (Yaksh et al. 2003; Kroin et al. 2003): both compounds decreased HR. Based on the known pharmacokinetic profile of fadolmidine and clonidine (Houston 1982; Pettinger 1980), the infusion dose of clonidine (Castro &

Eisenach 1989; Yaksh et al. 1994) that was needed to maintain analgesia could lead to the drug's accumulation in the body during a 24-h continuous i.t. infusion.

In addition, the combination of a local anaesthetic bupivacaine and fadolmidine i.t. decreased HR but not MAP in dogs. In humans, bupivacaine alone has been shown to evoke dose-dependent cardiovascular adverse effects, such as hypotension (Patro et al. 2016; Russell 1982). Fadolmidine i.t. has been reported to induce vasoconstriction (Eisenach et al. 1999; Lehtimäki et al. 2008) and to reduce spinal cord blood flow in sheep (Eisenach et al. 1999) and this could have an effect on the distribution of co-administered spinal bupivacaine or vice versa. However, the pharmacokinetic results of i.t. administration fadolmidine in rats as tested with the analgesic effect or by its effects on MAP, sedation and BT following the combination administration in dogs indicated that either most of the drug remained at the site of application or there was rapid clearance after the drug reached the systemic circulation.

After i.c.v. administration of fadolmidine, the decrease in BP was smaller than that of dexmedetomidine, indicating that since fadolmidine is a more polar compound than dexmedetomidine, it does not transfer out of the ventricle as rapidly as dexmedetomidine. These results are line with the findings of Xu et al (2000a) where fadolmidine evoked a sedative effect following injection into LC but not if administered at 1-2 mm rostal to the LC whereas dexmedetomidine had sedative effects after injection into both areas.

The receptor binding profile indicated that fadolmidine has binding affinity also to the human serotonin 5-HT_3 receptor. The serotonin 5-HT_3 receptor is a ligand gated ion channel expressed in the periphery and CNS. It regulates the release of various neurotransmitters e.g. serotonin, acetylcholine and noradrenaline, thus acting as a neuromodulator in many tissues and organs having potential effects on nociception and/or cardiovascular effects (Cortes-Altamirano et al. 2018; Hayashida et al. 2012; Ramage & Villalon 2008). However, faldolmidine did not exert any 5-HT_3 related effects on BP and HR after i.v. dosing when evaluated *in vivo* Bezold-Jarisch reflex measurements in anaesthetised rats. In these phenomena, the afferent fibers originating in the heart and lungs react to the injection of substances such as serotonin by producing profound reductions in arterial BP and HR (Sévoz et al. 1997; Verberne & Guyenet, 1992).

In summary, fadolmidine injection induced only minor cardiovascular effects at analgesic i.t. and epidural doses, i.e. an increase and decrease on MAP concomitantly with a decrease in HR, whereas after clonidine and dexmedetomidine (only i.t. tested), hypotension was evident following both i.t. and epidural administrations.

6.6 Supplementary *in vivo* safety studies

The activation of presynaptic α_2-adrenergic receptors is known to mediate several responses in the GI tract. Dexmedetomidine and clonidine have been reported to inhibit GI transit both in rodents and in humans (Iirola et al. 2011). Fadolmidine inhibited GI transit dose-dependently after i.t. administration in rats, but most clearly above doses needed for analgesic effects whereas both dexmedetomidine and clonidine inhibited GI-transit times already at the analgesic doses; these are a recognized adverse effect of dexmedetomidine (Iirola et al. 2011) and clonidine (Ferder et al. 1987) in humans. Dexmedetomidine and clonidine administered i.t. inhibited GI transit as has been reported following their systemic administration (Asai et al. 1997; Asai et al. 1997; Zádori et al. 2007) in rats. This parasympathetic effect is a characteristic of α_2-adrenoceptors agonists and is probably mostly peripheral, although part of it is believed to have a central origin (Asai et al. 1997; Fülöp et al. 2005). Furthermore, in rats, clonidine delivered i.v. was reported to reduce the gastric motility via the activation of presynaptic α_2-adrenoceptors (Zádori et al. 2007).

Adrenergic α_2-adrenoceptor agonists are known to induce a centrally mediated increase in urine output by lowering antidiuretic hormone and vasopressin secretion. In addition, peripherally α_2-adrenoceptor agonists mediated increase in sodium excretion in the kidney (Cabral et al. 1998; Miller et al. 2001, Sinclair 2003). In humans, clonidine decrease plasma renin activity (Vahabi & Kazemi 2011) and renovascular resistance (Nguyen et al. 2017) increasing urine output and electrolyte excretion. I.t. fadolmidine in rats increased dose-dependently urine output, sodium ion concentration and excretion, whereas i.v. doses increased only sodium ion concentration and excretion. The increase in sodium excretion is at least may be partially mediated by central modulation of α_2-adrenergic agonists since fadolmidine given i.t. was more potent than when delivered via i.v. doses. In anaesthetised rats, fadolmidine interrupted volume-evoked voiding cycles and induced urine over flow incontinence at high concentrations; in the analgesic dose range, the effects were mild. The effects of α_2-adrenoceptor agonists on micturition reflex were reported also in mice (Aro et al. 2015) and rats (Kontani et al. 2000; Streng et al. 2010). In the analgesic dose range (in rat tail-flick tests) both i.t. fadolmidine and clonidine appeared to be only slightly or moderately inhibitory whereas the effects of dexmedetomidine i.t. were more profound. As a comparison, morphine i.t inhibited the micturition cycles totally already at a dose below the ED_{50} analgesic dose. In the clinic, it is known that morphine inhibits micturition and can cause urine retention (Fernandez et al. 2014; Kleinmann & Wolter 2017).

The results *in vivo* studies of Gi motility, kidney and urodynamic functions indicate that the effects of fadolmidine on those parameters were seen at the higher that at the analgesic i.t. dose.

7 Summary

In summary, fadolmidine is a potent full α_2-adrenoceptor agonist of all three human α_2-adrenenoceptor subtypes and is being developed for spinal analgesia. In the present studies, fadolmidine induced dose-dependent analgesia in acute pain models following i.t. administration e.g. in a rat tail-flick test, and i.t. and epidural administration as well as during 24-h continuous i.t. infusion in a dog skin twitch test. When injected epidurally, the antinociceptive potency of fadolmidine was weaker than when administered i.t. Furthermore, fadolmidine as an adjuvant to a local anaesthetic bupivacaine i.t. evoked a synergistic analgesic effect and prolonged the duration of bupivacaine's sensory motor block in both rats and dogs. Spinally administered fadolmidine induced sedation and hypothermia, decreased heart rate and the respiratory rate, and initially increased and then decreased blood pressure. The results are summarized in Table 16. In addition, there was evidence that i.t. fadolmidine could inhibit gastrointestinal transit, interrupt volume-evoked voiding cycles and induce urine over-flow incontinence as well as evoke a mydriatic response. However, those typical α_2-adrenoceptors mediated adverse effects of fadolmidine were only present at rather high spinal doses/concentrations and mainly doses above the doses (ED_{50} values) that were needed to observe analgesic effects (ratio >1). In contrast, two established α_2-adrenoceptors agonists, dexmedetomidine and clonidine, exerted those adverse effects already at analgesic doses (ratio ≤1). In addition to the potency of these compounds at the α_2-adrenoceptor, the differences in their effects are attributable at least in part to the physicochemical and pharmacokinetic properties of fadolmidine. In contrast to clonidine and dexmedetomidine, fadolmidine is a polar compound and does not pass well across the blood-brain barrier and further distribute into the CNS following spinal administration. Furthermore, the pharmacokinetic results in rats indicate that either most of the i.t. administered fadolmidine remained at the site of application or was rapidly eliminated after reaching the systemic circulation.

These results support the hypothesis that i.t. administered fadolmidine could have potential use as a spinal analgesic evoking only with minor subraspinal or spinal adverse effects at analgesic doses. Furthermore, an i.t. infusion of fadolmidine could be a way to provide long-term antinociception with less of the known use-limiting

adverse effects such as hypotension associated with clonidine. In addition, fadolmidine as an adjuvant to bupivacaine was able to enhance the latter's analgesic effect without hypotension and thus these agents could represent a suitable combination for spinal anaesthesia.

Table 16. Potency of spinal (analgesic) and some supraspinal and peripheral *in vivo* pharmacological effects (i.e. sedation, hypothermia, mydiatic response, hypotension, hypertension and inhibition of GI motility) of i.t. and epidural fadolmidine, clonidine and dexmedetomidine in rats (I, II, IV) and dogs (III, IV).

PARAMETER	FADOLMIDINE	CLONIDINE	DEXMEDETOMIDINE
Analgesia [a]			
- **rat, i.t.**	0.73[I)], 1.0*[,IV)]	6.4[I)]	2.2[I)]
- **Dog, i.t.**	67[III)], ≤60*[,IV)]	78[III)]	-
- **Dog, epidural**	128[III)]	51[III)]	-
Sedation[b]			
- **rat, i.t.**	19[I)], 30*[,II)], 10*[,IV)]	4.0[I)], 10*[,II)]	0.63[I)]
- **Dog, i.t.**	100[III)], >60*[,IV)]	100[III)]	-
- **Dog, epidural**	100[III)]	100[III)]	-
Hypothermia[c]			
- **Rat, i.t.**	10[I)], 3.0[II)],10[IV)]	4.0[I)], 10[II)]	1.4[I)]
- **Dog, i.t.**	300[III)], >60[IV)]	100[III)]	-
- **Dog, epidural**	100[III)]	100[III)]	-
Mydriatic response[D], **rat, i.t.**	10[I)]	3[I)]	0.3[I)]
Hypotension[E]			
- **rat, i.t.**	1.0[I)], 10[II)]	<0.3[I)], 10[II)]	<0.1[I)]
- **dog, i.t.**	100[III)], >60[IV)]	30[III)]	-
- **dog, epidural**	30[III)]	30[III)]	-
Hypertension[F]			
- **rat, i.t.**	3.0[I)], 10[II)]	N/A[I)], 30[II)]	N/A[I)]
- **dog, i.t.**	100[III)], >60[IV)]	N/A[III)]	-
- **dog, epidural**	100[III)]	N/A[III)]	-
inhibition of GI motility[G], **rat, i.t.**	18[I)]	4.9[I)]	1.3[I)]
ratio			
sedation / analgesia			
- **rat, i.t.**	10–26	0.6–1.6	0.3
- **Dog, i.t.**	1.5	1.3	-
- **dog, epidural**	0.8	2.0	-
hypothermia / analgesia			
- **rat, i.t.**	10–14	0.6	0.6
- **dog, i.t.**	4.5	1.3	-
- **dog, epidural**	0.8	2.0	-
Mydriatic response / analgesia, rat, i.t.	14	0.5	0.1

PARAMETER	FADOLMIDINE	CLONIDINE	DEXMEDETOMIDINE
Hypotension / Analgesia			
- **rat, i.t.**	1.4–13.7	1.6	N/A
- **Dog, i.t.**	1.5	0.4	-
- **dog, epidural**	0.2	0.6	-
hypertension / analgesia			
- **rat, i.t.**	4.1–13.7	4.7	N/A
- **dog, i.t.**	1.5	N/A	-
- **dog, epidural**	0.8	N/A	-
inhibition of GI motility / analgesia, rat, i.t.	25	0.8	0.6

i.t. = intrathecal, N/A = not applicable, [*)] Tested dose (µg/animal), [A] Tail-flick test in rats and skin twitch test in dogs, ED_{50} (µg/animal), [B] Decrease in motor activity (I, II) or rotarod performance (IV) in rats [ED_{50} (µg/animal)], and muscle tone (score) in dogs, [C] Estimated -1°C in BT in rats and dogs [D] No statistical significant dose in mydriatic response in rats, [E] No statistical significant dose in hypotension in rats and dogs, [F] Initial increase in BP (hypertension) in rats and dogs, [G] Inhibition of gastrointestinal (GI) transit in rats, [I), II), III), IV)] Original publications I, II, III, IV, respectively.

Acknowledgements

This study was carried out in Orion Corporation, Orion Pharma, Research and Development, Turku with collaboration from the Department of Biomedicine, Pharmacology, Drug Development and Therapeutics, University of Turku.

My greatest thanks to my supervisors Professor Antti Haapalinna from Research, Orion Corporation and Professor Ullamari Pesonen from the Department of Biomedicine, University of Turku for their guidance, experience and support in this thesis. I wish to thank my supervisor Raimo Virtanen PhD and Professor Antti Haapalinna for the possibility to undertake my dissertation work while doing my full-time work at Orion. Your enthusiastic commitment to research, your support and mentoring during my research and the inspiring atmosphere in Orion have been very important to me. Furthermore, Raimo and Antti, I am very grateful that I was able to visit Professor Tony Yaksh's laboratory in the University of California where I obtained valuable experience and know-how on how to performing spinal and epidural catheterizations as well as on the perioperative care of experimental animals. Professor Yaksh is a very inspiring teacher. In addition, thanks to Kjersti Horais BSc for her valuable tips on how to carry out these experiments. I thank my supervisors Timo Lotta PhD and Eija Aho PhD for allowing me to participate in the scientific meetings organized by the Drug Research Doctoral Programme during the last years.

I wish to thank the members of my thesis steering group, Professor Ullamari Pesonen, Professor Markku Koulu and Professor Antti Haapalinna for all the feedback and support that they have given me throughout this PhD work. Your guidance, experience and valuable positive feedback have always been very encouraging

Professor Heikki Ruskoaho and Adjunct Professor Vesa Kontinen are warmly acknowledged for their critical review of this thesis, comments and suggestions to improve this work.

I want to express my sincere thanks to my other co-authors Jyrki Lehtimäki, Timo Viitamaa, Jarmo S. Salonen PhD and Ari Koivisto PhD. Your different scientific backgrounds, expertise and knowledge have been very inspiring. I have learnt a lot from you. A special thank you to Jyrki for our scientific discussions and

other talks over the years as well as co-operation and support. I also want to thank Ewen MacDonald Pharm D for the valuable comments and for revising the language of the thesis. I send a big vote of thanks to Pasi Hakulinen for professional discussions and advice with the statistical analysis.

Very special thanks to all my long-term colleagues in Orion pharmacology/ safety research for your expertise, knowledge, scientific and technical support and friendships. Thanks to all of you, Tarja Kuosmanen, Merja Ojala, Kirsti Nieminen, Lauri Nieminen, Stefan Karlsson, Marja-Leena Toivonen and the others.

In addition, I would like to thank Anne Alatupa, Päivi Saikkonen, Mari Hassinen, Leena Hellman, Leena Berg, Raili Harvanto, Leila Hytönen, Seija Knuutila, Leena Yli-Alho, Liisa Erkkilä and Auli Vähämäki for their superb technical assistance. I thank the Drug Research Doctoral Programme and Eeva Valve for organizing the interesting scientific meetings.

Finally, thanks to my nearest and dearest, to my husband Kalle who has had **endless patience** and has supported me throughout this thesis and I am also grateful for our children, Saara and Otto who have been my joy during these years. Your support, encouragement and understanding have been extremely important to me.

Research costs are financed Orion Corporation, Orion Pharma.

Piikkiö, 24th November 2020
Tiina Leino

References

Aantaa, R. & Scheinin, M., 1993. Alpha2-adrenergic agents in anaesthesia. *Acta Anaesthesiol Scand,* 37(5), p. 433–448.

Abdolmohammadi, S., Hétu, P.O., Héron, A. & Blaise, G., 2015. Efficacy of an intrathecal multidrug infusion for pain control in older adults and in end-stage malignancies: a report of three cases. *Pain Res Manag*, 20(3), p. 118–122.

Adolfsson, P.I., Dahle, L.O., Berg, G. & Svensson, S.P., 1998. Characterization of alpha2-adrenoceptor subtypes in pregnant human myometrium. *Gynecol Obstet Invest*, 45(3), p. 145–150.

Aghajanian, G.K. & Van der Maelen, C.P., 1982. Alpha 2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: intracellular studies in vivo. *Science*, 215(4538), p. 1394–1396.

Aho, M.S., Erkola, O.A., Scheinin, H., Lehtinen, A.M. & Korttila, K.T., 1991. Effects of intravenously administerd dexmedetomidine on pain after laparoscopic tubal ligation. Anest Analg, 73(2), p. 112–118.

Alam, M.N. & Mallick, B.N., 1994. Role of lateral preoptic area alpha-1 and alpha-2 adrenoceptors in sleep-wakefulness and body temperature regulation. *Brain Res Bull,* 35(2), p. 171–177.

Allen, J.W. & Yaksh, T.L., 2004. Assessment of acute thermal nociception in laboratory animals. *Methods Mol Med,* 99, p. 11–23.

Almeida, T.F., Roizenblatt, S. & Tufik, S., 2004. Afferent pain patways: a neuroanatomical review. *Brain Res*, 1000(1–2), p. 40–56.

Ansah, O.B. & Pertovaara, A., 2007. Peripheral suppression of arthritic pain by intraarticular fadolmidine, an α_2-adrenoceptor agonist, in the rat. *Anesth Anal*, 105(1), p. 245–250.

Ansonoff, M.A. & Etgen, A.M, 2001. Receptor phosphorylation mediates estradiol reduction of α_2-adrenoceptor coupling to G protein in the hypothalamus of female rats. *Endocrine*, 14(2), p. 165–174.

Antkiewicz-Michaluk, L., Romańska, I., Wasik, A. & Michaluk, J., 2017. Antidepressant-like effect of endogenous neuroprotective amines, 1MeTIQ in clonidine-induced depression: behavioral and neurochemical studies in rats. *Neurotox Res*, 32(1), p. 94–106.

Arima, J., Kubo, C., Ishibashi, H. & Akaike, N., 1998. Alpha2-adrenoceptor-mediated potassium currents in acutely dissociated rat locus coeruleus neurones, *J Physiol*, 508(Pt 1), p. 57–66.

Aro, E., Bastman, S., Andersson, K.E. & Streng, T., 2015. Is there a peripheral site of action contributing to the voiding effects of α_2-adrenoceptor agonists and antagonists? *World J Urol*, 33(3), p. 433–440.

Asai, T., Mapleson, W.W. & Power, I., 1997. Different effects of clonidine and dexmedetomidine on gastric emptying and gastrointestinal transit in the rat. Br J Anaesth, 78(3), p. 301–307.

Asai, T., Vickers, M.D. & Power, I., 1997. Clonidine inhibits gastric motility in the rat. *Eur J Anaesthesiol*, 14(3), p. 316–319.

Asano, T, Dohi, S., Ohta, S., Shimonaka, H. & Ida, H., 2000. Antinociceptin by epidural and systemic alpha2-adrenoceptor agonists and their binding affinity in rat spinal cord and brain. *Anesth Analg*, 90(2), p. 400–407.

Atchison, S.R., Durant, P.A.C. & Yaksh, T.L., 1986. Cardiorespiratory effects and kinetics of intrathecally injected D-Ala^2-D-Leu^5-enkephalin and morphine in unanaesthetized dog. *Anesthesiology*, 65(6), p. 609–616.

Bahari, Z. & Meftahi, G.H., 2019. Spinal α_2-adrenoceptors and neuropathic pain modulation; therapeutic target. *Br J Pharmacol*, 176(14), p. 2366–2381.

Bajic, D., Van Bockstaele, E.J. & Proudfit, H.K., 2001. Ultrastructural analysis of ventrolateral periqueductal gray projectons to A7 catecholamine cell group. Neuroscience, 104(1), p. 181–197.

Bajwa, S.J.S., Bajwa, S.K., Kaur, J., Singh, A., Singh, A. & Parmar, S.S., 2012. Prevention of hypotension and prolongation of postoperative analgesia in emergency ceasarean sections: a randomized study with intrathecal clonidine. *Int J Crit Illn Inj Sci*, 2(2), p. 63–69.

Baldini, A., Von Korff, M. & Lin, E.H., 2012. A review of potential adverse effects of long-term opioid theraphy: A practioner's guide. Prim *Care Companion CNS Disord*, 14(3). PCC.

Balldin, J., Berggren, U., Eriksson, E., Lindstedt, G. & Sundkler, A., 1993. Guanfacine as an alpha-2 agonist inducer of growth hormone secretion – a comparison with clonidine. *Psychoneuroendocrinology*, 18(1), p. 45–55.

Bannon, A.W. & Malmberg, A.B., 2007. Models of nocipetion: hot-plate, tail-flick, and formalin test in rodents. *Curr Protoc Neurosci,* 41(1), p. 8.9.1–8.9.16.

Barrot, M., 2012. Test and models of nociception and pain in rodents. *Neuroscience*, 211, p. 39–50.

Basbaum, A.I., Bautista, D.M., Scherrer, G. & Julius, D., 2009. Cellular and molecular mechanisms of pain. *Cell*, 139(2), p. 267–284.

Basbaum, A. & Fields, H.L., 1984. Endogenous pain control systems: brainstem spinal pathways and endorphine circuitry. *Annu Rev Neurosci*, 7, p. 309–338.

Bedder, M.D., Kozody, R., Palahniuk, R.J., Cumming, M.O. & Pucci, W.R., 1986. Clonidine prolongs canine tetracaine spinal anaesthesia. *Can Anaesth Soc J*, 33(5), p. 591–596.

Belleville, J.P., Ward, D.S., Bloor, B.C. & Maze, M., 1992. Effects of intravenous dexmedetomidine in humans. I sedation, ventilation, and metabolic rate. *Anesthesiology*, 77(6), 1125–1133.

Berger, A.C. & Whistler, J.L., 2010. How to design an opioid drug that causes reduced tolerance and dependence. *Ann Neurol*. 67(5), p. 559–569.

Berry, H. & Hutchinson, D.R., 1988. Tizanidine and ibuprofen in acute low-back pain: results of a double-blind multicenter study in general practice. *J Int Med Res*, 16(2), p. 83–91.

Blandizzi, C., Natale, G., Colucci, R., Carignani, D., Lazzeri, G. & Del Tacca, M., 1995. Characterization of alpha 2-adrenoceptor subtypes involved in the modulation of gastric acid secretion. *Eur J Pharmacol*, 278(2), p. 179–182.

Bloor, B.C., Frankland, M., Alper, G., Raybould, D., Weitz., J. & Shurtliff, M., 1992. Hemodynamic and sedative effects of dexmedetomidine in dog. *J Pharmacol Exp Ther*, 263(2), p. 690–697.

Bloor, B.C., Ward, D.S., Belleville, J.P. & Maze, M., 1992. Effects of intravenous dexmedetomidine in humans. II hemodynamic changes. *Anesthesiology*, 77(6), p. 1134–1142.

Bouret, S. & Sara S.J., 2005. Network reset: a simplified overarching theory of locus coeruleus noradrenaline function. *Trends Neurosci*, 28(11), p. 574–582.

Brummett, C.M., Hong, E.K., Janda, A.M., Amodeo, F.S. & Lydic, R.L., 2011. Perineural dexmedetomidine added to ropivacaine for sciatic nerve block in rats prolongs the duration of analgesia by blocking the hyperpolarization-activated cation current. *Anesthesiology*, 115(4), p. 836–843.

Burke, J.A & Potter, D.E., 1986. Ocular effects of a relatively selective α2 agonist (UK-14, 304-18) in cats, rabbits and monkey. Curr Eye Res, 5(9), p. 665–676.

Burton, S.A., Lemke, K.A., Ihle, S.L. & Mackenzie, A.L., 1997. Effects of medetomidine on serum insulin and plasma glucose concentrations in clinically normal dogs. *Am J Vet Res*, 58(12), 1440–1442.

Bylund, D.B., 2007. Alpha- and beta-adrenergic receptors: Ahlquist's landmark hypothesis of a single mediator with two receptors. *Am J Physiol Endocrinol Metab*, 293(6), p. E1479–E1481.

Bylund, D.B., Blaxall, H.S., Iversen, L.J., Caron, M.G., Lefkowitz, R.J. & Lomasney, J.W., 1992. Pharmacological characteristics of alpha 2-adrenergic receptors: comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning. *Mol Pharmacol*, 42(1), p. 1–5.

Cabral A.D., Kapusta D.R., Kenigs V.A. & Varner K.J., 1998. Central alpha2-receptor mechanisms contribute to enhanced renal responses during ketamine-xylazine anesthesia. *Am J Physiol*, 275(6), p. R1867–1874.

Carroll, D., Jadad, A., King, V., Wiffen, P., Glynn, C. & McQuay, H., 1993. Single-dose, randomized, bouble-blind, bouble-dummy cross-over comparison of extradural and i.v. clonidine in chronic pain. *Br J Anaesth*, 71(5), p. 665–669.

Castro, M.I. & Eisenach, J.C., 1989. Pharmacokinetics and dynamics of intravenous, intrathecal, and epidural clonidine in sheep. *Anesthesiology*, 71(3), p. 418–425.

Chan, A.K., Cheung, C.W. & Chong, Y.K., 2010. Alpha-2 agonists in acute pain management. Expert *Opin Pharmacother*, 11(17), p. 2849–2868.

Chiari, A., Lorber, C., Eisenach, J.C., Wildling, E., Krenn, C., Zavrsky, A., Kainz, C., Germann, P. & Klimscha, W., 1999. Analgesic and hemodynamic effects of intrathecal clonidine as the sole analgesic agent during first stage of labor. *Anesthesiology*, 91(2), p. 388–396.

Clark, F.M. & Proudfit, H.K., 1993. The projections of noradrenergic neurons in the A5 cathecolamine cell group to the spinal cord in the rat: anatomical evidence that A5 neurons modulate nociception. *Brain Res,* 616(1–2), p. 200–210.

Cohen, M., Zuk, J., McKay, N., Erickson, M., Pan, Z. & Galinkin, J., 2017. Intrathecal morphine versus extended-release epidural morphine for postoperative pain control in pediatric patients undergoing posterior spinal fusion. *Anesth Analg*, 124(6), p. 2030–2037.

Cooper, J.R., Bloom, F.E. & Roth R.H., 1991. 9. Norepinephrine and epinephrine, 10. Dopamine. *In* The Biochemical Basis of Neuropharmacology, Oxford University Press, New York & Oxford, p. 220–337.

Cornish, L.A., 1988. Guanfacine hydrochloride: a centrally acting antihypertensive agent. *Clin Pharm*, 7(3), p. 187–197.

Correa-Sales, C., Rabin, B. & Maze, M., 1992. A hypnotic response to dexmedetomidine, an α_2 agonist, is mediated in the locus coeruleus in rats. *Anesthesiology*, 76(6), p. 948–952.

Cortes-Altamirano J.L., Olmos-Hernandez A., Jaime H.B., Carrillo-Mora P., Bandala C., Reyes-Long S., Alfaro-Rodríguez A., 2018. Review: 5-HT1, 5-HT2, 5- HT3 and 5-HT7 receptors and their role in the modulation of pain response in the central nervous system. *Curr Neuropharmacol*, 16(2), 210–221.

Cruickshank, M., Henderson, L., MacLennan, G., Faser, C., Campbell, M., Blackwood, B., Gordon, A. & Brazzel, M., 2016. Alpha-2 agonists for sedation of mechanically ventilated adults in intensive care units: a systematic review. *Health Technol Assess*, p. 20(25), p. 1–117.

Davis, M., Commissaris, R.L., Yang, S., Wagner, K.R., Kehne, J.H., Cassella, J.V. & Boulis, N.M., 1989. Spinal vs. supraspinal sites of action of the α_2-adrenergic agonists clonidine and ST-91 on the acoustic startle reflex. *Pharmacol Biochem Behav*, 33(1), p. 233–240.

Day, H.E., Campeau, S., Watson Jr, S.J. & Akil, H., 1997. Distribution of alpha 1a-, alpha 1b- and alpha 1d- adrenergic receptor mRNA in the rat brain and spinal cord. *J Chem Neuroanat*, 13(2), 115–139.

Deer, T.R., Malinowski, M, Varshney, V. & Pope, J., 2019. Choice of intrathecal drug in the treatment of neuropathic pain – new research and opinion. Expert Rev Clin Pharmacol, 12(10), p. 1003–1007.

Deer, T.R., Pope, J.E., Hayek, S.M., Lamer, T.J., Veizi, I.E., Erdek, M., Wallace, M.S., Grider, J.S., Levy, R.M., Prager, J., Rosen, S.M., Saulino, M., Yaksh, T.L., De Andrès, J.A., Gonzales, D.A., Vesper, J., Schu, S., Simpson, B. & Mekhail, N., 2017. The polyanalgesic consensus conference (PACC): recommendations for intrathecal drug delivery: guidance for improving safety and mitigating risks. *Neuromodulation*, 20(2), p. 96–132.

De Kock, M., Gautler, P., Fanard, L., Hody, J.L & Lavand'homme, P., 2001. Intrathecal ropivacaine and clonidine for ambulatory knee arthroscopy. *Anesthesiology*, 94(4), p. 574–578.

Delaney, A.J., Crane, J.W. & Sah, P., 2007. Noradrenaline modulates transmission at a central synapse by a presynaptic mechanism. *Neuron*, 56(5), p. 880–892.

De Sarro, G.B., Ascioti, C., Froio, F., Libri, V. & Nisticò, G., 1987. Evidence the locus coeruleus is the site where clonidine and drugs acting at alpha 1- and alpha 2- adrenoceptors affect sleep and arousal mechanisms. *Br J Pharmacol,* 90(4), p. 675–685.

Detweiler, D.J., Eisenach, J.C., Tong, C. & Jackson., 1993. A cholinergic interachtion in alpha 2 adrenoceptor-induced antinociception in sheep. *J Pharmacol Exp Ther*, 265(2), p. 536–542.

D'Mello, R. & Dickenson, A.H., 2008. Spinal cord mechanism of pain. *Br J Anaesth*, 101(1), p. 8–16.

Dobrydnjov, I., Axelsson, K., Samarütel, J. & Holmström, B., 2002. Postoperative pain relief following intrathecal bupivacaine combined with intrathecal or oral clonidine. *Acta Anaesthesiol Scand*, 46(7), p. 806–814.

Doody, O. & Bailey, M.E., 2019. Understanding pain physiology and its application to person with intellectual disability. *J Intellect Disabil,* 23(1), 5–18.

Drew, G.M., Gower, A.J. & Marriott, A.S., 1979. Alpha 2-adrenoceptors mediate clonidine-induced sedation in the rat. *Br J Pharmacol*, 67(1), p. 133–141.

Durant, P.A.C. & Yaksh, T.L., 1986. Distribution in cerebrospinal fluid, blood, and lymph of epidurally injected morphine and inulin in dogs. *Anesth Analg,* 65(6), p. 583–592.

Eisenach, J.C., De Kock, M. & Klimscha, W., 1996. α_2-adrenergic agonists for regional anesthesia: a clinical review of clonidine (1984–1995). *Anesthesiology*, 85(3), p. 655–674.

Eisenach, J.C. & Dewan, D.M., 1990. Intrathecal clonidine in obstetrics: sheep studies. *Anesthesiology*, 72(4), p. 663–668.

Eisenach, J.C., Dewan, D.M., Rose, J.C. & Angelo, J.M., 1987. Epidural clonidine produces antinociception, but not hypotension, in sheep. *Anesthesiology*, 66(4), p. 496–501.

Eisenach, J.C. & Grice, S.C., 1988. Epidural clonidine does not decrease blood pressure or spinal cord blood flow in awake sheep. *Anesthesiology*, 68(3), p. 335–340.

Eisenach, J.C., Lavand'homme, P., Tong, C., Cheng, J.K., Pan, H.L., Virtanen, R., Nikkanen, H. & James, R., 1999. Antinociceptive and hemodynamic effects of a novel α_2-adrenergic agonist, MPV-2426, in sheep. *Anesthesiology*, 91(5), p. 14255–1436.

Eisenach, J.C., Rauck, R.L, Buzzanell, C. & Lysak, S.Z., 1989. Epidural clonidine analgesia for intractable cancer pain: phase I. *Anesthesiology*, 71(5), p. 647–652.

Eisenach, J.C., Shafer, S.L., Bucklin, B.A., Jackson, C. & Kallio, A., 1994. Pharmacokinetics and pharmacodynamics of intraspinal dexmedetomidine in sheep. *Anesthesiology*, 80(6), p. 1349–1359.

Eisenach, J.C. & Tong, C.Y., 1991. Site of hemodynamic effects of intrathecal alpha 2-adrenergic agonists. *Anesthesiology,* 74(4), p. 766–771.

Engelman, E. & Marsala, C., 2013. Efficacy of adding clonidine to intrathecal morphine in acute postoperative pain: meta-analysis. *Br J Anaesth*, 110(1), p. 21–27.

Erdine, S. & De Andrés, J., 2006. Drug delivery systems. *Pain Pract,* 6(1), p. 51–57.

Fagerholm, V., Haaparanta, M. & Scheinin, M., 2011. α2-Adrenoceptor regulation of blood glucose homeostasis. *Basic Clin Pharmacol Toxicol*, 108(6), p. 365–370.

Feldman, H.S. & Covino, B.G., 1981. A chronic model for investigation of experimental spinal anesthesia in the dog. *Anesthesiology*, 54(2), p. 148–152.

Ferder, L., Inserra, F, Medina, F., 1987. Safety aspects of long-term antihypertensive therapy (10 years) with clonidine. *J Cardiovasc Pharmacol*, 10(Supp 12), p. 104–108.

Fernandez, M.A., Karthikeyan, S., Wyse, M. & Foguet, P., 2014. The incidence of postoperative urinary retention in patients undergoing elective hip and knee arthroplasty. *Ann R Coll Surg Engl,* 96(6), p. 462–465.

Fields, H.L., Heinricher, M.M. & Mason, P., 1991. Neurotransmitters in nociceptive modulatory circuits. *Annu Rev Neurosci*, 14, p. 219–245.

Filos, K.S., Goudas, L.C., Patroni, O. & Polyzou, V., 1994. Hemodynamic and analgesic profile after intrathecal clonidine in human. *Anesthesiology*, 81(3), p. 591–601.

Fülöp, K., Zádori, Z., Rónai, A.Z. & Gyires, K., 2005. Characterisation of alpha2-adrenoceptor subtypes involved in gastric emptying, gastric motility and gastric mucosal defence. *Eur J Pharmacol*, 528(1–3), p. 150–157.

Fürst, S., 1999. Transmitters involved in antinociception in the spinal cord. *Brain Res Bull*, 48(2), p. 129–141.

Gabriel, J.S. & Gordin, V., 2001. Alpha 2 agonists in regional anesthesia and analgesia. *Curr Opin Anaesthesiol*, 14(6), p. 751–753.

Ganesh, M. & Krishnamurthy, D., 2018. A comparative study of dexmedetomidine and clonidine as an adjuvant to intrathecal bupivacaine in lower abdominal surgeries. *Anesth Essays Res*, 12(2), p. 539–545.

Gáspár, R., Gál, A., Gálik, M., Ducza, E., Minorics, R., Kolarovski-Sipiczki, Z., Klukovits, A. & Falkay, G., 2007. Different roles of α_2-adrenoceptor subtypes in non-pregnant and late-pregnant uterine contractility in vitro in the rat. *Neurochem Int*, 51(5), p. 11–18.

Geneen, L.J., Moore, R.A., Clarke, C., Martin, D., Colvin, LA & Smith, B.H., 2017. Physical activity and exercise for chronic pain in adults: an overwiew of cochrane reviews. *Cochrane Databse Syst Rev,* 1(1):CD011279.

Ghafoor, V.L., Epshteyn, M., Carlson, G.H., Terhaar, D.M., Charry, O. & Phelps, P.K., 2007. Intrathecal drug theraphy for long-term pain management. *Am J Health Syst Pharm,* 64(23), p. 2447–2461.

Giovannitti Jr, J.A., Thoms, S.M. & Crawford, J.J., 2015. Alpha-2 adrenergic receptor agonists: a review of current clinical applications. *Anesth Prog*, 62(1), p. 31–38.

Giovannoni, M.P., Ghelardini, C., Vergelli, C. & Dal Piaz, V., 2009. Alpha2-gonists as analgesic agents. *Med Res Rev*, 29(2), p. 339–368.

Gowing, L., Farrell, M., Ali, R. & White, J.M., 2016. Alpha2-adrenergic agonists for management of opoid withdrawal. *Cochrane Database Syst Rev*, 2016(5), CD002024.

Grangier, L., Martinez de Tejada, B., Savoldelli, G.L., Irion, O. & Haller, G., 2020. Adverse side effects and route of administration of opioids in combined spinal-epidural analgesia for labour: a meta-analysis of randomised trials. *Int J Obstet Anesth,* 41, p. 83–103.

Greenfield, D.S., Liebmann, J.M. & Ritch, R., 1997. Brimonidine: a new alpha2-adrenoreceptor agonist for glaucoma treatment. *J Glaucoma*, 6(4), p. 250–258.

Gregory, J. & McGowan, L., 2016. An examination of the prevalence of acute pain for hospital adult patients: a systematic review. *J Clin Nurs*, 25(5–6), p. 583–598.

Grimsrud, K.N., Ait-Oudhia, S., Durbin-Johnson, B.P., Rocke, D.M., Mama, K.R., Rezende, M.L., Stanley, S.D. & Jusko, W.J., 2015. Pharmacokinetic and pharmacodynamic analysis comparing diverse effects of detomidine, medetomidine, and dexmedetomidine in the horse: a population analysis. *J Vet Pharmacol Ther*, 38(1), p. 24–34.

Groh, A., Krieger, P., Mease, R.A. & Henderson, L., 2018. Acute and chronic pain processing in the thalamocortical system of humans and animal models. *Neuroscience*, 387, p. 58–71.

Guo, T.Z., Jiang, J.Y., Buttermann, A.E. & Maze, M., 1996. Dexmedetomidine injection into the locus ceruleus produces antinociception. *Anesthesiology*, 84(4), p. 873–881.

Guyenet, P.G., Stornetta, R.L., Riley, T., Norton, F.R., Rosin, D.L. & Lynch, K.R., 1994. Alpha2A-adenergic receptors are present in lower brainstem catecholaminergic and serotonergic neurons innervating spinal cord. *Brain Res*, 638(1–2), p. 285–294.

Gyires, K., Zádori, Z.S., Török, T. & Mátyus, P., 2009. α_2-Adrenoceptor subtypes-medeated physiological, pharmacological actions. *Neurochem Int,* 55(7), p. 447–453.

Haapalinna, A., Leino, T. & Heinonen, E., 2003. The alpha 2-adrenoceptor antagonist atipamezole potentiates anti-Parkinsonian effects and can reduce the adverse cardiovascular effects of dopaminergic drugs in rats. *Naynyn Schmiedebergs Arch Pharmacol*, 368(5), p. 342–352.

Haapalinna, A., Viitamaa. T., MacDonald, E., Savola, J.M, Tuomisto, L., Virtanen, R. & Heinonen, E., 1997. Evaluation of the effects of a specific α_2-adrenoceptor antagonist, atipamazole, on α_1- and α_2-adrenoceptor subtype binding, brain neurochemistry and behavior in comparison with yohimbine. *Naunyn Schmiedebergs Arch Pharmacol,* 356(5), 570–582.

Hammond, D.L., Tyce, G.M. & Yaksh, T.L., 1985. Efflux of 5-hydroxytryptamine and noradrenaline into spinal cord superfusates during stimulation of the rat medulla. *J Physiol*, 359, p. 151–162.

Harada, T. & Constantinou, C.E., 1993. The effect of alpha2 agonists and antagonists on the lower urinary tract of the rat. *J Urol*, 149(1), p. 159–164.

Harrison, R. & Kaufmann, C.S., 1977. Clonidine. Effects of a topically administered solution in intraocular pressure and blood pressure in open-angel glaucoma. *Arch Ophthalmol*, 95(8), p. 1368–1373.

Hassenbusch, S.J., Gunes, S., Wachsman, S. & Willis, K.D., 2002. Intrathecal clonidine in the treatment of intractable pain: a phase I/II study. *Pain Med*, 3(2), p. 85–91.

Hayashida K., Kimura M., Yoshizumi M., Hobo S., Obata H., Eisenach J.C., 2012. Ondansetron reverses antihypersensitivity from clonidine in rats after peripheral nerve injury: role of γ-aminobutyric acid in α2-adrenoceptor and 5-HT3 serotonin receptor analgesia. *Anesthesiology*, 117(2), p. 389–398.

Hayes, A.G., Skingle, M. & Tyers, M.B., 1986. Alpha-adrenoceptors-mediated antinociception and sedation in the rat and dog. *Neuropharmacology*, 25(4), p. 391–396.

Hayhurst, C. J. & Durieux, M.E., 2016. Differential opioid tolerance and opioid-induced hyperalgesia. *Anesthesiology*, 124(2), p. 483–488.

Heal, D.J., Prow, M.R., Butler, S.A. & Buckett, W.R., 1995. Mediation of mydriasis in conscious rats by central postsynaptic alpha2-adrenoceptors. *Pharmacol Biochem Behav*, 50(2), p. 219–224.

Herrero, J.F., Laird, J.M.A., Lopez-Garcia, J.A., 2000. Wind-up of spinal cord neurons and pain sensation: much ado about something? *Prog Neurobiol,* 61(2), p. 169–203.

Hisa, H., Araki, S., Tomura, Y., Hayashi, Y. & Satoh, S., 1989. Effects of alpha adrenoceptors blockade on renal nerve stimulation-induced norepinephrine release and vasoconstrction in the dog kidney. *J Pharmacol Exp Ther*, 248(2), 725–757.

Honkavaara, J.M., Restitutti, F., Raekallio, M.R., Kuusela, E.K. & Vainio, O., 2011. The effects of increasing doses of MK-467, a peripheral alpha(2)-adrenergic receptor antagonist, on the cardiopulmonary effects of intravenous dexmedetomidine in conscious dogs. *J Vet Pharmacol ther*, 34(4), p. 332–327.

Horváth, G., Brodacz, B. Holzer-Petsche, U., 2002. Blood pressure changes after intrathecal co-administration of calcium channel blocker with morphine or clonidine at the spinal level. *Naunyn Scmiedebergs Arch Pharmacol,* 366(3), 270–275.

Horváth, G., Joo, G., Dobos, I., Klimscha, W., Toth, G. & Benedek, G., 2001. The synergistic antinociceptive interaction of endomorphin-1 with dexmedetomidine and/or S(+)-ketamine in rats. *Anesth Analg,* 93(4), p. 1018–1024.

Horváth, G., Kovács, M., Szikszay, M & Benedek, G., 1994. Mydriatic and antinociceptive effects of intrathecal dexmedetomidine in conscious rats. *Eur J Pharmacol*, 253(1–2), p. 61–66.

Howorth, P.W., Teschemacher, A.G. & Pickering, A.E., 2009. Retrograde adenoviral vector targeting of nociresponsive pontospinal noradrenergic neurons in the rat in vivo. *J Comp Neurol,* 512(2), p. 141–157.

Houston, M.C., 1982. Clonidine hydrochloride. *South Med* J, 75(6), p. 713–719.

Huang, C.Y., Chen, Y.L., Li, A.H., Lu, J.C. & Wang, H.L., 2014. Minocycline, a microglial inhibitor, blocks spinal CCL2-induced heat hyperalgesia and augmentation of glutamatergic transmission in substantia gelatinosa neurons. *J Neuroinflammation*, 11:7.

ICH S7A quidelines, US FDA, S7A (2001).

Iirola, T., Vilo, S., Aantaa, R., Wendelin-Saarenhovi, M., Neuvonen, P.J., Scheinin, M. & Olkkola, K.T., 2011. Dexmedetomidine inhibits gastric emptying and oro-caecal transit in healty volunteers. *Br J Anaesth*, 106(4), p. 522–527.

Intengan, H.D. & Smyth, D.D., 1996. Clonidine-induced increase in osmolar clearance and free water clearance via activation of two distinct α_2-adrenoceptor sites. *Br J Pharmacol,* 119(4), p. 663–670.

Jaakkola, M.L., Salonen, M., Lehtinen, R. & Scheinin, H., 1991. The analgesic action of dexmedetomidine – a novel α_2-adrenoceptor agonist – in healthy volunteers. *Pain*, 46(3), p. 281–285.

Jin, S., Liang, D.D., Chen, C., Zhang, M. & Wang, J., 2017. Dexmedetomidine prevent postoperative nausea and vomiting on patients during general anesthesia: a PRISMA – compliant meta analysis of randomized controlled trials. *Medicine (Baltimore),* 96(1), e5770.

Joó, G., Horváth, G., Klimscha, W., Kekesi, G., Dobos, I., Szikszay, M. & Benedek, G., 2000. The effects of ketamine and its enantiomers on the morphine- or dexmedetomidine-induced antinociception after intrathecal administration in rats. *Anesthesiology*, 93(1), p. 231–241.

Jorm, C.M. & Stamford, J.A., 1993. Actions of the hypnotic anaesthetic, dexmedetomidine, on noradrenaline release and cell firing in rat locus coeruleus slices. *Br J Anaesth*, 71(3), p. 447–449.

Kallio, A., Scheinin, M., Koulu, M., Ponkilainen, R., Ruskoaho, H., Viinamäki, O. & Scheinin, M., 1989. Effects of dexmedetomidine, a selective alpha 2-adrenoceptor agonist, on hemodynamic control mechanisms. *Clin Pharmacol Ther*, 46(1), p.33–42.

Kalso, E.A., Pöyhiä, R. & Rosenberg, P.H., 1991. Spinal antinociception by dexmedetomidine, a highly selective alpha 2-adrenergic agonist. *Pharmacol Toxicol*, 68(2), p. 140–143.

Kamibayashi, T. & Maze, M., 2000. Clinical uses of alpha2-adrenergic agonists. *Anesthesiology*, 93(5), p. 1345–1349.

Kawamata, T., Omote, K., Kawamata, M., Iwasaki, H. & Namiki, A., 1997. Antinociceptive interaction of intrathecal alpha2-adrenergic agonists, tizanidine and clonidine, with lidocaine in rats. *Anesthesiology*, 87(2), p. 436–448.

Kawamata, T., Omote, K., Yamamoto, H., Toriyabe, M., Wada, K & Namiki, A., 2003. Antihyperalgesia and side effects of intrathecal clonidine and tizanidine in a rat model of neuropathic pain. *Anesthesiology*, 98(6), p. 1480–1483.

Kawamoto, S., Hirakata, H., Sugita, N. & Fukuda, K., 2015. Bidirectional effects of dexmedetomidine on human platelet function in vitro. *Eur J Pharmacol*, 766, p. 122–128.

Kawasaki, Y., Kumamoto, E., Furue, H. & Yoshimuta, M., 2003. Alpha 2 adrenoceptor-mediated presynaptic inhibition of primary afferent glutamatergic transmission in rat substantia gelatinose neurons. *Anesthesiology*, 98(3), p. 682–689.

Keating, G.M., 2015. Dexmedetomidine: a review of its use for sedation in the intensive care setting. *Drugs*, 75(10), p. 1119–1130.

Kehne, J.H., Gallager, D.W. & Davis, M., 1981. Strychnine: brainstem and spinal mediation of excitatory effects on acoustic startle. *Eur J Pharmacol*, 76(2–3), p. 177–186.

Kibaly, C., Loh, H.H. & Law, P.Y., 2016. A mechanistic approach to the development of gene therapy for chronic pain. *Int Rev Cell Mol Biol*, 327, p. 89–161.

Kiem, D.T., Bartha, L., Hársing Jr, L.G. & Makara, G.B., 1991. Reevaluation of the tole of alpha2-adrenoreceptors in morphine-stimulated release of growth hormone. Neuroendocrinology, 53(5), p. 516–522.

Kleinmann, B. & Wolter, T., 2017. Intrathecal opioid therapy for non-malignant chronic pain: a long-term perspective. *Neuromodulation*, 20(7), p. 719–726.

Klimscha, W., Chiari, A., Krafft, P., Plattner, O., Taslimi, R., Mayer, N., Weinstabl, C., Schneider, B. & Zimpfer, M., 1995. Hemodynamic and analgesic effects of clonidine added repetitively to continuous epidural and spinal blocks. *Anesth Analg,* 80(2), p. 322–327.

Knaus, A.E., Muthig, V., Schickinger, S., Moura, E., Beetz, N., Gilsbach, R. & Hein, L., 2007. Alpha2-adrenoceptor subtypes – unexpected functions for receptors and ligands derived from gene-targeted mouse models. *Neurochem Int*, 51(5), p. 277–281.

Kontani, H., Tsuji, T. & Kimura, S., 2000. Effects of adrenergic α2-adrenoceptor agonists on urinary bladder contraction in conscious rats. *Jpn J Pharmacol,* 84(4), p. 381–390.

Kroin, J.S., Buvanendran, A., Beck, D.R., Topic, J.E., Watts, D.E. & Tuman, K.J., 2004. Clonidine prolongation of lidocaine analgesia after sciatic nerve block in rats is mediated via hyperpolarization-activated cation current, not by α-adrenoceptors. *Anesthesiology*, 101(2), p. 488–494.

Kroin, J.S., McCarthy, R.J., Penn, R.D., Lubenow, T.J. & Ivankovich, A.D., 1996. Intrathecal clonidine and tizanidine in conscious dogs: comparison of analgesic and hemodynamic effects. *Anesth Analg*, 82(3), p. 627–635.

Kroin, J.S., McCarthy, R.J., Penn, R.D., Lubenow, T.J. & Ivankovich, A.D., 2003. Continuous intrathecal clonidine and tizanidine in conscious dogs: analgesic and hemodynamic effects. *Anesth Analg*, 96(3), p. 776–782.

Kumar, A., Maitra, S., Khanna, P. & Kumar Baidya, D., 2014. Clonidine for management of chronic pain: a brief review of the current evidences. *Saudi J Anaesth*, 8(1), p. 92–96.

Kumar, V.M., Vetrivelan, R. & Mallick, H.N., 2007. Noradrenergic afferents and receptors in the medial preoptic area: Neuroanatomical and neurochemical links between the regulation of sleep and body temperature. *Neurochem Int*, 50(6), p. 783–790.

Kuner, R., 2010. Central mechanisms of pathological pain. *Nat Med*, 16(11), p. 1258–1266.

Kwiat, G.C. & Basbaum, A.I., 1990. Organization of typosine hydroxylase- and serotonin-immunoreactive brainstem neurons with axon collaterals to the periaqueductal gray and the spinal cord in the rat. *Brain Res*, 528(1), p. 83–94.

Lamer, T.J., Deer, T.R. & Hayek, S.M., 2016. Advanced innovations for pain. *Mayo Clin Proc*, 91(2), p. 246–258.

Langer, S.Z., 1997. 25 years since the discovery of presynaptic receptors: present knowledge and future perspectives. *Trends Pharmacol Sci,* 18(3), p. 95–99.

Lanz, E., Theiss, D., Riess, W. & Sommer, U., 1982. Epidural morphine for postoperative analgesia: a double-blind study. *Anesth Analg,* 61(3), p. 236–240.

Lehtimäki, J., Haapalinna, A., Korhonen, T., Leino, T., Viitamaa, T., Wurster, S., Savola, J.M. & Virtanen, R., 1999. MPV-2426, a novel α_2-adrenergic agonist for spinal analgesia. *Fund Clin Pharmacol*, 13(Suppl 1), 380.

Lehtimäki, J., Leino, T., Koivisto, A, Viitamaa, T., Lehtimäki, T., Haapalinna, A., Kuokkanen, K. & Virtanen, R., 2008. In vitro and in vivo profiling of fadolmidine, a novel potent α_2-adrenoceptor agonist with local mode of action. *Eur J Pharmacol,* 599(1–3), p. 65–71.

Li, X. & Eisenach, J.C., 2001. Alpha2A-adrenoceptor stimulation reduces capsaicine-induced glutamate release from spinal cord synaptosomes. *J Pharmacol Exp Ther*, 299(3), p. 939–944.

Li, J.N. & Sheets, P.L., 2018. The central amygdala tp periaqueductal gray pathway compises intrinsically distinct neurons differentially affected in a model of inflammatory. *J Physiol*, 596(24), p. 6289–6305.

Link, R.E., Desai, K., Hein, L., Stevens, M.E., Chruscinski, A., Bernstein, D., Barsh, G.S. & Kobilka, B.K., 1996. Cardiovascular regulation in mice lacking α_2-adrenergic receptor subtypes b and c. *Science*, 273 (5276), p. 803–805.

Lirk, P., Picardi, S. & Hollman, M.W., 2014. Local anaesthetics: 10 essentials. *Eur J Anaesthesiol*, 31(11), p. 575–585.

Liu, S., Zhao, P., Cui, Y., Lu, C., Ji, M., Liu, W., Jiang, W., Zhu, Z. & Sun, Q., 2020. Effects of 5 μg dose of dexmedetomidine in combination with intrathecal bupivacaine on spinal anesthesia: a systematic review and meta-analysis. *Clin Ther*, 42(4), p. 676–690.

Livingston, A., Low, J. & Morris, B., 1984. Effects of clonidine and xylazine on body temperature in the rat. *Br J Pharmacol*, 81(1), p. 189–193.

MacDonald, E., Haapalinna, A., Virtanen, R., Lammintausta, R., 1989. Effects of acute administration of medetomidine on the behaviour, temperature and turnover rates of brain biogenic amines in rodents and reversal of these effects by atipamezole. *Acta Vet Scand*, 85, p. 77–81.

MacDonald, E., Scheinin, H. & Scheinin, M., 1988. Behavioural and neurochemical effects of medetomidine, a novel veterinary sedative. *Eur J Pharmacol,* 158(1–2), p. 119–127.

MacDonald, E. & Scheinin, M., 1995. Distribution and pharmacology of alpha 2-adrenoceptors in the central nervous system. *J Physiol Pharmacol*, 46(3), p. 241–258.

MacLennan, S.J., Luong, L.A., Jasper, J.R., To, Z.P. & Eglen, R.M., 1997. Characterization of alpha2-adrenoceptors mediated contraction of dog saphenous vein: identity with the human alpha 2A subtype. *Br J Pharmacol*, 121(8), p. 1721–1729.

Mahendru, V., Tewari, A., Katyal, S., Grewal, A., Singh, M.R. & Katyal, R., 2013. A comparison of intrathecal dexmedetomidine, clonidine, and fentanyl as adjuvants to hyperbaric bupivacaine for lower limb surgery: a double blind controlled study. J Anaesthesiol Clin Pharmacol, 29(4), p. 496–502.

Malcolm, A., Camilleri, M., Kost, L., Burton, D.D., Fett, S.L & Zinsmeister, A.R., 2000. Towards identifying optimal doses for alpha-2 adenergic modulation of colonic and rectal motor and sensory function. *Aliment Pharmacol Ther*, 14(6), p. 783–793.

Mastenbroek, T.C., Kramp-Hendriks, B.J., Kallewaard, J.W. & Vonk, J.M., 2017. Multimodal intrathecal analgesia in refractory cancer pain. *Scand J Pain*, 14, 39–43.

Maze, M. & Tranquili, W., 1991. Alpha2-adrenoceptor agonist: defining the role in clinical anesthesia. *Anesthesiolog*y, 74(3), p. 581–605.

McCarthy, R.J., Kroin, J.S., Lubenow, T.R., Penn, R.D. & Ivankovich, A.D., 1990. Effect of intrathecal tizanidine on antinociception and blood pressure in the rats. *Pain*, 40(3), p. 333–338.

Mensink, F.J., Kozody, R., Kehler, C.H. & Wade, J.G., 1987. Dose-response relationship of clonidine in tetracaine spinal anesthesia. *Anesthesiology*, 67(5), p. 717–721.

Mercadante, S., Porzio, G. & Gebbia, V., 2012. Spinal analgesia for advanced cancer patients: an update. *Crit Rev Oncol Hematol,* 82(2), p. 227–232.

Merighi, A., 2018. The histology, physiology, neurochemistry and circuitry of the substantia gelatinosa Rolandi (lamina II) in mammalian spinal cord. *Prog Neurobiol*, 169, p. 91–134.

Michel, M.C., Bond, R.A. & Summers, R.J., 2019. Adrenoceptors – New roles for old players. *Br J Pharmacol*, 176(14), p. 2339–2342.

Millan, M.J., 2002. Descending control of pain. *Prog Neurobiol*, 66(6), p. 355–474.

Millan, M.J., Dekeyne, A., Newman-Tancredi, A., Cussac, D., Audinot, V., Milligan, G., Duqueyroix, D., Girardon, S., Mullot, J., Boutin, J.A., Nicolas, J.P., Renouard-Try, A., Lacoste, J.M. & Cordi, A., 2000. S18616, a highly potent, spiroimidazoline agonist at α_2-adrenoceptors: I. receptor profile, antinociceptive and hypoyhermic actions in comparison with dexmedetomidine and clonidine. *J Pharmacol Exp Ther*, 295(3), p. 1192–1205.

Miller, J.H., McCoy, K.D., Coleman, A.S., 2001. Renal actions of the alpha2-adrenoceptor agonist, xylazine, in the anaesthetised rat. *N Z Vet J*, 49(5), p.173–180.

Minor, B.G., Danysz, W., Jonsson, G., Mohammed, A.K., Post, C. & Archer, T., 1989. Adaptive changes in alpha-2 adrenoceptor mediated responses: analgesia, hypothermia and hypoactivity. *Pharmacol Toxicol*, 65(2), p. 143–151.

Müllner. K., Gyires, K. & Furst, S., 2001. Involvement of the opioid system in the central antisecretory action of alpha-2 adrenoceptor agonists in rat. *J Physiol Paris*, 95(1–6), p. 209–214.

Müllner, K., Rónai, A.Z., Fülöp, K., Fürst, S. & Gyires, K., 2002. Involvement of central K(ATP) channels in the gastric antisecretory action of alpha2-adrenoceptor agonists and beta-endorphin in rats. *Eur J Pharmacol*, 435(2–3), p. 225–229.

Myers, R.D., Beleslin, D.B. & Rezvani, A.H., 1987. Hypothermia: role of α1- and α2-noradenergic receptors in the hypothalamus of the cat. *Pharmacol Biochem Behav*, 26(2), p. 373–379.

Nag, S. & Mokha, S.S., 2016. Activation of the trigeminal α2-adrenoceptor produces sex-specific, estrogen dependent thermal antinociception and antihyperalgesia using an operant pain assay in the rat. *Behav Brain Res,* 314, p. 152–158.

Nagappa, S., Kalappa, S. & Sridhara, R.B., 2018. Clonidine as an adjuvant to caudal epidural ropivacaine for lumbosacral spine surgeries. *Anesth Essays Res*, 12(1), p. 240–245.

Nagasaka, H. & Yaksh, T.L., 1990. Pharmacology of intrathecal adrenergic agonists: cardiovascular and nociceptive reflexes in halothane-anesthetized rats. *Anesthesiology*, 73(6), p. 1198–1207.

Nalepa, I., Vetulani, J., Borghi, V., Kowalska, M., Przewlocka, B & Pavone, F., 2005. Formalin hindpaw injection induces changes in the [^{3}H]prazosin binding to α_1-adrenoceptors in specific region of the mouse brain and spinal cord. *J Neural Transm*, 112, p. 1309–1319.

Niemi, L., 1994. Effects of intrathecal clonidine on duration of bupivacaine spinal anaesthetisia, haemodynamics, and postoperative analgesia in patients undergoing knee arthroscopy. *Acta Anaesthesiol Scand*, 38(7), p. 724–728.

Niemi, R., Huuskonen, J., Laine, K. & Järvinen, T., 2005. Systhesis, hydrolysis, and intraocular pressure lowering effects of fadolmidine prodrugs. *Int J Pharm*, 295(1–2), p. 121–127.

Nguyen, V., Tiemann, D., Park, E. & Salehi, A., 2017. Alpha-2 agonists. *Anesthesiology Clin*, 35(2), p. 233–245.

Nishiyama, T. & Hanaoka, K., 2004. Intrathecal clonidine and bupivacaine have synergistic analgesia for acute thermally or inflammatory-induced pain in rats. *Anesth Analg,* 98(4), p. 1056–1061.

Okada, S., Miyai, Y., Sato, K., Masaki, Y., Higuchi, T., Ogino, Y. & Ota, Z., 1986. Effects of clonidine on insulin secretion: a case report. *J Int Med Res*, 14(6), 299–302.

Ong, W.Y., Stohler, C.S. & Herr, D.R., 2019. Role of the prefrontal cortex in pain processing. *Mol Neurobiol*, 56(2), p. 1137–1166.

Onttonen, T., Kalmari, J. & Pertovaara, A., 2000. Selective and segmentally restricted antinociception induced by MPV-2426, a novel alpha-2-adrenoceptor agonist, following intrathecal administration in the rat. *Acta Anaesthesiol Scand,* 44(9), p. 1077–1082.

Onttonen, T. & Pertovaara, A., 2000. The mechanical antihyperalgesic effect of intrathecally administered MPV-2426, a novel α2-adrenoceptor agonist, in a rat model of postoperative pain. *Anesthesiology*, 92(6), p. 1740–1745.

Ouyang, H., Bai, X., Huang, W., Chen, D., Dohi, S. & Zeng, W., 2012. The antinociceptive activity of intrathecally administered amiloride and its interactions with morphine and clonidine in rats. *J Pain,* 13(1), 41–48.

Ozawa, H., Chen, C.S., Watanabe, H. & Uematsu, T., 1977. Effects of clonidine on blood pressure, heart rate and body temperature in conscious rats. *Jnp J Pharmacol*, 27(1), p. 47–54.

Patro, S.S., Deshmukh, H., Ramani, Y.R. & Das, G., 2016. Evaluation of dexmedetomidine as an adjuvant to intrathecal bupivacaine in infraumbilical surgeries. *J Clin Diagn Res*, 10(3), p. UC13–16.

Peniche, A., Poree, L., Schumacher, M. & Yu, X., 2018. Integrating patient-controlled analgesia using implanted intrathecal pumps for postoperative pain management: A case report. *A A Pract*, 10(11), p. 285–287.

Penning, J. P., & Yaksh, T. L., 1992. Interaction of intrathecal morphine with bupivacaine and lidocaine in the rat. *Anesthesiology*, 77(6), p. 1186–2000.

Pertovaara, A., 1993. Antinociception induced by alpha-2-adrenoceptor agonists, with special emphasis on medetomidine studies. *Prog Neurobiol,* 49(6), p. 691–709.

Pertovaara, A., 2004. Antinociceptive properties of fadolmidine (MPV-2426), a novel alpha2-adrenoceptor agonist. *CNS Drug Rev*, 10(2), p. 117–126.

Pertovaara, A., 2006. Noradrenergic pain modulation. *Prog Neurobiol*, 80(2), p. 53–83.

Pertovaara, A., 2013. The noradrenergic pain regulation system: a potential target for pain therapy. *Eur J Pharmacol*, 716(1–3), p. 2–7.

Pertovaara, A., Hämäläinen, M.M., Kauppila, T., Mecke, E. & Carlson, S., 1994. Dissociation of the α_2-adenergic antinociception from sedation following microinjection of medetomidine into the locus coeruleus in rats. *Pain*, 57(2), p. 207–215.

Pertovaara, A. & Kalmari, J., 2003. Comparison of the visceral antinociceptive effects of spinally administered MPV-2426 (Fadolmidine) and clonidine in the rat. *Anesthesiology*, 98(1), p. 189–194.

Pertovaara, A. & Wei, H., 2000. Attenuation of ascending nociceptive signals to the rostroventromedial medulla induced by a novel α_2-adrenoceptor agonist, MPV-2426, following intrathecal application in neuropathic rats. *Anesthesiology*, 92(4), p. 1082–1092.

Peterhoff, M., Sieg, A., Brede, M., Chao, C.M., Hein, L & Ullrich, S., 2003. Inhibition of insulin secretion via distinct signaling pathways in alpha2-adrenoceptors knockout mice. *Eur J Endocrinol*, 149(4), p. 343–350.

Pettinger, W.A., 1980. Pharmacology of clonidine. *J Cardiovasc Pharmacol*, 2(suppl. 1), p. S21–S28.

Pinthong, D., Songsermsakul, P., Rattanachamnong, P. & Kendall, D.A., 2004. The effects of imidazoline agents on the aggregation of human platelets. *J Pharm Pharmacol*, 56(2), p. 213–220.

Pohjanoksa, K., Jansson, C.C., Luomala, K., Marjamäki, A., Savola, J.M & Scheinin, M., 1997. α_2-Adrenoceptor regulation of adenylyl cyclase in CHO cells: dependence on receptor density, receptor subtype and current activity of adenylyl cyclase. *Eur J Pharmacol*, 335(1), p. 53–63.

Pol, O., Valle, L., Ferrer, I. & Puig, M.M., 1996. The inhibitory effects of α_2-adrenoceptor agonists on gastrointestinal transit during croton oil-induced intestinal inflammation. *Br J Pharmacol*, 119(8), p. 1649–1655.

Post, C., Gordh, T., Minor, B.G., Archer, T. & Freedman, J., 1987. Antinociceptive effects and spinal cord tissue concentrations after intrathcal injection of guanfacine or clonidine into rats. *Anesth Analg*, 66(4), p. 317–324.

Prager, J., Deer, T., Levy, R., Bruel, B., Buchser, E., Caraway, D., Cousins, M., Jacobs, M., McGlothlen, G., Rauck, R., Staats, P. & Stearns, L., 2014. Best practices for intrathecal drug delivery for pain. *Neuromodulation*, 17(4), p. 354–372.

Pöpping, D.M., Elia, N., Marret, E., Wenk, M. & Tramèr, M.R., 2009. Clonidine as an adjuvant to local anesthetics for peripheral nerve and plexus blocks. *Anesthesiology*, 111(2), p. 406–415.

Ramage, A.G., Villalón, C.M., 2008. 5-hydroxytryptamine and cardiovascular regulation. *Trends Pharmacol Sci*, 29(9), p. 472–481.

Reiz, S., Ahlin, J., Ahrenfeldt, B., Andersson, M & Andersson, S., 1981. Epidural morphine for postoperative pain relief. *Acta Anaesthesiol Scand,* 25(2), p. 111–114.

Remko, M., Swart, M. & Bickelhaupt, F.M., 2006. Theoretical study of structure, pKa, lipophilicity, solubility, absortion, and polar surface area of some centrally acting antihypertensives. *Bioog Med Chem,* 14(6), p. 1715–1728.

Restitutti, F., Raekallio, M., Vainionpää M, Kuusela, E., & Vainio, O., 2012. Plasma glucose, insulin, free fatty acids, lactate and cortisol concentrations in dexmedetomidine-sedated dogs with or without MK-467: a peripheral α2 –adrenoceptors antagonist. *Vet J*, 193(2), 481–485.

Rosin, D.L., Talley, E.M., Lee, A, Stornetta, R.L., Gaylinn, B.D., Guyenet, P.G. & Lynch, K.R., 1996. Distribution of alpha 2C-adrenergic receptors-like immunoreactivity in the rat central nervous system. *J Comp Neurol*, 372(1), p. 135–165.

Rugino T.A., 2018. Effect on primary sleep disorders when children with ADHD are adminstered guanfacine extended release. *J Atten Disord*, 22(1), p. 14–24.

Ruohonen, S.T., Ruohonen, S., Gilsbach, R., Savontaus, Eriika, Scheinin, M. & Hein, L., 2012. Involvement of α2-adrenoceptor subtype A and C in glucose homeostasis and adrenaline-induced hyperglycaemia. *Neuroendocrinology*, 96(1), p. 51–59.

Russell, I. F. 1982. Intrathecal bupivacaine 0.5% for caesarean section. *Anaesthesia*, 37(3), p. 346–347.

Sabbe, M.B., Penning, J.P., Ozaki, G.T. & Yaksh, T.L., 1994. Spinal and systemic action of the alpha 2 receptor agonist dexmedetomidine in dogs. Antinociception and carbon dioxide response. *Anesthesiology*, 80(5), p. 1057–1072.

Sakamoto, H., Fukuda, S., Minakawa, Y. & Sawamura, S., 2013. Clonidine induces sedation through acting on the perifornical area and the locus coeruleus in rats. *J Neurosurg Anesthesiol,* 25(4), p. 399–407.

Sallinen, J., Haapalinna, A., Viitamaa, T., Kobilka, B.K. & Scheinin, M., 1998. Adrenergic alpha2C-receptors modulate the acoustic startle reflex, prepulse inhibition, and aggression in mice. *J Neurosci*, 18(8), p. 3035–3042.

Sallinen, J., Höglund, M., Engström, M., Lehtimäki, J., Virtanen, R., Sirviö, J., Wurster, S., Savola, J.M. & Haapalinna, A., 2007. Pharmacological characterization and CNS effects of novel highly selective alpha2C-adrenoceptor antagonist JP-1302. *Br J Pharmacol*, 150(4), p. 391–402.

Samieirad, S., Sharifian-Attar, A., Eshghpour, M., Mianbandi, V., Shadkam, E., Hosseini-Abrishami, M. & Hashemipour, M-S., 2018. Comparison of ondansentron versus clonidine efficacy for prevention of postoperative pain, nausea and vomiting after orthognathic surgeries: A triple blind randomized controlled trial. *Med Oral Patol Oral Cir Bucal*, 23(6), p. 767–776.

Sato, J. & Perl, E.R., 1991. Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury. *Science*, 251(5001), p. 1608–1610.

Savola, J.M., 1989. Cardiovascular actions of medetomidine and their reversal by atipamezole. *Acta Vet Scand Suppl*, 85, p. 39–47.

Sawamura, S., Kingery, W.S., Davies, M.F., Agashe, G.S., Clark, J.D., Kobilka, B.K., Hashimoto, T. & Maze, M., 2000. Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of α_{2B} adrenoceptors. *J Neurosci*, 20(24), p. 9242–9251.

Scheinin, M., Kallio, A., Koulu, M., Viikari, J. & Scheinin, H., 1987. Sedative and cardiovascular effects of medetomidine, a novel selective alpha 2-adrenoceptor agonist, in healthy volunteers. *Br J Clin Pharmacol*, 24(4), p. 443–451.

Scheinin, M., Lomasney, J.W., Hayden-Hixson, D.M., Schambra, U.B., Caron, M.G., Lefkowitz, R.J. & Fremeau Jr., R.T., 1994. Distribution of α_2-adrenergic receptor subtype gene expression in rat brain. *Brain Res Mol Brain Res,* 21(1–2):133–149.

Scheinin, M., Sallinen, J. & Haapalinna, A., 2001. Evaluation of the α_{2C}-adrenoceptor as a neuropsychiatric drug target sudies in transgenic mouse models. *Life Sci*, 68(19–20), p. 2277–2285.

Scheinin, H., Virtanen, R., MacDonald, E., Lammintausta, R., Scheinin, M., 1989. Medetomidine – a novel alpha 2-adrenoceptor agonist: a review of its pharmacodynamic effects. *Prog Neurospychopharmacology Biol Psychiatry*, 13(5), p. 635–651.

Schiller, L.R., Santa Ana, C.A., Morawski, S.G. & Fordtran, J.S., 1985. Studies of the antidiarrheal action of clonidine. Effects on motility and intestinal absorption. *Gastroenterology*, 89(5), p. 982–988.

Schitysik, G., 1986. Animal pharmacology of guanfacine. *Am J Cardiol*, 57(9), p. 13E–17E.

Schwartz, D.D., Jones, W.G., Hedden, K.P. & Clark, T.P., 1999. Molecular and pharmacological characterization of the canine brainstem alpha-2A adrenergic receptor. *J Vet Pharmacol Ther*, 22(6), p.380–386.

Sen, J. & Sen, B., 2015. Response to low-dose intrathecal clonidine in septuagenarians undergoing sub-umbilical surgeries: A study. *Saudi J Anaesth*, 9(2), p. 142–147.

Sévoz, C., Callera, J.C., Machado, B.H., Hamon, M. & Laguzzi, R., 1997. Role of serotonin3 receptors in the nucleus tractus solitarii on the carotid cemoreflex. *Am J Physiol*, 272(3 Pt 2), p. 1250–1259.

Shockley, D.C., Wade, L.H. & Williams-Johnson, M.M., 1993. Effects of alpha-2-adrenoceptor agonists on induced diuresis in rats. *Life Sci*, 53(3), p. 251–259.

Sia, A.T., Kwek, K. & Yeo, G.S., 2005. The in vitro effects of clonidine and dexmedetomidine on human myometrium. *Int J Obstet Anesth*, 14(2), p. 104–107.

Sica, D.A., 2007. Centrally acting antihypertensive agents: an update. *J Clin Hypertens (Greenwich),* 9(5), p. 399–405.

Sinclair, M.D., 2003. A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. *Can Vet J,* 44(11), p. 885–897.

Smith, B.D., Baudendistel, L.J., Gibbons, J.J. & Schweiss, J.F., 1992. A comparison of two epidural alpha 2- agonists, guanfacine and clonidine , in regard to duration of antinociception, and ventilatory and hemodynamic effects in goats. *Anest Analg*, 74(5), 712–718.

Sng, B.L. & Sia, A.T.H., 2017. Maintenance of epidural labour analgesia: the old, the new and the future. *Best Pract Res Clin Anaesthesiol*, 31(1), p. 15–22.

Soini, S.L., Duzic, E., Lanier, S.M. & Akerman, K.E.O., 1998. Dual modulation of calcium channel current via recombinant alpha2-adrenoceptors in pheochromocytoma (PC-12) cells. *Pflugers Arch*, 435(2), p. 280–285.

Solomon, R.E., Brody, M.J. & Gebhart, G.F., 1989. Pharmacological characterization of alpha adrenoceptors involved in the antinociceptive and cardiovascular effects of intrathecally administerd clonidine. *J Pharmacol Exp Ther,* 251(1), p. 27–38.

Sonohata, M., Furue, H., Katafuchi, T., Yasaka, T., Doi, A., Kumamoto, E. & Yoshimura, M., 2004. Actions of noradrenaline on substantia gelatinosa neurons in the rat spinal cord revealed by in vivo patch recording. *J Physiol*, 555(Pt2), p. 515–526.

Staikou, C. & Paraskeva, A., 2014. The effects of intrathecal and systemic adjuvants on subarachnoid block. *Minerva Anesthesiol*, 80(1), p. 96–112.

Starke, K., 2001. Presynaptic autoreceptors in the third decade: focus on α_2-adrenoceptors. *J Neurochem*, 78(4), p. 685–693.

Stone, L.S., Broberger, C., Vulchanova, L., Wilcox, G.L., Hökfelt, T., Riedl, M.S. & Elde, R., 1998. Differential distribution of alpha2A and alpha2C adrenergic receptor immunoreactivity in rat spinal cord. *J Neurosci*, 18(15), p. 5928–5937.

Strebel, S., Gurzeler, J.A., Schneider, M.C., Aeschbach, A. & Kindler, C.H., 2004. Small-dose intrathecal clonidine and isobaric bupivacaine for orthopedic surgery: a dose-response study. *Anesth Analg*, 99(4), p. 1231–1238.

Streng, T., Santti, R. & Andersson, K.E., 2010. Voiding effects mediated by α_2-adrenoceptors in the anaesthetized male rat. *BJU Int,* 106(10), p. 1546–1549.

Sun, S., Wang, J., Bao, N., Chen, Y., & Wang, J., 2017. Comparison of dexmedetomidine and fentanyl as local anesthetic adjuvants in spinal anesthesia: a systematic review and meta-analysis of randomized controlled trials. *Drug Des Devel Ther*, 11, p. 3413–3424.

Swain, A., Nag, D.S., Sahu, S. & Samaddar, D.P., 2017. Adjuvants to local anesthetics: current understanding and future trends. *World J Clin Cases*, 5(8), p. 307–323.

Takano, Y. & Yaksh, T.L., 1993. Chronic spinal infusion of dexmedetomidine, ST-91 and clonidine: spinal alpha$_2$ adrenoceptor subtypes and intrinsic activity. *J Pharmacol Exp Ther,* 264(1), 327–335.

Takayanagi, I., Konno, F. & Kusunoki, M., 1985. Some pharmacological effects of tizanidine on smooth muscle organs and α_2-adrenoceptor. *Gen Pharmac*, 16(5), p. 501–503.

Talke, P., Xu, M., Paloheimo, M. & Kalso, E., 2003. Effects of intrathecally administered dexmedetomidine, MPV-2426 and tizanidine on EMG in rats. *Acta Anaesthesiol Scand,* 47(3), p. 347–354.

Tallarida, R.J., 1992. Statistical analysis of drug combinations for synergism. *Pain*, 49(1), p. 93–97.

Tallarida, R.J., Porreca, F. & Cowan, A., 1989. Statsitical analysis of drug-drug and site-site interactions with isobolograms. *Life Sci*, 45(11), p. 947–961.

Tanabe, M., Ono, H. & Fukuda, H., 1990. Spinal α_1- and α_2-adrenoceptors mediated facilitation and inhibition of spinal motor transmission, respectively. *Jpn J Pharmacol,* 54(1), p. 69–77.

Taneja, A., Luca Di lorio, V., Danhof, M. & Della Pasqua, O., 2012. Translation of drug effects from experimental models of neuropathic pain and analgesia to humans. *Drug Discov Today*, 17(15–16), p. 837–849.

Timmons, S.D., Geisert, E., Stewart, A.E., Lorenzon, N.M. & Foehring, R.C., 2004. Alpha2-adrenergic receptor-mediated modulation of calcium current in neocortical pyramidal neurons. *Brain Res*, 1014(1–2), p. 184–196.

Tonner, P.H., 2017. Additives used to reduce perioperative opioid consumption 1: Alpha2-agonists. *Best Pract Res Clin Anaesthesiol,* 31(4), p. 505–512.

Trendelenburg, A.U., Sutej, I., Wahl, C.A., Moderings, G.J. Rump, L.C. & Starke, K., 1997. A re-investigation of questionable subclassifications of presynaptic alpha2-autoreceptors: rat vena cava, rat atria, human kidney and guinea-pig urethra. *Naunyn Schmiedebergs Arch Pharmacol*, 356(6), p. 721–737.

Tsuda, M., Beggs, S., Salter, M.W. & Inoue, K., 2013. Microglia and intractable chronic pain. *Glia*, 61(1), p. 55–61.

Uusitalo, P., Al-Ramahi, D., Tilli, I., Aantaa, R.A., Scheinin, M. & Saari, T.I., 2018. Subcutaneously adminsterd dexmedetomidine is effectly absorbed and is associated with attenuated cardiovascular effects in healty volunteers. *Eur J Clin Pharmacol*, 74(8), p. 1047–1054.

Vahabi, S. & Kazemi, A.H., 2011. Effects of clonidine as premedication on plasma renin activity, serum and urine electrolytes and body fluids in general anesthesia. A randomized double blind placebo controlled clinical trial. *Middle East J Anaesthesiol*, 21(1), p. 71–76.

Verberne, A.J. & Guyenet, P.G., 1992. Medullary pathway of the Benzold-Jarisch reflex in the rat. *Am J Physiol*, 263(6 Pt 2), p. 1195–1202.

Viramontes, B.E., Malcolm, A., Camilleri, M., Szarka, L.A., McKinzies, S., Burton, D.D. & Zinsmeister, A.R., 2001. Effects of an alpha(2)-adrenergic agonist on gastrointestinal transit, colonic motility, and sensation in humans. *Am J Physiol Gastrointest Liver Physiol*, 281(6), p. G1468–1476.

Wang, J.K., Nauss, L.A. & Thomas, J.E., 1979. Pain relief by intrathecally applied morphine in man. *Anesthesiology*, 50(2), p. 149–151.

Wang, X.M., Zhang, Z.J., Bains, R. & Mokha, S.S., 2002. Effect of antisense knock-down of α2a- and α2c-adrenoceptors on the antinociceptive action of clonidine on trigeminal nociception in the rat. *Pain*, 98(1–2), p. 27–35.

Weatherall, M., Antaa, R., Conti, G., Garratt, C., Pohjanjousi, P., Lewis, M.A., Moore, N. & Perez-Gutthann, S., 2017. A multinational, drug utilization study to investigate the use of dexmedetomidine (Dexdor®) in clinical practice in the EU. Br J Clin Pharmacol, 83(9), p. 2066–2076.

Wei, H., Jyväsjärvi, E., Niinisalo, S., Hukkanen, M., Waris, E., Konttinen, Y.T. & Pertovaara, A., 2002. The influence of chemical sympathectomy on pain responsivity and α_2-adrenergic antinociception in neuropathic animals. *Neuroscience*, 114(3), p. 655–668.

Wei, H. & Pertovaara, A., 2013. Regulation of neuropathic hypersensitivity by α_2-adrenoceptors in the pontine A7 cell group. *Basic Clin Pharmacol Toxicol*, 112(2), p. 90–95.

Weiner, N. & Molonoff, P.B., 1994. Catecholamines. In: Siegel, J.G. (ed) Basic Neurochemistry. Raven Press, New York, p. 261–281.

Whiteside, G.T., Pomonis, J.D. & Kennedy, J.D., 2016. Preclinical pharmacological approaches in drug discovery for chronic pain. *Adv Pharmacol*, 75, p. 303–323.

Xia, F., Chang, X., Zhang, Y., Wang, L & Xiao, F., 2018. The effect of intrathecal dexmedetomidine on the dose requirement of hyperbaric bupivacaine in spinal anaesthesia for caesarean section: a prospective, double-blinded, randomized study. *BMC Anesthesiol*, 18(1), 74.

Xu, M., Kontinen, V.K. & Kalso, E., 2000b. Effects of radolmidine, a novel α2-adrenergic agonist compared with dexmedetomidine in different pain models in the rat. *Anesthesiology*, 93(3), p. 473–481.

Xu, M., Wei, H., Kontinen, V.K., Kalso, E. & Pertovaara, A., 2000a. The dissociation of sedative from spinal antinociceptive effects following administration of a novel alpha-2-adrenoceptor agonist, MPV-2426, in the locus coeruleus in the rat. *Acta Anaesthesiol Scand,* 44(6), p. 648–655.

Yaksh, T.L., 1985. Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. *Pharmacol Biochem Behav*, 22(5), p. 845–858.

Yaksh, T.L., 1986. The effects of intrathecally administered opioid and adrenergic agents on spinal function. In: Yaksh, T.L., ed. Spinal afferent processing. New York: Plenum Press; 1986:505–539.

Yaksh, T.L., Fisher, C.J., Hockman, T.M. & Wiese, A.J., 2017. Current and future issues in the development of spinal agents for the management of pain. *Curr Neuropharmacol*, 15(2), p. 232–259.

Yaksh, T.L., Horais, K.A., Tozier, N.A., Allen, J.W., Rathbun, M., Rossi, S.S., Sommer, C., Meschter, C., Richter, P.J. & Hildebrand, K.R., 2003. Chronically infused intrathecal morphine in dogs. *Anesthesiology*, 99(1), p. 174–187.

Yaksh, T.L., Rathbun, M.L., Dragani, J.C., Malkmus, S., Boudeau, A.R., Richter, P., Powell, H., Myers, R.R & LeBel, C.P., 1997. Kinetic and safety studies on intrathecally infused recombinant-

methionyl human brain-derived neurotrophic factor in dogs. *Fundam Appl Toxicol*, 38(1), p. 89–100.

Yaksh, T.L., Rathbun, M., Jage, J., Mirzai, T., Grafe, M. Hiles, R.A., 1994. Pharmacology and toxicology of chronically infused epidural clonidine HCl in dogs. *Fundam Appl Toxicol*, 23(3), p. 319–335.

Yaksh, T.L. & Rudy, T.A., 1976. Chronic catheterization of the spinal subarachinoid space. *Physiol Behav*, 17(6), p. 1031–1036.

Yokota, S.I., Hikasa, Y. & Mizushima, H., 2013. Effects of imidazoline and non-imidatzoline α-adenergic agents on rabbit platelet aggregation. *Pharmacology*, 91(3–4), p. 135–144.

Yu, Y. & Koss, M.C., 2005. Rat clonidine mydriasis model: imidazoline receptors are not involved. *Autom Neurosci*, 117(1), p. 17–24.

Zádori, Z.S., Shujaa, N., Fülöp, K., Dunkel, P. Gyires, K., 2007. Pre- and postsynaptic mechanisms in the clonidine- and oxymetazoline-induced inhibition of gastric motility in the rat. *Neurochem Int*, 51(5), p. 297–305.

Zhang, C., Li, C., Pirrone, M., Sun, L. & Mi, W., 2016. Comparison of dexmedetomidine and clonidine as adjuvants to local anesthetics for intrathecal anesthesia: a meta-analysis of randomized controlled trials. *J Clin Pharmacol*, 56(7), p. 827–834.

UNIVERSITY OF TURKU

Painosalama Oy, Turku, Finland 2021

ISBN 978-951-29-8307-0 (PRINT)
ISBN 978-951-29-8308-7 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)