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PREMATURE EJACULATION – POTENTIAL ROLE OF α_2-ADRENOCEPTOR AGONISTS FOR SYMPTOMATIC TREATMENT

Studies of dexmedetomidine, fadolmidine and tasipimidine

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ABSTRACT

Ejaculation is a physiological process that results in the expulsion of semen from the male reproduction tract during a sexual climax and is considered an essential component of natural conception. Ejaculation is a delicately orchestrated process controlled by the peripheral nervous system and the central nervous system and involving sympathetic, parasympathetic and somatic neurons. The most common ejaculatory dysfunction is premature ejaculation being also the most common sexual dysfunction in men – with a prevalence rate up to 30% and not much affected by age. Premature ejaculation is associated with reduced satisfaction in sexual life, distress, anxiety, and depression and has a negative impact on relationships with sexual partners. The etiology of premature ejaculation is considered multifactorial, with psychological, relationship and biological factors. There are limited pharmacological treatment options available. Dapoxetine, an on-demand selective serotonin uptake inhibitor, is the first drug registered for premature ejaculation treatment. A locally administered eutectic mixture of prilocaine/lidocaine, a spray with a local anesthetic effect on the penis, is the other approved medication for premature ejaculation, while others have been used as off-label. Clinical data accumulated in recent years suggests that lifelong premature ejaculation may be associated with increased activity of the sympathetic nervous system. Selective α_2-adrenoceptors have a key role in regulating sympathetic tone in both the periphery and the CNS. Compounds that activate α_2-adrenoceptors decrease sympathetic tonus, and they are widely used in human and veterinary practice. This knowledge of pharmacology prompted us to study the role of α_2-adrenoceptor agonists dexmedetomidine, fadolmidine and tasipimidine in the symptomatic treatment of premature ejaculation. Our observations suggest that centrally acting α_2-adrenoceptor agonists might provide symptomatic relief for premature ejaculation by prolonging ejaculation latency without affecting any other parameter of sexual behaviour or sexual incentive motivation. Whether the effects observed in male rats are directly transferable to men can only be determined through clinical studies.

KEYWORDS: α2-adrenoceptor agonist, premature ejaculation, dexmedetomidine, fadolmidine, tasipimidine

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TIIVISTELMÄ

Ejakulaation eli siemensyöksyn seurauksena siemeneste kulkeutuu ulos uroksen siemenjohtimesta seksuaalisen huipentuman aikana ja sitä pidetään olennaisena osana luonnollista hedelmöitystä. Ejakulaatiota säätelevät sekä ääreishermosto että keskushermosto sympaattisten, parasympaattisten ja somaattisten hermojen välityksellä. Yleisin ejakulaation häiriö on ennenaikainen siemensyöksy, joka on myös yleisin miesten seksuaalinen toimintahäiriö – esiintyvyysaste voi olla jopa 30 %, eikä se juurikaan riipu iästä. Ennenaikaiseen siemensyöksyyn liittyy tyypillisesti monia psykososiaalisia haittoja kuten ahdistusta ja masennusta, mikä voi vaikuttaa kielteisesti seksuaaliterveyteen ja parisuhteeseen. Ennenaikaisen siemensyöksyn etiologia on psykologisten, biologisten ja sosiaalisten tekijöiden summa. Ennenaikaiseen siemensyöksyn hoitoon on tarjolla vain rajoitetusti hoitovaihtoehtoja. Dapoksetiini, nopeasti vaikuttava selektiivinen serotoniinin takaisinoton estäjä, on ensimmäinen tähän indikaatioon rekisteröity lääke. Paikallisesti penikseen annosteltava puudute, prilokaiini-/lidokaiininsuihke, on toinen hyväksytty lääke ennenaikaiseen siemensyöksyyn, kun taas muita lääkkeitä on käytetty vain kokeellisesti. Kliiniset tutkimukset viittaavat siihen, että elinikäinen ennenaikainen siemensyöksy voisi liittyä sympaattisen hermoston lisääntyneeseen aktiivisuuteen. Selektiivisillä α_2-adrenergisilla reseptoreilla on keskeinen rooli sympaattisen hermoston aktiivisuuden säätelyssä sekä ääreis- että keskushermostossa. Yhdisteet, jotka aktivoivat α_2-adrenergisia reseptoreita, vähentävät sympaattista tonusta, ja ne ovat kliinisessä käytössä ihmisille ja eläimille. Nämä fysiologiset ja farmakologiset löydökset innostivat meitä tutkimaan α_2-adrenergisten agonistien, deksmedetomidiinin, fadolmidiinin ja tasipimidiinin, roolia ennenaikaisen siemensyöksyn oireellisessa hoidossa. Havaintomme viittaavat siihen, että keskushermostoon vaikuttavat α_2-adrenergiset agonistit saattavat tarjota oireellista helpotusta ennenaikaiseen siemensyöksyyn pidentämällä siemensyöksyn latenssia vaikuttamatta kuitenkaan samalla muihin seksuaalikäyttäytymisen tai seksuaalisen motivoitumisen parametreihin. Se, ovatko nämä rotilla havaitut vaikutukset toistettavissa miehillä, voidaan selvittää vain kliinisissä tutkimuksissa.

AVAINSANAT: α_2-adrenoreseptori, ennenaikainen siemensyöksy, dexmedetomidiini, fadolmidiini, tasipimidiini.

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Abbreviations

5-HT	5-hydroxytryptamine
ANOVA	Analysis of variance
BAPTA	Glycine,N,N'-[1,2-ethanediylbis(oxy-2,1-phenylene)]bis[N-[2-[(acetyloxy)methoxy]-2-oxoethyl]]-, bis[(acetyloxy)methyl] ester
BP	Blood pressure
BT	Body temperature
Ca^{2+}	Calcium ion
$[Ca^{2+}]$	Calcium ion concentration
CHO	chinese hamster ovary
CNS	Central nervous system
CRO	Contract research organisation
DMEM	Dulbecco's Modified Eagle Medium
EDTA	Ethylenediaminetetraacetic acid
HEPES	N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid
HR	Heart rate
GDP	guanosine diphosphate
GPCR	G protein-coupled receptor
i.t.	Intrathecal
i.v.	Intravenous
K^+	Potassium ion
LC	Locus coeruleus
MAP	Mean arterial pressure
MPOA	medial preoptic area
NA	Noradrenaline
Na^+	Sodium ion
$[Na^+]$	Sodium ion concentration
nPGI	Nucleus paragigantocellularis
PVN	Paraventricular nucleus of hypothalamus
s.c.	Subcutaneous
SSRI	selective serotonin reuptake inhibitor

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 Lehtimäki J, Leino T, Koivisto A, Lehtimäki T, Haapalinna A, Kuokkanen K, Virtanen R. *In vitro* and *in vivo* profiling of fadolmidine, a novel potent α2-adrenoceptor agonist with local mode of action. Eur. J. Pharmacol., 2008; 599: 65-71.

II Snoeren EMS, Lehtimäki J, Ågmo A. Effect of dexmedetomidine on ejaculatory behavior and sexual motivation in intact male rats. Pharmacol. Biochem. Behav., 2012; 103: 345-352.

III Leino T, Lehtimäki J, Koivisto A, Haapalinna A, Pesonen U. Fadolmidine – Favourable adverse effects profile for spinal analgesia suggested by *in vitro* and *in vivo* models. Eur. J. Pharmacol., 2020; 882: 173-298.

IV Lehtimäki J, Ventura-Aquino E, Chu X, Parades RG, Ågmo A. Sexual Incentive Motivation and Copulatory Behavior in Male Rats Treated with the Adrenergic α_2-adrenoceptor agonists Tasipimidine and Fadolmidine: Implications for treatment of premature ejaculation. J. Sex. Med., 2021; 18: 1677-1689.

V Lehtimäki J, Jalava N, Unkila K, Aspegren J, Haapalinna A, Pesonen U. Tasipimidine—the pharmacological profile of a novel orally active selective α_{2A}-adrenoceptor agonist. Eur J Pharmacol. 2022 Apr 8; 923:174949.

1 Introduction

Ejaculation is a physiological process that results in the expulsion of semen from the male reproduction tract during a sexual climax and is considered an essential component of natural conception. The ejaculation process consists of two phases: during the emission phase, sperm moves from the testes and the epididymis to the beginning of the urethra – after which, during the expulsion phase, the semen is expelled through the urethra and out of the body. Ejaculation is a delicately orchestrated synchronised process controlled by the peripheral nervous system and the central nervous system (CNS) and involving sympathetic, parasympathetic and somatic neurons. Hence, there are several types of ejaculation disturbances.

The most common ejaculatory dysfunction is premature ejaculation. It is also the most common sexual dysfunction in men – with a prevalence rate up to 30%, which is not much affected by age (Laumann et al. 2009; Porst et al. 2007). The exact number for the prevalence rate of premature ejaculation is difficult to define since there have been various definitions for premature ejaculation over time:

- Diagnostic and Statistical Manual of Mental Disorders, fifth edition, by the American Psychiatric Association (2013)
- International Statistical Classification of Disease, 11th edition, by the World Health Organization (2019)
- Guideline on Disorders of Ejaculation by the American Urological Association/Sexual Medicine Society of North America (2020)
- The International Society for Sexual Medicine

Although there are subtle differences in the definitions, the consensus is that premature ejaculation is clinically considered a dysfunction if a patient has recurrent ejaculation within one minute of vaginal penetration and before the person wishes it, or if ejaculation occurs shortly after vaginal penetration, the problem has lasted for several months and is associated with clinically significant distress.

The exact course of lifelong premature ejaculation is unknown. However, clinical data accumulated in recent years suggests lifelong premature ejaculation may be associated with increased activity of the sympathetic nervous system. In Rowland's (2010) study, men diagnosed with premature ejaculation had faster and

larger increases in heart rate (HR) during exposure to a pornographic video combined with vibrotactile stimulation of the penis compared to men who did not have premature ejaculation. In their study of reactive hyperaemia, Francomano et al. (2013) reported increased sympathetic activity associated with premature ejaculation by using peripheral arterial tonometry measurements. Along the same lines, Xia et al. (2014) showed in their study that men suffering from premature ejaculation had enhanced sympathetic skin response on the penis, whereas Turan and Gürel (2020) observed that HR recovery after intense exercise was slower in men diagnosed with premature ejaculation compared to men without premature ejaculation, again suggesting sympathetic overactivity. Furthermore, Erbay and Ceyhun's (2021) heart rate variability analyses, used to measure changes in autonomic nervous system activity, indicated that the low frequency/high frequency ratio, reflecting sympathetic/parasympathetic activity ratio, was significantly higher in men with premature ejaculation compared to a control group. Xia et al. (2020) provided further evidence by demonstrating that serum noradrenalin (NA) concentration is increased significantly in patients with premature ejaculation compared to healthy men, while serum 5-hydroxytryptamine (5-HT) concentration is decreased. These observations are in line with animal data demonstrating that male rats with short ejaculation latencies also have higher serum NA concentrations compared to rats with long ejaculation latencies (Xia et al. 2018; Zhang et al. 2020). It has also been shown that agonists of mu-opioid and gamma-aminobutyric acid (GABA) receptors in the paraventricular nucleus of the hypothalamus (PVN) inhibit ejaculation in male rats by weakening sympathetic nerve activity (Zhang et al. 2020).

Selective serotonin reuptake inhibitors (SSRI) such as paroxetine and citalopram) have been used for off-label symptomatic treatment of premature ejaculation, with relatively satisfactory results in chronic use (Ciocanel et al. 2019; Waldinger 2007a,b; Zhang et al. 2019). However, to avoid the adverse effects of chronic use of SSRIs, an on-demand SSRI, dapoxetine, was developed for symptomatic treatment of premature ejaculation. Dapoxetine is the first drug registered for the treatment of premature ejaculation in the European Union (Yue et al. 2015). The locally administered eutectic mixture of prilocaine/lidocaine, a spray with a local anaesthetic effect, is the other approved medication for premature ejaculation, with all others being used off-label. Among other putatively efficient drugs for premature ejaculation are the opioid agonist tramadol (Kirby et al. 2015) and phosphodiesterase-5 inhibitors (PDE5 inhibitor) (Jin et al. 2018). Also, selective α_{1A}-adrenoceptor antagonists have been studied for premature ejaculation treatment (Sato et al. 2017).

Presynaptically located α_2-adrenergic autoreceptors on noradrenergic nerve cells inhibit noradrenaline (NA) release and thus diminish the activity of noradrenergic pathways, resulting in sympatholytic effects in the CNS and periphery. These α_2-

adrenoceptors are also expressed on noradrenergic nerve endings anatomically involved in seminal emission and ejaculation. Hypothetically, activation of these α_2-adrenoceptors might result in delayed or reduced peripheral responses involved in seminal emission, which makes the receptors possibly attractive drug targets for the symptomatic treatment of premature ejaculation. Since primary premature ejaculation greatly impacts the mental health of patients, causing anxiety and depression (Liu et al. 2021), one could speculate that known central sympatholytic and anxiolytic effects of α_2-adrenoceceptor agonists could provide additional benefits in the treatment of premature ejaculation.

Past research experience in the α_2-adrenoceceptor field has led to the hypothesis that α_2-adrenoceceptor agonists could be beneficial for symptomatic treatment of premature ejaculation. It has been suggested that short ejaculation latencies in male rats are analogous to premature ejaculation symptoms in men (Chan et al. 2008; Pattij et al. 2005a; Pattij et al. 2005b). Therefore, it is reasonable to assume that rats can be used as a research model to evaluate drug efficacy in the symptomatic treatment of premature ejaculation in men. This prompted us to study the effects of three α_2-adrenoceceptor agonists developed by Orion – dexmedetomidine, tasipimidine and fadolmidine – on sexual incentive motivation and copulatory behaviour in male rats.

Dexmedetomidine has been in clinical use for years and its pharmacological and pharmacokinetic properties are well known. Thus, it was selected as a prototypic α_2-adrenoceceptor agonist to study the potential beneficial effects of α_2-adrenoceceptor agonists on sexual motivation and copulatory behaviour.

Tasipimidine is an orally active α_{2A}-selective α_2-adrenoceceptor agonist developed for situational anxiety and fear in dogs and is currently being investigated for insomnia indication in human patients. The oral bioavailability of dexmedetomidine is limited (Anttila et al. 2003), and consequently, oral dosing is not an optimal dosing route in clinical settings. Thus, we speculated that tasipimidine, as an orally active compound, could provide additional value regarding convenience in use. As a part of this thesis work, we characterised the in vitro and in vivo pharmacological properties of tasipimidine in rat and mice models (Lehtimäki et al. 2022) and studied its effects on sexual motivation and copulatory behaviour in male rats (Lehtimäki et al. 2021).

Fadolmidine is a selective α_2-adrenoceceptor agonist with a local mode of action. It was originally developed as a spinal analgesic but is not currently in clinical use. Based on its chemical structure, fadolmidine demonstrates poor penetration of the blood–brain barrier (BBB) and is metabolised relatively fast in the bloodstream. Peripheral α_2-adrenoceptors are mainly located in the vascular and smooth muscle prejunctional terminals, where they inhibit NA release (Doherty 1998). Thus, α_2-adrenoceptor agonists might reduce sympathetic responses in sexual organs

innervated by the sympathetic nervous system. Peripherally acting α_2-adrenoceptor agonists might provide an attractive treatment option to avoid the centrally mediated effects of α_2-adrenoceptors, such as sedation and hypotension. In this thesis work, the pharmacological profile of fadolmidine was thoroughly characterised using multiple in vitro, ex vivo and in vivo models (Lehtimäki et al. 2008; Leino et al. 2020). Further, its effects on sexual motivation and copulatory behaviour in male rats were studied (Lehtimäki et al. 2021).

Premature ejaculation reduces sexual satisfaction and often weakens the quality of relationships (Tan et al. 2012). It also greatly impacts patients' mental well-being by causing anxiety and depression. On some occasions premature ejaculation can be a learned pattern developed and maintained by anxiety about sexual failure (Althof 2006; Lu et al. 2020; McCabe & Connaughto 2014; Mourikis et al. 2015; Rajkumar & Kumaran 2015; Strassberg et al. 1990). Psychotherapeutic approaches have been shown to provide positive effects, especially when combined with pharmacological treatment and particularly for acquired premature ejaculation (Cooper et al. 2015). However, medical treatment seems to be the more attractive option, especially for men with lifelong premature ejaculation. Consequently, several drugs are used for the symptomatic treatment of premature ejaculation, delaying ejaculation.

2 Review of the Literature

2.1 Physiology and anatomy of ejaculation

Ejaculation is considered the climax of the male sexual cycle. As a physiological process, ejaculation results in the expulsion of semen from the male reproduction tract and, in that sense, is considered an essential component of natural conception. Physiologically, ejaculation is a delicately orchestrated process controlled by peripheral nerves and the CNS, with the involvement of sympathetic, parasympathetic and somatic neurons.

Antegrade ejaculation comprises two distinct phases, namely emission and expulsion, which are orchestrated by the spinal ejaculatory generator located in the lumbar region, specifically at the L3–L4 level. The emission phase is a sympathetic reflex governed by the lumbar secretory center, spanning from T10 to L2 within the spinal cord. During this emission phase, contractions occur in the epididymis, vas deferens, seminal vesicles, and prostate, facilitating the deposition of sperm and seminal fluids into the posterior urethra (Birowo et al. 2010; Gil-Vernet et al. 1994; Master & Turek 2001). Cholinergic stimulation during the process of erection leads to an increase in secretion, while adrenergic stimulation enhances emission by triggering contractions in the vas deferens, seminal vesicles, and the smooth muscles of the prostate and bladder neck. Subsequently, the expulsion phase is initiated by the accumulation of seminal fluid in the urethra. A rise in pressure in the prostatic urethra, caused by adrenergic activity and rhythmic somatic stimulation of sacral muscles (Carro-Juárez & Rodríguez-Manzo 2000; Everaert et al. 2010; McKenna et al. 1991), prompts the expulsion of seminal fluid. Concurrently, the bladder neck undergoes closure to prevent retrograde ejaculation, while the detrusor muscle of the urinary bladder relaxes. The spinal mechanical centre (S2–S4) controls the expulsion phase by inducing contractions of bulbospongiosus, bulbocavernosus and perineal muscles. Everaert et al. (2010) concluded that in men, undamaged postganglionic parasympathetic fibres are essential for emission, whereas intact postganglionic sympathetic fibres are required for successful ejaculation.

Lumbar spinothalamic neurons within the spinal cord, which transmit signals to the thalamus, exhibit significant activation during ejaculation in male rats (Kozyrev & Coolen 2015; Staudt et al. 2012; Truitt & Coolen 2002; Truitt et al. 2003) and in

men (Chéhensse et al. 2013, 2017). These spinothalamic neurons receive both excitatory and inhibitory inputs from higher brain regions, including the medial preoptic area (MPOA), nucleus paragigantocellularis (nPGI), and the PVN, reflecting the subjective arousal and inhibition processes accordingly (Markowski et al. 1994; Marson & McKenna 1994; McKenna 2022; Yells et al. 1992). Some of these descending inputs are monoaminergic, particularly serotonergic and noradrenergic. Additionally, sensory neurons in the spinal cord, especially those originating from the genital area, provide excitatory and inhibitory connections. When the level of excitatory inputs exceeds inhibitory inputs, lumbar spinothalamic cells activate the spinal generator responsible for ejaculation, initiating a coordinated neural pattern that influences the sexual organs, ultimately leading to ejaculation (Golowasch 2019; McKenna 2022; Steuer & Guertin 2019). The spinal generator of ejaculation is recognized as a typical central pattern generator, which refers to a biological neural circuit capable of generating rhythmic outputs without requiring rhythmic input. The spinal generator integrates signals from sexually-related afferent nerves, orchestrates both sympathetic and parasympathetic activities, and coordinates motor output from the CNS (Allard et al. 2005). Lumbar spinothalamic cells have two primary outputs: one projection connects to sympathetic and parasympathetic preganglionic neurons that innervate the pelvic organs, while the other projection extends to the thalamus (Figure 1).

The supraspinal centres play an important role in ejaculation physiology. Sexually related somatosensory inputs are conducted from the genital areas to the sensory spinal nuclei, as well as the cerebral cortex. Somatosensory inputs and external sexual clues, such as visual, auditory and olfactory signals, are forwarded to the thalamus (subparafascicular nucleus) and hypothalamus (MPOA, paraventricular nucleus), which integrates genital and other sensory inputs and activates relay stations in the midbrain (periaqueductal grey) and pons (nPGI) with direct projections into the spinal cord. The MPOA summarises sexually related stimuli and generates coherent outputs relevant to sexual responses. Inhibitory neurons from the nPGI, causing tonic inhibition of ejaculation, terminate in the region of the pudendal motor neurons in the ventral part of the lumbosacral spinal cord. Anatomical studies have shown that the main sympathetic innervations to accessory sex glands are the lumbar splanchnic nerve, the caudal mesenteric plexus, the hypogastric nerve and the pelvic plexus, as well as its branches (Giuliano 2011; Kihara et al. 1998) (Figure 2).

The two components of sexual climax, pelvic activation and the subjective experience, are related to spinal and supraspinal mechanisms accordingly (McKenna 2022). It has been reported that men with complete spinal cord lesions can achieve ejaculation by genital stimulation without the supraspinal component (Alexander &

Marson 2018a,b; Chéhensse et al. 2013). The psychological components of orgasm are generated by supraspinal sites.

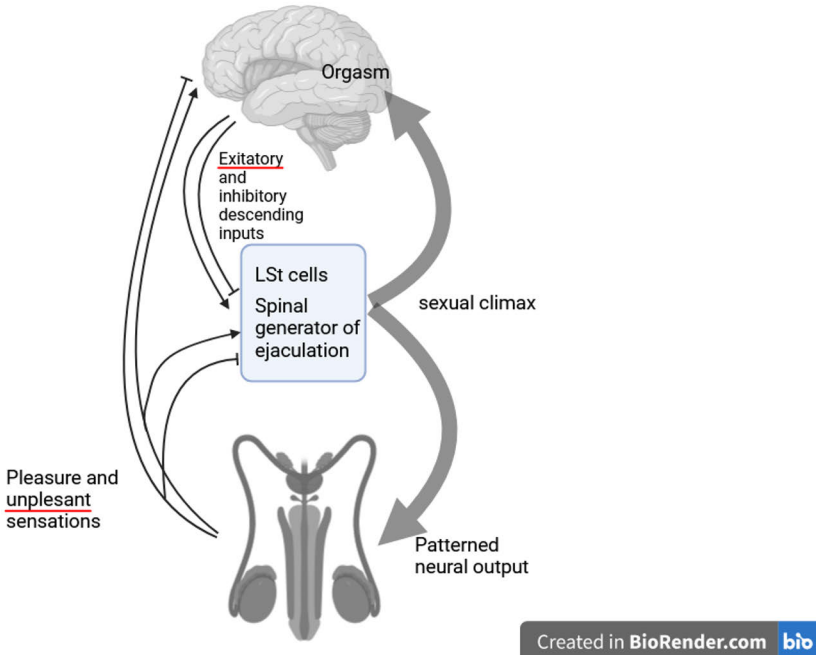


Figure 1. Schematic presentation of the ejaculation control. Ejaculation is generated by the lumbar spinothalamic neurons cells (LSt) in the spinal generator of ejaculation in the lumbar spinal cord, evoking the pelvic responses characteristic of ejaculation and activating the subjective experience of orgasm in supraspinal sites. The activity of LSt cells is controlled by spinal sensory inputs and excitatory and inhibitory projections from supraspinal sites. LSt cells are also regulated by monoaminergic neuromodulatory inputs. Modified from McKenna (2022).

Research conducted on male rats with spinal cord transections under urethane anesthesia by Carro-Juárez and Rodríguez-Manzo (2006) has shown that adrenergic innervation plays a significant role in influencing the function of the spinal ejaculation generator. In this experimental model, the activation of α_1-adrenoceptors by methoxamine and the blocking of α_2-adrenoceptors by yohimbine are involved in initiating and regulating the rhythmic genital motor pattern associated with ejaculation. In contrast, clonidine, an α_2-adrenoceptor agonist, and prazosin, an α_1-adrenoceptor antagonist, are capable of inhibiting the activity of the spinal generator responsible for ejaculation without affecting other genital motor functions (Carro-Juareza & Rodríguez-Manzo 2003, 2006). Consequently, yohimbine was found to induce the ejaculatory genital motor pattern in Carro-Juareza and Rodríguez-Manzo's (2003) study involving a depleted coital reflex model. Courtois et al. (2013)

also pointed out that men experiencing ejaculation exhibit characteristic signs of sympathetic activation, including hypertension, tachycardia, and rhythmic involuntary muscle contractions. Collectively, these findings suggest that noradrenergic compounds have the potential to modulate the spinal generator responsible for controlling ejaculation.

The supraspinal circuitry that regulates sexual function receives noradrenergic innervation from either the lateral tegmental area or the locus coeruleus (LC). Notably, the pudendal nucleus of motoneurons, which supplies the striated genital muscles involved in ejaculation, contains a particularly dense population of noradrenergic cell groups (Kojima et al. 1985; Lyons et al. 1989; Rajaofetra et al. 1992). The brain regions thought to contribute to the noradrenergic control of ejaculation include the nPGI, LC, and PVN. α_2-adrenoreceptors are widely distributed throughout the CNS (Alburges et al. 1993; Wamsley et al. 1992), and their presence has been confirmed in these brain regions, along with noradrenergic connections to other parts of the brain. Consequently, it is reasonable to hypothesize that compounds capable of penetrating the brain and acting on α_1- and α_2-adrenoceptors could play a role in the supraspinal modulation of ejaculation.

Clinical observations of men who have undergone retroperitoneal lymphadenectomy for testicular cancer or resection for rectal cancer provide further evidence supporting the involvement of sympathetic pathways in ejaculation. Anejaculation and retrograde ejaculation have been observed as unintended consequences of the inadvertent disruption of sympathetic efferent nerves in these patients. In contrast, surgical procedures that preserve sympathetic innervation are associated with a lower incidence of ejaculatory dysfunction. Furthermore, in paraplegic men unable to ejaculate during sexual intercourse, electrical stimulation of the hypogastric plexus, which contains postganglionic sympathetic neurons, can trigger emission (Brindley et al. 1989).

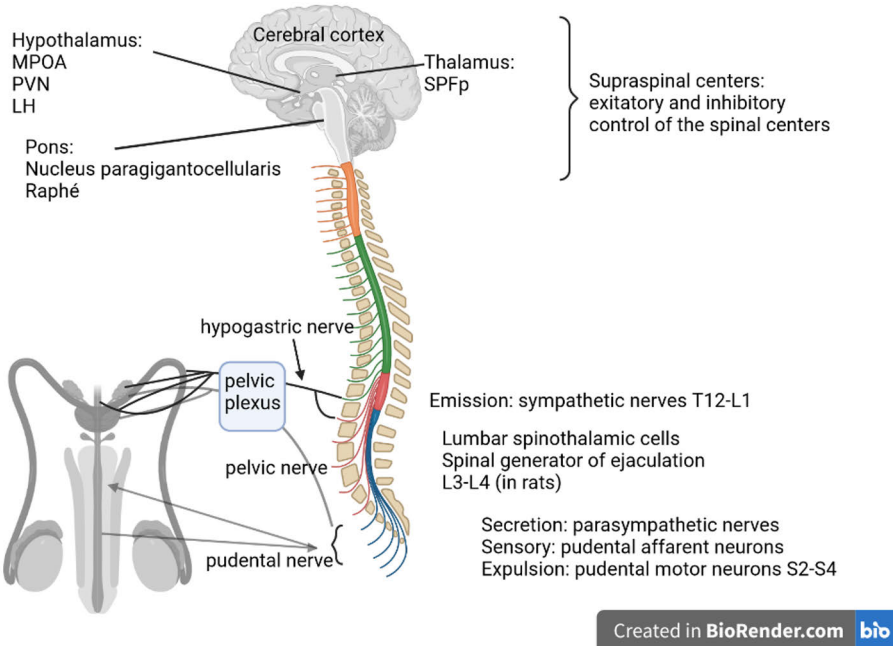


Figure 2. Schematic presentation of the neurophysiology of ejaculation. Modified from Celigoj et al. (2016) and Giuliano (2013). MPOA = medial preoptic area, PVN = paraventricular nucleus of hypothalamus, SPFp = parvocellular subparafascicular nucleus, LH = lateral hypothalamus.

2.2 Premature ejaculation

Definitions for premature ejaculation have evolved over time from authority-based expert opinions to more scientifically objective and subjective concepts (Althof et al. 2022). Evidence-based research aims to establish definitions with the most relevant criteria to yield the fewest diagnostic errors, either a false-positive or a false-negative diagnosis. However, clinical practice requires some degree of freedom over the established diagnostic criteria to provide help to men complaining of premature ejaculation but having an atypical presentation or not fitting exactly into the classification. Diagnosis of premature ejaculation is based on the medical and sexual history that classifies premature ejaculation as lifelong or acquired and determines whether premature ejaculation is consistent or situational. Specific attention is given to the duration of ejaculation, the degree of sexual stimulus, the impact on sexual activity and quality of life, and drug use or abuse (Salonia et al. 2021). There are various criteria for premature ejaculation in use by different bodies, with some minor differences.

Table 1. Various criteria for the premature ejaculation by different authors

AUTHOR	CRITERIA FOR PREMATURE EJACULATION
Diagnostic and Statistical Manual of Mental Disorders, fifth edition (2013)	A persistent or recurrent pattern of ejaculation occurring during partnered sexual activity within approximately 1 min following vaginal penetration and before the individual wishes. The symptom must be present for at least 6 months and must be experienced on almost all or all (approximately 75%–100%) occasions of sexual activity, causing clinically significant distress. The sexual dysfunction is not better explained by a nonsexual mental disorder or as a consequence of severe relationship distress or other significant stressors and is not attributable to the effects of a substance/medication or another medical condition.
International Statistical Classification of Disease, 11th edition (2019)	Male early ejaculation is characterised by ejaculation that occurs prior to or within a very short duration of the initiation of vaginal penetration or other relevant sexual stimulation, with no or little perceived control over ejaculation. Early ejaculation occurs episodically or persistently over a period of at least several months and is associated with clinically significant distress.
American Urological Association/Sexual Medicine Society of North America's Guideline on Disorders of Ejaculation (2020)	Lifelong premature ejaculation is defined as poor ejaculatory control, associated bother and ejaculation within about 2 min of initiation of penetrative sex that has been present since sexual debut. Acquired premature ejaculation is defined as consistently poor ejaculatory control, associated bother and ejaculation latency that is markedly reduced from prior sexual experience during penetrative sex.
The International Society for Sexual Medicine 2013 (Althof et al. 2014)	A male sexual dysfunction characterised by ejaculation which always or nearly always occurs prior to or within about one minute of vaginal penetration, and the inability to delay ejaculation on all or nearly all vaginal penetrations, with negative personal consequences such as distress, bother, frustration and/or the avoidance of sexual intimacy

According to McMahon et al. (2008), the concepts that are essential for the definition of premature ejaculation are rapidity of ejaculation, perceived self-efficacy and control, and negative personal consequences. Accordingly, the three-pronged diagnostic criteria for premature ejaculation are as follows (Rowland et al. 2022):

- short ejaculatory latency upon minimal stimulation
- the inability to control, delay or postpone ejaculation
- negative consequences such as bother or distress about the condition

The lack of self-efficacy regarding ejaculatory control is considered the central concept for both the diagnosis of premature ejaculation and the perceived treatment benefits.

Premature ejaculation is the most common sexual dysfunction among men, with prevalence rates of 20%–30% (Althof 2006; Hatzimouratidis et al. 2010; Porst et al. 2007). Patrick et al. (2009) data suggests that the prevalence of lifelong premature ejaculation, based on an intravaginal ejaculatory latency of 1–2 minutes, is 2%–5%. The prevalence of premature ejaculation is not affected by age (Montorsi 2005; Porst et al. 2007).

Lifelong premature ejaculation occurs by definition in all or nearly all vaginal penetration occurrences, beginning from the first sexual encounter. In comparison, acquired (secondary) premature ejaculation develops after previous sexual experiences without ejaculatory problems and can result from psychological/relationship or pathophysiological changes, such as pelvic trauma, disease and medication. The focus of this thesis is on lifelong premature ejaculation.

2.2.1 Pathophysiology

The aetiology of premature ejaculation is considered multifactorial, with psychological, relationship and biological aspects being underpinning factors.

Psychological factors

Self-reports by men having premature ejaculation suggest that ejaculation latency is affected by various psychological, behavioural, partner-related and/or situational contextual factors. While biological factors may set a predetermined range for ejaculation latency, psychological variables such as sexual desire, expectations, anxiety, attention, and arousal contribute to individual variations within this range. Additionally, psychological and biological processes can interact in a reciprocal manner: anxiety can hinder sexual response, and experiences of sexual failure may negatively impact self-esteem and increase anxiety, exacerbating the issue (Buvat 2011).

Relationship and cultural factors

Premature ejaculation typically affects both partners in a relationship. The partner may share the distress, self-doubt, and sexual dissatisfaction associated with the short duration of the sexual act. Furthermore, if the partner also has their own sexual issues, it can worsen the problem or interfere with treatment. Relationship dynamics and cultural factors, such as societal expectations regarding gender roles, can

significantly influence how a couple perceives the problem and their willingness to seek and engage in treatment (Buvat 2011; Saitz & Serefoglu 2015).

Biological factors

Biological factors contributing to premature ejaculation can be categorized as either physiological or pathophysiological. Physiological factors are related to the neurophysiology of the reflexive components of ejaculation and can have genetic or epigenetic origins. However, no specific anomalies in genotype, sensorimotor response, neurotransmitter function, or pathophysiological conditions have been definitively identified in men with lifelong premature ejaculation or in animal models. It's worth noting that men with erectile dysfunction often have an increased risk of experiencing premature ejaculation, although determining the primary underlying cause can be complex (Rowland 2021). Certain genetic factors have been suggested to contribute to intravaginal ejaculation latency time, including genes related to the 5-HT_{1A} and 5-HT_{2C} receptor subtypes, as well as the dopamine transporter gene polymorphism (Eltonsi et al. 2017 and Jern & Ventus 2017). Additionally, Nikoobakht et al. (2005) reported that men diagnosed with premature ejaculation tend to have a significantly lower semen-to-serum magnesium ratio, and they found a reverse relationship between body mass index and premature ejaculation.

Sympathetic nervous system overactivity

Krüger et al. (1998) and Wiedeking et al. (1979) have suggested a connection between plasma NA levels during sexual activity in men and the quality of erection stages. These studies indicate that the concentration of NA transiently increases, up to 12 times higher than baseline levels, during ejaculation. This increase is attributed to heightened neurotransmitter release, which is a result of increased activity in the sympathetic nervous system. Recent research has been accumulating evidence suggesting a potential link between premature ejaculation and elevated sympathetic nervous system activity. Rowland (2010) found that men experiencing premature ejaculation exhibited faster and more pronounced increases in heart rate when exposed to pornographic videos combined with vibrotactile stimulation of the penis compared to men without diagnosed premature ejaculation. These early findings led to the speculation that premature sympathetic activation might contribute to early ejaculation. Along the same lines, Zorba et al. (2012) reported heightened sympathetic activity in men with lifelong premature ejaculation, as measured through a 24-hour heart rate variability analysis. Furthermore, Erbay and Ceyhun (2020) observed a higher ratio of sympathetic to parasympathetic activity in men

with premature ejaculation compared to controls, indicating an imbalance in the autonomic nervous system. Increased sympathetic activity was also evident in men diagnosed with premature ejaculation when measuring reactive hyperemia using peripheral arterial tonometry (Francomano et al. 2013). Making the evidence even more compelling, Xia et al. (2014) noted enhanced sympathetic skin responses on the penises of men diagnosed with premature ejaculation. This was further supported by Turan and Gürel's (2020) study, which indicated that heart rate recovery after intense exercise was slower in men with premature ejaculation compared to healthy controls. Additionally, compared to healthy men, the levels of NA were significantly higher in patients with premature ejaculation, while serum serotonin levels were decreased (Xia et al. 2020). Consequently, primary symptoms of premature ejaculation can potentially be alleviated by SSRIs, which inhibit the sympathetic nervous system (Xia et al. 2014).

Observations in men, considering the role of heightened sympathetic activity as the underlying cause of premature ejaculation, align with findings from studies involving rats. These studies have revealed that male rats with shorter ejaculation latencies exhibit higher serum concentrations NA compared to rats with longer ejaculation latencies (Xia et al. 2018; Zhang et al. 2020). Given that serum NA primarily originates from the peripheral nervous system, it can be hypothesised that increased sympathetic nervous system activity is also associated with rapid ejaculation in rats. Additionally, research has demonstrated that agonists of mu-opioid and gamma-aminobutyric acid receptors in the PVN can inhibit ejaculatory behaviours in male rats by dampening sympathetic nerve activity (Zhang et al. 2020). The PVN plays a crucial role in integrating ejaculatory reflexes and regulating sympathetic outflow to the intermediolateral column of the spinal cord (Marson & McKenna 1994; Zhong et al. 2008). Li et al. (2001) have shown that N-methyl-D-aspartic acid (NMDA) receptors in the PVN control sympathetic nerve discharge. These NMDA receptors are believed to influence ejaculatory responses by affecting sympathetic outflow, and ejaculatory latency may be linked to the baseline sympathetic nervous system activity during sexual activity. NMDA facilitates ejaculation, and a specific NMDA receptor antagonist, D(-)-2-amino-5-phosphonopentoic acid, delays ejaculation in rats (Xia et al. 2017). Accordingly, the upregulation of NMDA receptors in the PVN reduces ejaculation latency in rats (as suggested by Yang et al. in 2021).

Blocking α_2-adrenoreceptors stimulates sexual behaviour in rats, while activation of these receptors inhibits copulation (Benelli et al. 1993; Clark 1991; Clark et al. 1984; Clark et al. 1985; Clark & Smith 1990). Stimulation of α_1-adrenoceptors may also exert an inhibitory effect on sexual behaviour (Clark 1991). Recent studies indicate that the sympathetic pathway of ejaculation primarily operates through α_{1A}-adrenoreceptors. NA induces contractions of the vas deferens

smooth muscle by predominantly binding to α_{1A}-adrenoreceptors, which is necessary for subsequent sperm ejaculation (Sanbe et al. 2007). The expulsion phase of ejaculation is triggered by the accumulation of seminal fluid in the urethra, leading to an increase in urethral pressure to its maximum level (Carro-Juárez & Rodríguez-Manzo 2000). It is plausible that heightened sympathetic activation in premature ejaculation results in an early rise in urethral pressure, thereby inducing ejaculation. Consequently, α_{1A}-adrenoreceptor antagonists may hold therapeutic promise for the symptomatic treatment of premature ejaculation (Hsieh et al. 1998; Hsieh et al. 1999; Hsieh et al. 2014; Sato et al. 2017).

In essence, the concept of short ejaculation latency in male rats is considered analogous to premature ejaculation in men (Chan et al. 2008; Pattij et al. 2005). Several drugs that influence copulatory behaviour in rats have also demonstrated efficacy in humans. For example, SSRIs inhibit copulatory behaviour (Chan et al. 2008; Waldinger 2007a,b, 2007b), while sildenafil stimulates copulation and facilitates ejaculation (Giuliani et al. 2002; Ottani et al. 2002; Steidle et al. 2007). Therefore, it is reasonable to assume that compounds affecting ejaculation in rats will also have similar effects in men, and rat models can be utilized to assess drugs for the treatment of premature ejaculation.

2.2.2 Treatment

Premature ejaculation is associated with reduced satisfaction in sexual life, distress, anxiety, embarrassment and depression and has a negative impact on relationships with sexual partners (Kempeneers et al. 2018; Tan et al. 2012). Nevertheless, most men having premature ejaculation do not seek help. Porst et al. (2007) reported as low a number as 9.0% of men with premature ejaculation consulting a physician for the symptoms, and 91.5% reported little or no improvement after the visit.

Data on psychotherapeutic approaches suggests they might have some positive effects, especially on acquired premature ejaculation and when combined with drug treatment (Cooper et al. 2015). However, particularly for patients with lifelong premature ejaculation, pharmacological treatment appears to be more effective. Accordingly, the European Association of Urology's guidelines state that pharmacotherapy must be considered the first-line treatment for patients with lifelong premature ejaculation (Salonia et al. 2021).

Various drugs are currently used for premature ejaculation with the aim of delaying ejaculation. Specific SSRIs have been used for off-label symptomatic treatment of premature ejaculation for several years. Satisfactory results have been reported for paroxetine and citalopram, as well as other SSRIs (Ciocanel et al. 2019; Zhang et al. 2019). However, the known shortcomings of classical SSRIs limit their use for premature ejaculation: they must be taken chronically, and they inhibit other

sexual responses, such as desire and erection (Waldinger 2007a,b). These shortcomings prompted studies exploring on-demand SSRIs. Dapoxetine was the first drug registered as a treatment for premature ejaculation in the European Union (Yue 2015). A locally administered eutectic mixture of prilocaine/lidocaine, a spray with a local anaesthetic effect on the penis, is the other approved medication for premature ejaculation. Others have been used as off-label.

Mu-opioid receptor agonists inhibit ejaculatory behaviours in male rats by weakening sympathetic nerve activity (Olivier et al. 2016; Zhang et al. 2020). Accordingly, the opioid agonist tramadol (used on demand) has been reported to increase intravaginal ejaculatory latency time and to improve subjective measures of satisfaction in men diagnosed with premature ejaculation (Kirby et al. 2015).

PDE5 inhibitors have shown efficacy by increasing intravaginal ejaculation latency time, sexual satisfaction and ejaculatory control in men diagnosed with premature ejaculation when used alone or combined with SSRIs (Jin et al. 2018; Martyn-St James et al. 2017). It is assumed that PDE5 inhibitors act peripherally by relaxing smooth muscles in the prostate, seminal vesicles, vas deferens and urethra, as well as centrally by reducing sympathetic output (Mattos et al. 2008; Wang et al. 2006). Erectile dysfunction is a common comorbidity with premature ejaculation, and in clinical practice, it is recommended that erectile dysfunction should always be treated first.

Selective α_{1A}-adrenoceptor antagonists have also been studied for premature ejaculation treatment. Sato et al. (2017) reported that silodosin increases intravaginal ejaculation latency time in patients with acquired premature ejaculation and alleviates premature ejaculation symptoms. Other medical treatments, such as 5-HT_{1A} receptor antagonists (Migliorini et al. 2021) and pregabalin (El Najjar et al. 2020), have been suggested for premature ejaculation but with less established efficacy. In a review article, Abdel-Hamid (2005) discussed an alternative approach to developing drugs acting directly upon accessory sex organs, emphasising smooth muscle cell relaxation with α_1-adrenoceptor antagonists, PDE5 inhibitors, calcium channel blockers and potassium channel openers.

2.3 α_2-adrenoceptors

2.3.1 Structure and function at the cellular level

α_2-adrenoceptors belong to the seven transmembrane receptor superfamily, referring to a group of membrane proteins containing seven membrane-spanning helices with an amino terminus outside and a carboxyl terminus inside the cells. Seven transmembrane receptors are also referred to as G protein–coupled receptors (GPCRs), as they are coupled into heterotrimeric guanine nucleotide–binding

regulatory proteins (G proteins) on the intracellular side of the cell membrane and, in the case of α_2-adrenoreceptors, to the inhibitory Giα subtype. G proteins belong to a group of enzymes called GTPases. They are heterotrimeric complexes made up of alpha (α), beta (β) and gamma (γ) subunits. The activity of G proteins is regulated by factors controlling the α unit's ability to bind to and hydrolyse guanosine triphosphate (GTP) to guanosine diphosphate (GDP) (Wettschureck & Offermanns 2005).

α_2-adrenoceptors are activated by the physiological agonists adrenaline and NA. At the second messenger level, activated α_2-adrenoceptors inhibit the activity of adenylyl cyclase (AC), resulting in reduced levels of cyclic adenosine monophosphate and causing hyperpolarisation of noradrenergic neurons by the opening of K^+ channels by the α_{2B}- and α_{2C}-adrenoceptor subtypes (Arima et al. 1998) and inhibition of Ca^{2+} influx (Soini et al. 1998; Timmons et al. 2004). At the molecular level, the binding of an agonist ligand to an α_2-adrenoceptor induces dissociation of a GDP molecule from the α-subunit of the heterodimeric Gi protein and replacement of the molecule with a GTP molecule. Consequently, the α-subunit dissociates from the βγ-dimer and inhibits AC on the inner surface of the cell membrane. GTP is hydrolysed to GDP by the α-subunit's GTPase activity. The heterodimeric G protein reassociates and is recycled to the receptor again (Figure 3).

Overactivation of α_2-adrenoceptors results in receptor desensitisation by a β-arrestin pathway. Receptor activation leads to interaction between the βγ-subunits of the G protein and G protein-coupled receptor kinases, thus enabling the phosphorylation of α_2-adrenoceptors. Afterwards, β-arrestin binds to the phosphorylated α_2-adrenoceptors, resulting in α_2-adrenoceptor endocytosis (DeGraff et al. 1999; Diverse-Pierluissi et al. 1996).

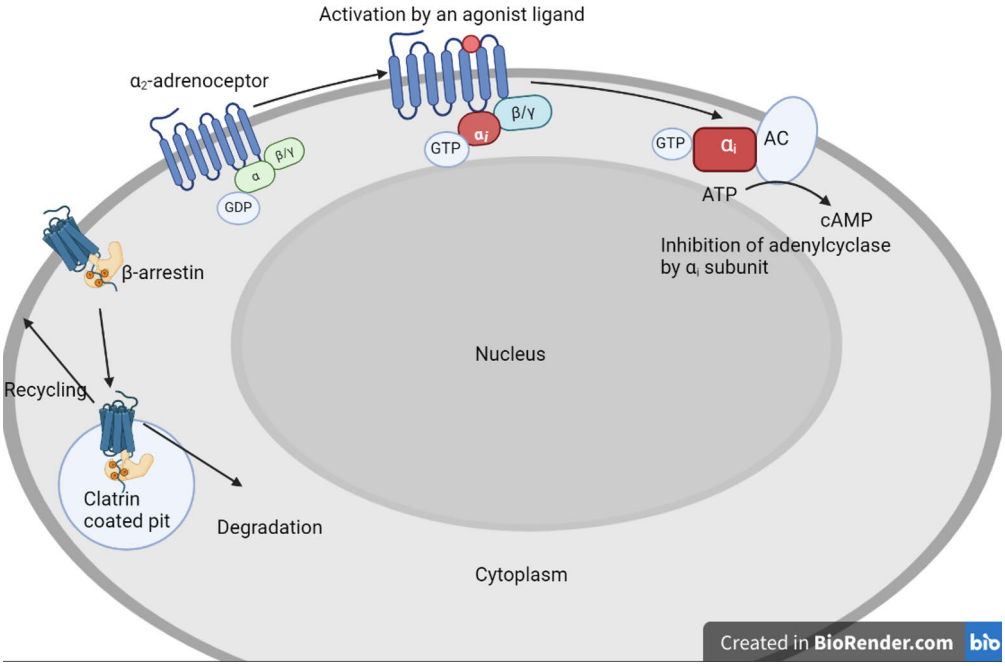


Figure 3. Schematic presentation of α_2-adrenoceptor activation, second messenger engagement and recycling. ATP = adenosine triphosphate, AC = adenylate cyclase, cAMP = cyclic adenosine monophosphate, GDP = guanosine diphosphate GTP = guanosine triphosphate

Presynaptic α_2-adrenoceptors on noradrenergic nerve cells function as autoreceptors inhibiting the release of NA and some other neurotransmitters, thereby reducing the activity of noradrenergic pathways. In nonnoradrenergic nerve cells, NA inhibits the release of acetylcholine from parasympathetic neurons by acting on α_2-adrenergic heteroreceptors. Some of the α_2-adrenoceptors are located on postsynaptic cells and mediate the effects of catecholamines released from sympathetic nerves, such as vasoconstriction (Philipp et al. 2002).

2.3.2 Physiology

From a physiological perspective, α_2-adrenoceptors play a role in mediating various effects of endogenous catecholamines both in the CNS and in the peripheral regions of the body. Activation of α_2-adrenoceptors leads to pharmacodynamic responses, including sedation, analgesia (Yaksh 1985), bradycardia, and hypertension caused by peripheral vasoconstriction, followed by subsequent hypotension (Dabire et al.1986; Laubie et al. 1985). Additionally, α_2-adrenoceptor activation can result in

hypothermia, as evidenced by research conducted by Bill et al. (1989) and Minor et al. (1989).

Based on pharmacological and molecular cloning investigations, human α_2-adrenoceptors are categorized into three distinct subtypes: α_{2A}, α_{2B}, and α_{2C} (Bylund et al. 1992). The rodent α_{2D}-adrenoceptor is an orthologue of the human α_{2A}-adrenoceptor. The α_{2A}-subtype primarily mediates most of the physiological actions attributed to α_2-adrenergic activity, while the α_{2B} and α_{2C} receptor subtypes are generally viewed as regulators with more subtle effects. For instance, in vascular smooth muscle, stimulating α_{2B}-adrenoceptors induces vascular contraction and hypertensive responses, opposing the hypotensive effects resulting from the activation of central α_{2A}-adrenoceptors (Link et al. 1996). The α_{2C}-adrenoceptor subtype is linked to various centrally mediated effects, including the startle reflex, stress response, and locomotion (Civantos Calzada et al. 2001). Interestingly, the α_{2C}-adrenoceptor subtype exhibits atypical subcellular localization in vascular smooth muscle cells and fibroblasts at normal body temperature. However, lower temperatures stimulate its transport to the cell surface (Filipeanu 2015).

2.3.3 α_2-adrenoceptor agonists in clinical use

Based on the various important physiological functions of α_2-adrenoceptors, α_2-adrenoceptor agonists have many potential clinical applications (Giovannitti et al. 2015; Malinovsky et al. 2003; Nguyen et al. 2017; Schuman 2000; Wolraich et al. 2019) (Table 2). It is known that α_2-adrenergic agonists potentiate the anaesthesia efficacy of opiates in acute and chronic pain indications and thus have potential as opiate-sparing agents (Tonner 2017). However, the clinical use of α_2-adrenoceptor agonists is limited because of their physiological effects (sedation, hypotension and bradycardia), which are challenging to avoid. Even with intrathecal (i.t.) dosing, the lipophilic compounds cause typical α_2-adrenoceptor-related adverse effects that limit their use (Giovannoni et al. 2009).

Table 2. Clinically used α_2-adrenoceptor agonists with the intended indications

DRUG NAME	CLINICAL USE
CLONIDINE	Hypertension ADD/ADHD Chronic pain Withdrawal symptoms Postoperative shivering
TIZANIDINE	Muscle spasm and cramps in CNS disorders Myofascial pain disorder of the head and neck Spasticity of cerebral palsy
GUANFACINE	ADHD Hypertension
BRIMONIDINE	Persistent erythema of rosacea Reduce intraocular pressure in glaucoma
DEXMEDETOMIDINE	ICU sedation Procedural sedation Sedative and analgesic in veterinary medicine Noise-associated acute anxiety and fear in dogs
TASIPIMIDINE	Situational anxiety and fear in dogs

* ADD = attention-deficit disorder, ADHD = attention-deficit/hyperactivity disorder, CNS = central nervous system, ICU = intensive care unit

2.3.4 Role of α-adrenoceptors in ejaculation

The first part of ejaculation, the emission phase, is especially controlled by the activity of the sympathetic nervous system. Emission is initiated by activation of α_1-adrenoceptors in the distal epididymis. It continues with contractions of the vas deferens, seminal vesicles and prostate and is driven by α_1-adrenoceptor stimulation (Sanbe et al. 2007). Accordingly, Sato et al. (2017) reported that a selective α_{1A}-adrenoceptor antagonist, silodosin, improves premature ejaculation symptoms and increases intravaginal ejaculation latency in patients with acquired premature ejaculation. Silodosin mainly acts on peripheral adrenoceptors, suggesting that these receptors might be a feasible target for the pharmaceutical treatment of premature ejaculation.

Various studies have shown that presynaptic α_2-adrenoceptors are expressed on noradrenergic nerve endings, which are anatomically involved in seminal emission and ejaculation (Altman et al. 1999; Michel & Vrydag 2006; Trendelenburg et al. 2003). Physiologically, activation of α_2-adrenoceptors might result in delayed or reduced peripheral responses involved in seminal emission. Accordingly, α_2-adrenoceptor agonists such as dexmedetomidine, clonidine and tizanidine reduce the contractile response of the vas deferens in ex vivo setups (Altman et al. 1999; Drew

1977; Takayanagi et al. 1984). Yonezava et al. (1986) showed that intracerebroventricularly administered clonidine selectively inhibits the ejaculatory response in rats by stimulating central α_2-adrenoceptors, suggesting an important role of CNS-located α_2-adrenoceptors. This finding has been further supported by studies of the effect of clonidine on ejaculation in dogs (Yonezawa et al. 1992). In Yonezawa et al.'s (1992) study, chronic and acute treatment with clonidine decreased the amount of ejaculate. The acute effect was inhibited by yohimbine but not by DG-512 (an α_2-adrenoceptor antagonist that poorly penetrates the BBB), suggesting the role of CNS-located α_2-adrenoceptors. Taken together activation of both peripheral and CNS-located α_2-adrenoceptors can contribute to increasing ejaculation latency.

3 Aims of the study

Clinical and experimental animal data indicates sympathetic overactivation as a potential cause of lifelong premature ejaculation. Orion's experience in the field of α_2-adrenoceptor pharmacology prompted us to study the possible role of α_2-adrenoceptor agonists in the symptomatic treatment of premature ejaculation. Short ejaculation latencies in male rats are considered comparable to premature ejaculation in men, and accordingly, drugs acting on copulatory behaviour in rats also do so in men. Therefore, it was reasonable to presume that compounds delaying ejaculation in rats would also do so in men and that rat models can be used to evaluate drugs for the symptomatic treatment of premature ejaculation. It was hypothesised that both central and peripheral α_2-adrenoceptor activation might be beneficial for the symptomatic treatment, as well as the psychological component, of premature ejaculation.

The well-characterised α_2-adrenoceptor agonist dexmedetomidine was selected to test the hypothesis. It is also known that dexmedetomidine has low oral bioavailability, which might compromise the oral dosing option. Thus, the efficacy of a novel orally active α_2-adrenoceptor agonist tasipimidine was tested in the premature ejaculation model to circumvent that challenge. Since α_2-adrenoceptor activation in the CNS might cause unwanted side effects, such as sedation, we were also interested in testing whether the peripheral mode of action would be enough for the symptomatic treatment. Novel α_2-adrenoceptor agonist fadolmidine, with a local mode of action, was selected to test this hypothesis. Fadolmidine poorly penetrates the BBB; therefore, when dosed peripherally most effects are thought to be mediated through peripheral α_2-adrenoceptors. The prerequisite for these premature ejaculation studies with the abovementioned novel α_2-adrenoceptor agonists was the thorough pharmacological profiling of fadolmidine and tasipimidine.

4 Materials and Methods

4.1 Animals used in ex vivo and in vivo studies

For the pharmacological profiling assays, male Sprague-Dawley rats (B&K, Sweden), male NMRI mice (B&K, Sweden), male Hsd:ICR (CD-1) mice (Harlan, UK), male HsdRCCHanWist rats (Harlan, UK) and male Dunklin-Hartley guinea pigs (B&K, Sweden) were used. The study animals were housed in groups of 5 or 10 in 22 ± 2°C at a relative humidity of 50% ± 10% and on a 12 h light/dark cycle, with free access to tap water and food (Special Diet Service, England). All experimentations 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.

For the sexual motivation and ejaculatory behaviour studies, experimentally and drug-naive male and female Wistar rats (Charles River Laboratories, Sulzfeld, Germany, and the Institute of Neurobiology, National Autonomous University of Mexico, Campus Juriquilla) were used. The rats were housed in groups of two to four in Macrolon® individually ventilated cages on a reversed 12 h light/dark cycle, in a room with controlled temperature (21 ± 1°C) and relative humidity (50% ± 10%). Standard rat pellets and tap water were available ad libitum. All experimental procedures were approved by the Norwegian Food Safety Authority (ID 3327) in agreement with the European Union Council Directive 2010/63/EU and according to the Reglamento de la Ley General de Salud en Materia de Investigación para la Salud, NOM-062-ZOO-1999, of the Mexican Health Ministry, which follows National Institutes of Health guidelines.

4.2 Test compounds

Atipamezole, dexmedetomidine, fadolmidine and tasipimidine were synthesised by Orion Corporation (Finland). The chemical structures of dexmedetomidine, tasipimidine and fadolmidine are presented in Figure 4.

Clonidine, 2-methylserotonin and tetrodotoxin were purchased from Research Biochemicals International (USA). Ondansetron was obtained from Glaxo UK Ltd.

(England) and prazosin from Pfizer (USA). Acetylcholine was purchased from Sigma (USA) and morphine from Leiras (Finland).

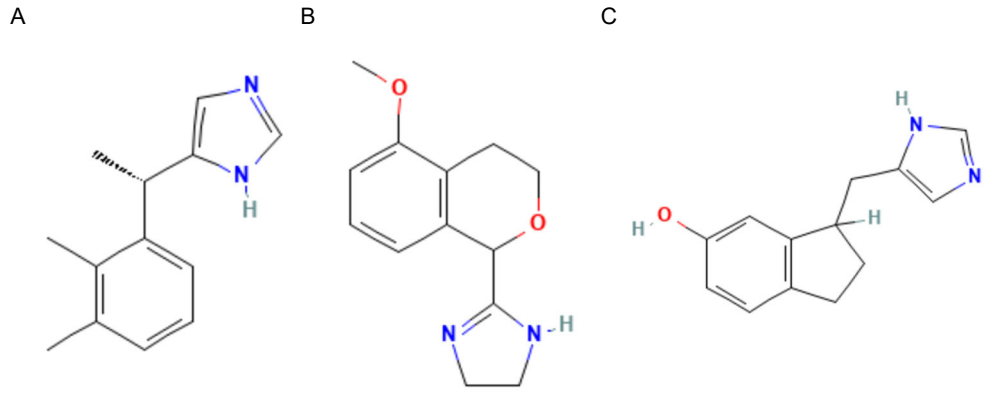


Figure 4. Chemical structure of A. dexmedetomidine, B. tasipimidine and C. fadolmidine.

4.3 In vitro studies

4.3.1 Selectivity profile (original publications III and V)

A selectivity profile covering binding and functional assays for 121 receptors, ion channels and enzymes was performed for fadolmidine and tasipimidine at 10 µM concentration by a contract research organisation (CRO), Cerep (Celle-Lévescault, France). Results of the selectivity profile warranted further functional in vitro studies in the cell-based assays on 5-HT_3 receptors for fadolmidine and 5-HT_{1A}, 5-HT_{1D} and 5-HT_7 receptors for tasipimidine.

4.3.2 Ligand-binding assays (original publications I and V)

The binding affinity of fadolmidine for the human α_2-adrenoceptor subtypes (α_{2A}, α_{2B} and α_{2C}) was measured against [^{3}H]rauwolscine using cell membrane fractions of the Chinese hamster ovary (CHO) cells transfected with each of the α_2-adrenoceptors subtypes. Membranes, [^{3}H]rauwolscine and nine concentrations of fadolmidine, covering a concentration range of five log units (0.1 nM- 1µM), were incubated for 30 min at room temperature in 50 nM KH_2PO_4, pH 7.5. Each concentration was tested in duplicates in one 96-well plate, and two independent assay plates were tested. Maximal binding of [^{3}H]rauwolscine was tested, performing the incubation without the test compound and nonspecific binding by

incubating membranes and [^{3}H]rauwolscine in the presence of 100 μM adrenaline. Incubation was stopped by rapid vacuum filtration through a glass fibre filter, followed by washing three times with ice-cold incubation buffer (5 ml). Radioactivity on the dried filters was evaluated using a scintillation counter (BetaPlate, Wallac, Turku, Finland). Specific binding was calculated by subtracting nonspecific counts from the total activity counts, and the IC50 values were calculated by a nonlinear least square curve fitting and further converted to Ki values using the Cheng–Prusoff equation (GraphPad Prism version 4.00).

Binding affinity measurements for tasipimidine on the human α_1- and α_2-adrenoceptor subtypes were conducted by Cerep (Celle-Lévescault, France). Cell membrane fractions of the CHO cells expressing either of the three human α_1-adrenoceptor (α_{1A}, α_{1B} and α_{1D}) or α_2-adrenoceptor subtypes (α_{2A}, α_{2B} and α_{2C}) were incubated for 60 min at room temperature with five concentrations of tasipimidine (from 0.1 nM to 1 μM) and a fixed concentration of a ^{3}H-labelled receptor ligand, prazosin (for the α_1-adrenoceptors) or RX 821002 (for the α_2-adrenoceptors). Displacement of the ^{3}H-labelled ligand by tasipimidine was considered an indication of competitive binding to the receptor. IC50 values were calculated by nonlinear least square curve fitting and converted to Ki values using the Cheng–Prusoff equation.

4.3.3 Biochemical [^{35}S]GTPγS binding assay (original publication V)

Functional effects of tasipimidine on the human α_2-adrenoceptor subtypes were studied on a biochemical [^{35}S]GTPγS binding assay by a CRO (Ricerca, Taipei, Taiwan). Membranes of the insect Sf9 cells were used for the α_{2A}- and α_{2C}-adrenoceptor subtypes and CHO-K1 cells for the α_{2B}-receptor subtypes. Tasipimidine was pre-incubated in a buffer consisting of 20 mM HEPES (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA with membranes and 3 μM GDP for 20 min at 25°C. Afterwards, [^{35}S]GTPγS was added, and incubation continued for a further 15 min for Sf9 cells and 30 min for CHO-K1 cells. The agonist response was quantitated by scintillation counting as bound [^{35}S]GTPγS relative to the reference agonist UK-14304.

4.3.4 Functional cell assays

α_2-adrenoceptors (original publication I and V)

For the functional intracellular [Ca^{2+}] measurements CHO cells stably co-expressing either of the α_{2A}-, α_{2C}- and α_{2D}-adrenoceptors (Pohjanoksa et al. 1997) and pCEP-

Gα16 (Molecular Devices, CA, USA). For the human α_{2B}-adrenoceptors studies, stably transfected CHO cells with and mitochondrially targeted aequorin (Euroscreen, Charleroi, Belgium) were used. Cells were cultured at 37°C in a 5% CO_2 and 95% air atmosphere in Ham's F-12 medium supplemented with 10% fetal calf serum (FCS), 25 mM HEPES, 100 IU/ml penicillin, 100 µg/ml streptomycin, 500 µg/ml geneticin and 240 µg/ml hygromycin B. The cell culture reagents were obtained from Gibco. Cells were subcultured two to three times a week using 0.25% trypsin and 1 mM EDTA, with a subculture ratio of 1:5–1:40. The cells were plated into black-walled, clear-bottom 96-well plates, with a density of 35,000–45,000 cells/well, a day before the experiment.

Prior to the experiments, the growth medium was removed, and cells underwent one hour incubation at 37°C in the dark. During the incubation, cells were exposed to FLIPR Calcium assay reagents: Calcium 3 for α_{2B}, Calcium 4 for α_{2A} and or Calcium 5 for α_{2C} and α_{2D}-adrenoceptors (Molecular Devices, CA, USA). The incubation occurred in Probenecid-Ringer buffer composed of (in mM) 150 NaCl, 3 KCl, 1.2 $MgCl_2$, 1 $CaCl_2$, 5 glucose, 20 HEPES and 2.5 probenecid (pH 7.4 adjusted with 1.0 M NaOH). Osmolarity was adjusted to 322 using the Osmostat® OM-6020 osmometer (DIC Kyoto Daiichi Kagagu Co. Ltd, Kyoto, Japan).

Intracellular [Ca^{2+}] measurements were carried out utilising a fluorometric imaging plate reader equipped with an integrated fluid transfer unit (FLIPR, Molecular Devices, CA, USA). The experiments took place at 37°C. Agonists were dissolved in Probenecid-Ringer buffer and administered on the cells over a concentration range spanning from 0.01 nM to 10 µM, with half logarithmic increments. The cellular response was recorded for 1 min. The excitation wavelength of 470–495 or 485 nm was used, and emission was measured at 515–575 nm or 525 nm, depending on the assay reagent.

The values for the half-maximal effective concentration (EC50) of the agonists were fitted with the Michaelis–Menten equation with R linear correlation coefficients in XLfit4. The results are expressed as mean ± standard deviation. The intrinsic activities (IAs) of the compounds were compared to adrenaline (10 µM), which is recognised as a full α_2-adrenoceptor agonist.

α_1-adrenoceptors (original publication I and V)

For the assessment of α_{1A}-adrenoceptor functions the human lymph node carcinoma of the prostate (LNCaP) cells were employed, which endogenously express human α_{1A}-adrenoceptors. The cells were cultured in RPMI 1640 medium with 5% FCS, 2 mM GlutaMAX, 100 IU/ml penicillin and 100 µg/ml streptomycin. The growth medium was changed every two to three days. The cells were subcultured with a ratio of 1:5–1:40 biweekly using 0.25% trypsin and 1 mM EDTA, A day prior to the

experiments, the cells were seeded into black-walled, clear-bottom 96-well plates at a density of 30,000 cells/well.

Further, Millipore's G protein–coupled calcium-optimised FLIPR cell line (ChemiScreen™) having the α_{1A}-adrenoceptors stably transfected to Chem-1 host cells was used for the functional α_{1A}-adrenoceptor studies. These cells express high levels of Gα15 that force couples α_{1A}-adrenoceptors to intracellular calcium signalling. Cells were cultured at 37°C in a 5% CO_2 and 95% air atmosphere in DMEM supplemented with 10% FCS, 25 mM HEPES, 100 IU/ml penicillin, 100 µg/ml streptomycin and 500 µg/ml geneticin. The growth medium was changed every second or third day, and cells were subcultured two to three times a week at a ratio of 1:5–1:20 using 0.05% trypsin and 1 mM EDTA. A day before the experiment, the cells were transferred into black-walled, clear-bottom 96-well (30,000 cells/well) or 384-well plates (12,500 cells/well).

The CRO Cerep (Celle-Lévescault, France) conducted assessments of the functional effects of tasipimide on the human α_{1A}-adrenoceptors using an intracellular [Ca^{2+}] fluorimetry assay, employing human recombinant CHO cells following the protocol of Vicentic et al. (2002). Additionally, an inositol monophosphate accumulation assay performed over 30 min at 37°C, was conducted with human recombinant AequoZen frozen CHO cells. The quantification was done by homogeneous time-resolved fluorescence by a CRO (Eurofins Panlabs, Taipei, Taiwan).

Human embryonic kidney cells (HEK-293) were utilised for conducting studies on the human α_{1B} and rodent α_{1A}-/α_{1B}-adrenoceptor. Cells were cultured in DMEM containing 10% FCS, 25 mM HEPES, 100 IU/ml penicillin and 100 µg/ml streptomycin. Subculture was performed twice a week using 0.25% trypsin and 1 mM EDTA. To prepare for transfection, the cells were seeded onto 96-well plates a day in advance at a density of 30,000 cells/well in a normal growth medium. The cells were transiently transfected with the eukaryotic expression vectors encoding the full-length human adra-1b receptor (Origene Technologies, Inc., MD, USA), the rat adra-1a or the rat adra-1b receptor (both obtained from Cell Culture Service GmbH, Hamburg, Germany) at plasmid dose of 0.125 µg per well and lipofectamine (Invitrogen, MS, USA) 0.5 µl per well. The host vectors used were pCMV6-XL4 for the human adra-1b receptor and pcDNA3.1 for both the rat adra-1a and adra-1b receptors. After a five-hour incubation, the transfection medium was replaced with the normal full-growth medium, and assays were performed 24–28 h post-transfection.

Functional patch clamp studies for human 5-HT_3 receptors (original publication III)

Transfected cells stably expressing human 5-HT_{3A} and 5-HT_{3AB} receptors, (Molecular Biology laboratory, Helsinki, Orion Pharma) were cultured on six well

plates at 37°C in a 5% CO_2 and 95% air atmosphere They were maintained in DMEM with 10% heat-inactivated FCS, 10 IU penicillin, 10 µg streptomycin, 10 mM HEPES, and 0.2 mg/ml geneticin and 0.2 mg/ml hygromycin. For the experiments, cells adhered to a glass coverslip were employed.

Patch clamp experiments were conducted in a whole-cell, voltage-clamp configuration, with voltage clamped to −70 mV at room temperature, using the Axopatch 200B amplifier (Axon Instruments, CA, USA). Cell capacitance was compensated for, but series resistance compensation was not performed. Borosilicate glass pipettes (Clark Electromedical, England) with a resistance of 1–1.5 MOhm were used. The intracellular solution was composed of (in mM) 120 KCl, 5 BAPTA (tetrapotassium salt), 0.5 $CaCl_2$, 1 $MgCl_2$, 2 adenosine triphosphate (disodium salt) and 10 HEPES, with pH 7.2 (adjusted with KOH) and osmolarity of 290 mOsm (Osmostat OM-6020, DIC Kyoto Daiihi Kagagu Co. Ltd, Kyoto, Japan). The calculated free [Ca^{2+}] was 23 nM. The cells were exposed to continuous flow of 2 ml/min of an extracellular solution consisting of (in mM) 150 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 2.5 glucose and 10 HEPES with pH of 7.4 (adjusted with NaOH) and osmolarity adjusted to 305 mOsm. The test compounds were dissolved in the extracellular solution and applied using the RSC-200 rapid solution changer (BioLogic, Grenoble, France). The cells were perfused for 20 s with increasing concentrations of the test compounds, with washouts periods lasting from 90 to 100 second in between. Recordings were digitised using Digidata 1200 (Axon Instruments, CA, USA), sampled at a rate of 2 kHz, recorded with Clampex 8.0 and finally analysed with Clampfit 8.0 (Axon Instruments, CA, USA). The results were fitted using the Hill equation, and the analysis were carried out in SigmaPlot 4.01.

4.4 Ex vivo studies

4.4.1 Rat vas deferens preparations (original publications I and V)

The rats used in the studies were euthanised by using CO_2. Vas deferentia were dissected and then divided into prostatic and epididymal halves, specifically for the studies of α_2- and α_1-adrenoceptor, respectively. The preparations were allowed to equilibrate under a resting tension of 0.5 g for 5 min before measurements. The experiments were conducted in tissue chambers containing Krebs solution composed of (mM) 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 0.6 $MgSO_4$, 25 $NaHCO_3$ and 11.1 glucose. The solution was continuously aerated with 5% carbogen at 37°C and pH 7.4. To prevent interference from β2-adrenoceptors and the reuptake of released NA, propranolol at 260 µg/l and desipramine at 2 µg/ml were added to the chambers.

To study presynaptic α_2-adrenoceptors, electrical field stimulation was applied by delivering twin pulses: duration 2 ms, delay 5 ms, voltage 70 V, frequency 0.2 Hz (Grass Instruments Co., USA). Isometric contractions of the prostatic vas deferens preparations were measured using force-displacement transducers (Grass Instruments Co., USA). The test compounds were introduced cumulatively with half logarithmic increments at five-minute intervals, and inhibition of the electrically evoked contractions was measured. The point at which contractions were entirely inhibited represented the maximal effect.

The percentage of inhibitions was calculated and expressed as dose-response curves, with mean values ± standard error of the mean (SEM) determined using Microsoft Excel in the USA. The intrinsic activity (IA) and half-maximal effective concentration (EC50) values were calculated using GraphPad Prism 4.0 software (GraphPad Software Inc. in the USA).

Responses of the epididymal vas deferens preparations to the activation of postsynaptic α_1-adrenoceptors, were assessed by measuring isometric contractions. The preparations were pre-treated with a known α_1-adrenoceptor agonist, phenylephrine, at a concentration of 10 µM. Subsequently, phenylephrine was administered in a cumulatively with half logarithmic increments at two-minute intervals, thus creating a dose-response curve which after the preparations were washed, and dose–response curves for the test compounds were constructed accordingly. The contractions induced by the test compounds were quantified as percentages of the maximum contractions obtained with phenylephrine.

A CRO Eurofins (Taipei, Taiwan) conducted the functional ex vivo studies of tasipimidine on the α_1-adrenoceptors in the vas deferens and prostate preparations as well as α_2-adrenoceptors in the electrically stimulated rat prostate preparations. The preparations were incubated in 10 ml organ baths in Krebs solution (pH 7.4, 32°C). Isometric contractions of the vas deferens and prostate preparations were measured in response to α_1-adrenoceptor agonists using the known α_1-adrenoceptor agonists phenylephrine (2 µM) and NA (1 µM) as references, respectively. For the α_2-adrenoceptor agonists, the study involved the measurement of the inhibition of electrically induced contractions in prostate preparations, which were then compared to the results obtained with the established α_2-adrenoceptor agonist, clonidine (10 nM).

4.4.2 Guinea pig ileum preparation (original publication III)

Guinea pigs used in the experiments were euthanised using CO_2. The ileum was subdivided into 2 cm segments on a petri dish containing a physiological solution composed of (in 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.

A four-position Schuler organ bath (Hugo Sachs Elektronik, March, Germany) with a chamber volume of 10 ml at 37°C was employed. The ileum preparations were stabilised under a tension of 1 g for a duration of 30 min which involved three washing steps. Isometric contractions of the ileum preparations were measured using a force-displacement transducer (FT03, Grass Instruments, Quincy, MA, USA) connected to amplifiers (low-level preamplifier and driver amplifier 7DAG, Grass Instruments, Quincy, Mass., USA). The amplified signals were then recorded using an ink oscillograph (7WU 16F, Grass Instruments, Quincy, MA, USA).

To assess the maximum contractile capacity of the preparations, two administrations of acetylcholine (1 μM) were delivered at six-minute intervals, each separated by washout phases. The test compounds were introduced noncumulatively at 15-minute intervals with half logarithmic increments, the doses being separated by washes. The contact time of the test compound was two minutes. The measurements of isometric contractions of the preparations served as indicators of 5-HT_3-receptor agonism. A known selective 5-HT_3 receptor antagonist, ondansetron, was dosed 15 min before the test substance to antagonise any effects on 5-HT_3. Atipamezole and prazosin were applied into the incubation medium 16 min before the agonist to prevent any effects on presynaptic α_2-adrenergic and postsynaptic α_1-adrenergic receptors, respectively.

4.5 In vivo studies

4.5.1 I.t. cannulation (original publication III)

I.t. catheterisation for rats was conducted according to method outlined by Yaksh and Rudy (1976), with certain modifications. A sterile saline-filled polyethylene catheter (PE10, Intramedic, Becton Dickinson, NJ, USA) was inserted through the atlanto-occipital membrane into the spinal cavity, extending approximately 8 centimeters from the point where it was introduced right beneath the skull so that the catheter tip reached the rostral edge of the lumbar enlargement. The external tip of the catheter was sealed by melting. The procedure was carried out under anaesthesia induced by midazolam (5 mg/kg, Dormicum® 5 mg/ml, Roche, Basel, Switzerland) and fentanyl (0.25 mg/kg)-fluanisone (8 mg/kg) (Hypnorm®, Janssen, Buckinghamshire, UK) both given s.c. To confirm the accurate placement of the catheter tip, an intrathecal dose of 0.5 mg of lidocaine (Lidocain Pond® 50 mg/ml, Medipolar, Oulu, Finland) was administered on the third day following catheterization. Transient paralysis of both hind limbs was considered indicative of successful catheterisation. The operated rats were allowed to recover individually and were observed for a minimum of one week. Only those rats that displayed normal

neurological function based on visual assessments were included in the subsequent experiment.

4.5.2 Mydriasis measurements (original publication I)

Rats were anaesthetised using sodium pentobarbital (Mebunat® 60 mg/ml, Orion Corporation, Turku, Finland), 45 mg/kg i.p. Additional boluses of 0.05 ml were provided as necessary but not exceeding a total of 0.15 ml. To perform i.v. dosing, a 22G cannula was introduced to the lateral tail vein. Once anaesthesia reached a stable state, the baseline value for the pupil diameter was measured using a stereomicroscope. Test compounds were administered either i.v. (0.5 ml/kg) or s.c. (0.5 ml/kg). The i.v. boluses were administered cumulatively with half logarithmic increments at five-minute intervals. The mydriatic response was assessed before each subsequent dose. In the cases of s.c. injections, a single bolus was administered, and mydriatic response was observed every five minutes over a period of half an hour. The measurements of pupil diameter were manually recorded in the study log, means ± S.E.M. were calculated and the dose–response curves were constructed (Microsoft Excel).

4.5.3 Bezold–Jarisch reflex measurements (original publication III)

Rats were anaesthetised using sodium barbital (Mebunat 60 mg/ml, Orion Corporation, Turku, Finland) at a dose of 1.25 mg/kg i.v. A cannula was inserted into the left femoral artery and linked to a pressure transducer for the purpose of MAP. Additionally, the right femoral vein was cannulated for the i.v. administration of the test compounds. A series of three 0.2 ml boluses of the test compounds were given with intervals of 5 min. The first injection comprised of physiological salt solution to evaluate the volume-related effects on HR and/or MAP. The second injection introduced the antagonist of the 5-HT_3 receptor (ondansetron), α_1-adrenoceptors (prazosin), α_2-adrenoceptors (atipamezole) or a combination of both α-adrenoceptor antagonists. When no antagonist was employed, equal amounts of physiological solutions were used to compensate any potential volume-related effect. The third injection contained the actual test compound. Throughout the assay, both MAP and HR were continuously monitored and electronically recorded using pressure transducers (MP-15, Micron Instruments, USA) connected to an amplifier (Type 301, Hugo Sachs Elektronik, March, Germany). The obtained signal data was captured via an analogue-to-digital converter and stored in a computer-based data acquisition system, MP100WS (Biopac Systems Inc., CA, USA).

4.5.4 Kidney function measurements (original publication III)

Before the experiments, the rats underwent a15 h fast while having unrestricted access to tap water. At the beginning of the experiments, the rats were administered tap water (30 ml/kg) orally which after fadolmidine or saline were given immediately either i.v. (1 ml/kg) or i.t. (10 µl/rat). The diuretic compound furosemide (Furesis, Orion Corporation, Espoo, Finland) was given s.c. (2.0 mg/kg). Subsequently, the treated rats were placed in metabolic cages (Cod.170022, Tecniplast, Pontremoli, Italy) for urine collection. Throughout the collection period, the rats did not have access to food or water. After six hours, urine output was quantified, and the urine was centrifuged at 600 g for 7 min (Jouan Plasma 1000, France), followed by determination of urine osmolarity using Osmostat OM-6020 (DIC Kyoto Daiichi Kagagu Co. Ltd, Kyoto, Japan). The collected urine samples were preserved at −20°C and were subsequently analysed for [Na^+] and [K^+] (Yhtyneet Laboratoriot Oy, Helsinki, Finland).

4.5.5 Urodynamic measurements (original publication III)

The rats were anaesthetised with urethane at dose of 1.0–1.4 g/kg i.p. Subsequently, the urinary bladder was cannulated using a 22G injection needle for the infusion of physiological salt solution at a rate of 0.1 ml/min (Perfusor ED 2, B. Braun AG, Melsungen, Germany). Voiding volumes and intravesical pressure within the urinary bladder were measured using a pressure transducer (MP-15, Micron Instruments, USA). The signals were converted into digital format via an analogue-to-digital converter and were then recorded using the MP100WS system (Biopac Systems Inc., CA, USA). Only those rats exhibiting reproducible volume-evoked micturition cycles prior the dosing of the test compounds were chosen for the studies. The test compounds were dosed i.t. at a volume of 10 µl. While various test compounds were assessed in separate experiments, both fadolmidine and a saline control group was included all the studies for making comparisons.

4.5.6 Haemodynamic measurements (original publication I and III)

Pithed rat model

Peripheral vasopressor and sympatho-inhibitory effects of fadolmidine were studied in rats. Rats were administered atropine at a dose of 1 mg/kg s.c. and were subsequently pithed and connected to a respirator (BioScience, UK). The left femoral vein and artery were cannulated for i.v. drug administration and to measure blood

pressure (BP), respectively. BP was recorded using a Micron MP-15 pressure transducer (Micron Instruments, CA, USA) connected to a Grass 7D amplifier (Grass Instruments Co., MA, USA). The signals from the BP trace were also employed for HR measurements. The signals were recorded at a rate of 30 Hz via MP100A system (Biopac Systems Inc., CA, USA) which transformed the analogue input into digital format for further analysis and storage.

Each rat was given a minimum 20 min to stabilise before conducting the experiment, the body temperature (BT) was maintained at 37°C. Cumulative doses of fadolmidine were administered at 15 min intervals. The changes in MAP were recorded, and the dose required to raise the MAP by 50 mm Hg was determined. Sympatho-inhibitory effects were assessed by measuring inhibition of tachycardia induced by a train of 20 electrical pulses (square wave, duration 0.3 ms, frequency 0.3 Hz, supramaximal voltage) applied to sympathetic preganglionic nerves through the pithing electrode positioned at the C7–Th1 level in the spinal canal. Electrical stimuli were delivered 5 min after each injection of either saline or the test compound. Only rats demonstrating tachycardia of 60 beats/min or higher were used in the study. The dose that inhibited tachycardia by 50% of the maximal response was determined by constructing a logarithmic dose–response curve.

Anaesthetised rat model

Rats were anaesthetised using sodium pentobarbital (Mebunat® 60 mg/ml, Orion Corporation, Turku, Finland) at a dose of 75 mg/kg i.p. The left femoral vein was cannulated for drug injections and the left femoral artery for MAP and HR measurements. BT was maintained at 37 ± 0.5°C throughout the experiments. Only rats with a MAP of 70 mmHg or higher were included in the studies. Following a 10 to 20 min observation period, different doses of fadolmidine (1.0, 3.0 and 10.0 µg/kg), dexmedetomidine (1.0, 3.0 and 10.0 µg/kg) or saline (for the control group) was administered via slow i.v. bolus with a delivery rate of 1 ml over five minutes using an infusion pump (Perfusor®ED 2, B. Braun, Melsungen, Germany).

Telemetry measurement in conscious rats

Rats were habituated to an open-field environment through a series of four 3-minute training sessions conducted over two weeks prior to the telemetry implantation. The rats were anaesthetised with s.c. doses of midazolam 5 mg/kg (Dormicum®, Roche, Basel, Switzerland) and fentanyl (0.25 mg/kg)–fluanisone (8 mg/kg) (Hypnorm®, Janssen, Buckinghamshire, UK). The tip of a telemetry transmitter was implanted near the iliac bifurcation in the descending aorta through a midline abdominal incision and secured in place using cyanoacrylate glue and a cellulose fibre patch. A

telemetry transmitter was sutured to the muscle wall in the peritoneal cavity, and the skin was stitched using nonabsorbable sutures. The rats were given a minimum of one week to recover in individual cages before further experiments.

HR, MAP and BT were continuously recorded using the Dataquest i.v. telemetry system (Data Sciences, St. Paul, MN), which consists of monitoring transmitters (model TL11M2-C50-PTX), receivers (model RLA2000) and a consolidation matrix (BCM100). The BP and HR recordings sampled at rates of 500 Hz and 250 Hz, respectively. Data collection and storage were managed with the Dataquest LabPRO™ software.

Exploratory behaviour was assessed within 70 × 70 cm open-field arena constructed from non-reflective black plastic, with 38 cm walls. The ambulations were monitored by a video camera 220 cm above the arena and recorded by an image analyser (Poly-Track video tracking system, San Diego Instruments, CA, USA). The arena was divided into nine squares, each measuring 23 × 23 cm, using the Chromotrack software (Prototype Systems Ltd, MN, USA), and the number of ambulations was manually counted based on the recordings. All behavioural experiments were conducted undisturbed in a quiet, noise-free environment.

The rats were habituated for immobilisation and acclimated to the open-field system throughout four 3-minute sessions held over two days. On the test day, the rats were transferred to the experimental room for at least 1 h to habituate in individual cages. Rats were i.t. dosed with fadolmidine (1, 3, 10 and 30 μg/rat), clonidine (10, 30 and 100 μg/rat) or sterile water (10 μl) using a randomised cross-over design, with one dose administered each day and washout periods of 24 h for fadolmidine and 48 h for clonidine. Immediately after administration of the test compound, the rats were returned to their home cages for concurrent BP and HR recordings along with motor activity assessments.

4.5.7 Spontaneous motility in mice (original publication V)

The locomotor activity of the mice was assessed in a polypropylene animal enclosure (38 × 22 × 15 cm), that featured a floor layered with granulated aspen bedding. Photobeam frames (PAS, Cage Rack®, San Diego Instruments, San Diego, CA, USA) surrounded the enclosure, positioned at two different heights: 30 mm above the floor (consisting of three photocells with 95 mm spacing) and 60 mm above the floor (comprising eight photocells with 25 mm spacing). Crossing of alternate beams of the lower frame was recorded as ambulation (horizontal movement), while breaking of alternate beams of the upper frame was recorded as rearing (vertical movement). Before testing, the mice were weighed and transferred into the experimental room for at least 30 min in advance. The mice were s.c. administered either distilled water, dexmedetomidine (3 or 10 μg/kg), fadolmidine (10, 30, 100,

300, 1,000 or 3,000 µg/kg) or tasipimidine. Fifteen minutes after the injection, the mice were placed into the enclosure for 30 min to measure spontaneous motility. In cases of oral dosing, the mice were returned to their home cages for 30 min prior to the measurement. The spontaneous locomotor activity was recorded at 5 min intervals for 30–120 min.

4.5.8 Startle reflex measurements (original publication V)

Acoustic startle reflex experiments with rats were performed in startle boxes measuring 320 mm in length, 320 mm in width and 320 mm in height. Each box comprised animal restrainers of 225 mm in length, 80 mm in width and 85 mm in height, an integrated floor grid equipped with weight sensors, stimulus elements, a control unit, two special control interfaces and a startle response software package designed for Windows (TSE Systems GmbH, TSE Startle Response System V3.00, Bad Homburg, Germany). Acoustic stimuli of 110 dB intensity, 40 ms duration and a frequency of 25 kH were generated by two speakers integrated into the restrainers. The response of the rats to the acoustic stimulus was observed by monitoring their acceleration on the measuring platform.

Prior to the experiments, rats were allowed to acclimate to the experimental environment for a minimum of 30 min. Two distinct protocols were employed for the study. Protocol A was utilised to study the duration of the drug response. After the habituation of 90 s, the baseline activity was measured (30 s, four acoustic stimuli with 10 s intervals). This measurement was repeated one-hour-intervals up to four hours. The protocol B was used to examine the onset of the drug response. After the habituation period baseline activity was measured for 30 s by introducing acoustic stimuli. This stimulus was repeated 20 times with 60 s intervals. The startle responses were calculated using the formulas:

Onset of sedation = [MaxG from second test (each individual startle response) / Mean MaxG from 1. test]*100

Duration of sedation = (Mean MaxG from 3. 4. or 5. test / Mean MaxG from 1. test)*100.

The startle percentages were used for statistical comparisons.

4.5.9 Sexual motivation and ejaculation behaviour (original publications II and IV)

A pre-made solution of dexmedetomidine was diluted immediately prior to use with physiological saline to achieve required concentrations. The selection of doses 0.1 and 1 µg/kg i.p., was based on data from earlier studies (Bol et al. 1997; Millan et al. 2000a; Millan et al. 2000b; Viitamaa et al. 2006). The higher dose while reported to

be ineffective in terms of locomotor activity (Viitamaa et al. 2006) has demonstrated efficacy in certain analgesic models (Bol et al. 1997; Millan et al. 2000a) and aggression tests (Millan et al. 2000b). The lower dose anticipated to have no sedative effects.

Tasipimidine was dissolved in 0.05 M of citrate buffer (pH 4.4) shortly before use and administered s.c. Two doses were employed (100 and 200 µg/kg), the lower dose intended to remain below the sedative threshold and the higher dose expected to induce a mild sedative effect.

Fadolmidine was freshly prepared in physiological saline just before the experiments. Three doses (3, 30 and 100 µg/kg) were employed. Two lower doses have previously been demonstrated to have most of their effects in the periphery, while the highest dose was selected to exert central effects as well. Fadolmidine, or vehicle for the control group, was administered at a volume of 1 ml/kg 15 min before the initiation of behavioural observations.

To assure maximum receptivity and proceptivity, female rats underwent hormonal treatment. They were ovariectomised under isoflurane anaesthesia at least two weeks prior to use. Simultaneously, they received s.c. implant of 5 mm long capsule made of medical grade silastic tubing, 0.0625 in. inner diameter, 0.125 in. outer diameter (Degania Silicone, Degania Bet, Israel) filled with 10% solution of 17β-oestradiol (Sigma, St. Louis, MO, USA) in cholesterol (Sigma, St. Louis, MO, USA). The tips of the capsules were sealed with medical-grade adhesive silicone (NuSil Silicone Technology, Carpinteria, CA, USA). Additionally, female rats were administered 1 mg progesterone per rat (Sigma-Aldrich, St. Louis, MO, USA) dissolved in peanut oil (Apoteksproduskjon, Oslo, Norway) via s.c. injection with a volume of 0.2 ml 3.5 h before testing. Alternatively, for the fadolmidine study, sexual receptivity was induced through a sequential administration of oestradiol benzoate (25 µg/rat) and progesterone (1 mg/rat) (Sigma-Aldrich, St. Louis, MO, USA) dissolved in corn oil 125 µg/ ml and 5 mg/ml, respectively. These hormones were administered s.c. in a volume of 0.2 ml/rat, approximately 48 and 4 h prior to the tests, respectively. Both treatments protocols ensured the maximum receptivity and a high level of proceptivity.

Sexual incentive motivation tests were conducted in an oval arena measuring 100 × 50 cm, in dim white light with illumination of approximately of 5 lux in the arena. The arena was surrounded by 45 cm high walls with two diagonally opposed apertures measuring 25 × 25 cm each for the placement of wire mesh–separated cages housing the incentive rats, intact males and sexually receptive females. In front of these cages, a designated virtual region, the incentive zone, was established, covering an area of 21 × 30 cm. To monitor the subject's movements within the arena and the incentive zones, a video tracking system (Ethovision, Noldus, Wageningen, Netherlands) was employed, recording the subject's position at a rate of 5 Hz. The

subject was considered to be inside of the incentive zone when its center of gravity fell within that region. Several parameters were subsequently calculated:

- time spent in the incentive zone
- number of visits to the zone
- distance moved
- time spent moving
- speed of movement
- preference score: time spent in the incentive zone adjacent to the female incentive / (time spent in the incentive zone adjacent to the female incentive + the time spent in the incentive zone adjacent to the male incentive).

The observation of sexual behaviour took place in a separated room from the incentive motivation setup, under an illumination of 60 lux. This room was equipped with a rectangular arena measuring 60 mm in length, 40 mm width and 40 mm in height with a plexiglass front panel. The analysis of behavioural traits was analysed with Observer XT 12.5 software (Noldus). The following behavioural characteristics were recorded:

- mount latency (time from introduction of the female to the first mount with pelvic thrusting)
- intromission latency (time from introduction of the female to the first vaginal penetration, i.e. intromission)
- ejaculation latency (time from the first intromission until ejaculation)
- postejaculatory interval (time from ejaculation until the next intromission)
- number of mounts
- number of intromissions

From the measured parameters, the following indexes were calculated: interintromission interval (the ejaculation latency divided by the number of intromissions) and intromission ratio (the number of intromissions / [the number intromissions + the number of mounts]).

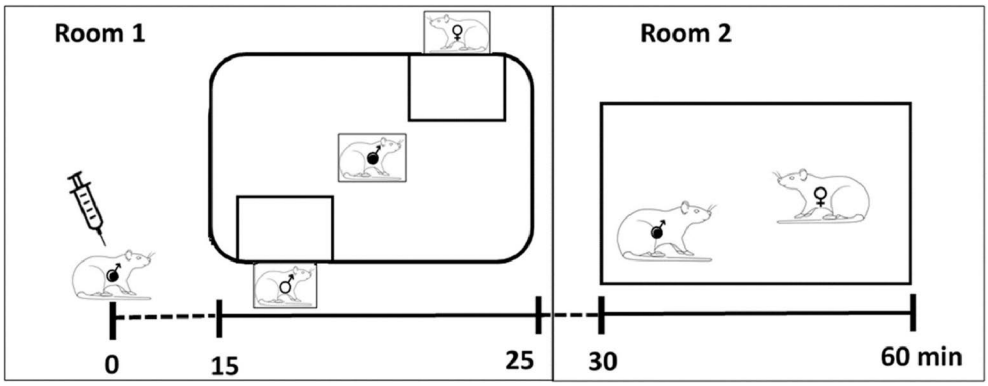


Figure 5. The sequence of events of the sexual motivation and ejaculation behaviour studies. Drug injection and the test for sexual incentive motivation were performed in the same room. After the experiment, the experimental subject was transferred to an adjacent room, where the test for copulatory behaviour was performed after 5 minutes of familiarisation with the observation cage. From the original publication V.

Male rats underwent three pre-experimental tests for sexual behaviour, which were stopped at the first postejaculatory interval. If the study object failed to ejaculate, the test was stopped 30 min after the first intromission. In the case the male rats did not perform intromissions, the test ended 15 min after the female was introduced. Only the males that successfully ejaculated in at least two of the tests were selected in the study.

After the pre-tests, the male rats underwent a familiarization session with the incentive motivation arena. Each of these three sessions lasted for 10 minutes and were spaced 48 hours apart. Before each test, the arena was carefully cleaned with a 0.1% solution of glacial acetic acid in water. The drug treatments were started one week after the final familiarisation session. The study subject was placed in the centre of the incentive motivation arena 15 min after drug injection. The experimenter promptly exited the room and did not return until the end of the 10 min observation period.

Following the test in the incentive motivation arena, the study subjects were transferred to a separate room for the immediate observation of sexual behaviour. They were placed in an observation arena, and a sexually receptive female was introduced five minutes later. Sexual behaviour was observed for a duration of 30 minutes.

Data from the sexual incentive motivation test were analysed with either a one-factor analysis of variance (ANOVA) for repeated measures (preference score, distance moved, time moving, speed of movement) or a two-factor ANOVA for repeated measures on both factors, incentive (male, female) and treatment. The

variables subjected to this analysis were time spent in the incentive zones, the number of visits to the incentive zones and the mean duration of each visit.

Sexual behaviour data was analysed either with one-factor or repeated measures ANOVA or, when the data deviated from normality according to the Shapiro–Wilk test, with Friedman's ANOVA. Post hoc comparisons were conducted with Tukey's honestly significant difference test or where appropriate, the Wilcoxon test with the Bonferroni correction for multiple comparisons.

5 Results

5.1 Pharmacological characterization of fadolmidine

5.1.1 Binding affinity and functional activity on human α_2- and α_1-adrenoceptors in cell-based assays (original publication I)

The binding affinity of fadolmidine for human α_1- and α_2-adrenoceptors was studied using cell membrane preparations, and functional activity was assessed on living cells. Fadolmidine exhibited high binding affinity and acted as a full agonist on all human α_2-adrenoceptor subtypes in the nanomolar range (Table 3) compared to the endogenous α_2-adrenoceptor agonist adrenaline. Moreover, when studied on human α_{1A}-adrenoceptors (LNCaP cells) and α_{1B}-adrenoreceptors (transfected HEK cells), fadolmidine demonstrated full agonist behaviour, with EC50 values of 22 nM and 3.4 nM, respectively. For comparison, a known α_2-adrenoceptor agonist, dexmedetomidine, displayed potent full agonism on all human α_2-adrenoceptor subtypes but exhibited no agonistic activity on the human α_{1A}-adrenoceptor at concentrations up to 10 μM. Dexmedetomidine displayed only partial agonism on the human α_{1B}-adrenoceptor, with an EC50 value of 370 nM and IA of 0.24.

Table 3. Binding affinity and agonist activity of fadolmidine compared to the well characterised α_2-adrenoceptor agonist dexmedetomidine

COMPOUND	α_2 SUBTYPE	AFFINITY Ki (nM)	AGONISM EC50 (nM)	INTRINSIC ACTIVITY
ADRENALIN	2A		4.7	1.0
	2B		37.0	1.0
	2C		11.2	1.0
FADOLMIDINE	2A	2.5	0.4	0.84
	2B	0.6	4.9	0.65
	2C	0.3	0.5	0.96
DEXMEDETOMIDINE	2A	4.9	1.5	0.95
	2B	3.8	1.1	1.0
	2C	3.8	1.6	1.0

From the original publication I.

5.1.2 Functional activity on rat vas deferens preparation (original publication I)

To assess the agonistic effects of fadolmidine on the rodent presynaptic α_{2D}-adrenoceptors and the postsynaptic α_1-adrenoceptors, experiments were conducted using rat (Sprague–Dawley) vas deferens preparations. Fadolmidine demonstrated a dose-dependent inhibition of electrically induced isometric contractions in the vas deferens preparation, with an EC50 value of 6.4 nM and an IA of 0.72 (Figure 6). In the epididymal segments of the vas deferens expressing α_1-adrenoceptors, fadolmidine elicited dose-dependent isometric contractions, with an EC50 value of 5.6 µM and IA of 0.9, compared to the maximum effect by phenylephrine (a known full agonist on α_1-adrenoceptors). For comparison, dexmedetomidine was a potent full agonist on both the rat α_{2D}-adrenoceptors (EC50 value of 2.6 nM and IA of 1.00) and the rat α_1-adrenoceptors (EC50 value of 0.78 µM and IA of 1.27).

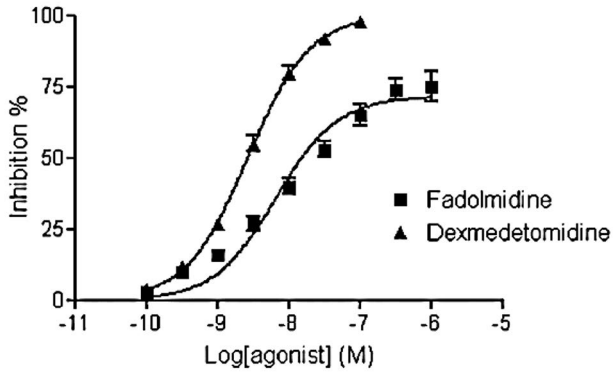


Figure 6. Inhibition of the electrically evoked contraction in Sprague–Dawley rat vas deferens ex vivo preparations by fadolmidine and dexmedetomidine (mean ± S.E.M.). From the original publication I.

5.1.3 Mydriatic effects (original publication I)

Agonist effects of fadolmidine on the central α_2-adrenoceptors were assessed by studying mydriasis response in anaesthetised rats (Sprague–Dawley) after i.v. and s.c. dosing. The i.v. doses of fadolmidine and dexmedetomidine increased pupil diameter dose-dependently, resulting in ED50 values of 45.6 µg/kg and 1.8 µg/kg, respectively (Figure 7). Much higher s.c. doses were needed for mydriasis by fadolmidine; 100 µg/kg and below had no mydriatic effects, whereas with 300 µg/kg profound mydriasis was observed. For comparison, s.c. dexmedetomidine induced profound mydriasis at a dose of 10 µg/kg, and the maximum response was achieved at 30 µg/kg (Figure 8).

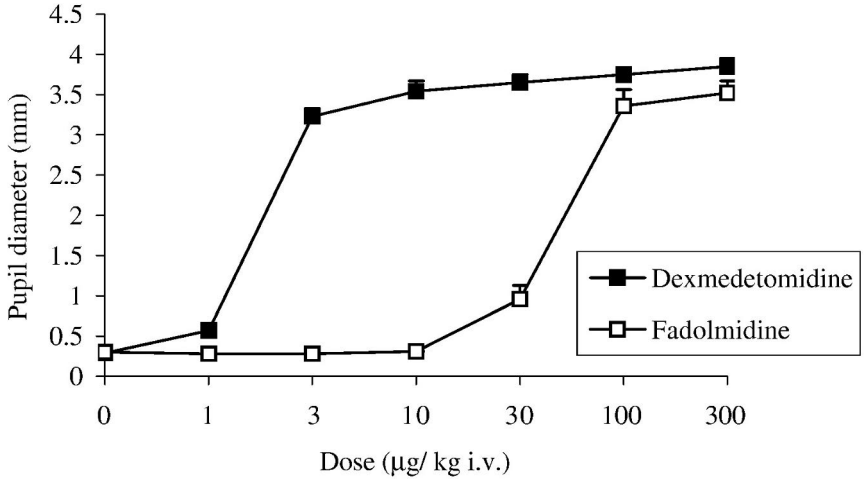


Figure 7. Mydriasis caused by intravenous doses of fadolmidine and dexmedetomidine in anaesthetised male Sprague–Dawley rats (mean ± S.E.M.). From the original publication I.

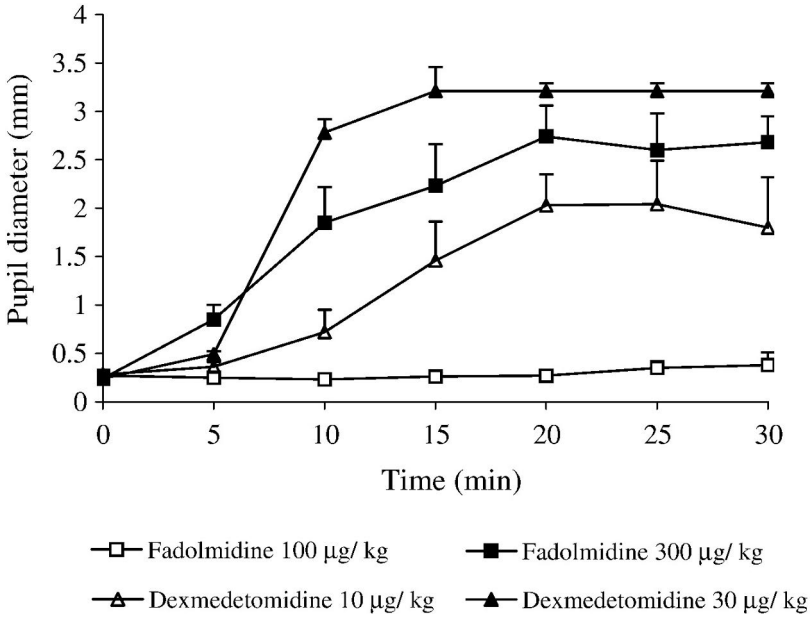


Figure 8. Mydriasis caused by subcutaneous doses of fadolmidine and dexmedetomidine in anaesthetized male Sprague–Dawley rats (mean ± S.E.M.). From the original publication I.

5.1.4 Cardiovascular effects (original publication I and III)

Peripheral cardiovascular effects of fadolmidine were studied in the pithed rat model and compared to those of dexmedetomidine. Both compounds increased MAP dose-dependently, resulting in an increase of 50 mmHg at 0.23 µg/kg for fadolmidine and 0.84 µg/kg for dexmedetomidine. Fadolmidine and dexmedetomidine also inhibited electrically induced tachycardia – with 50% response doses of 0.10 µg/kg and 0.47 µg/kg, respectively.

In anaesthetised rats slow i.v. infusion of fadolmidine at doses of 1, 3 and 10 µg/kg induced a transient dose-dependent peak of 53% in MAP at 10 µg/kg – followed by a long-lasting decrease in HR, with a maximum of 21%. For comparison, dexmedetomidine decreased both MAP and HR immediately after dosing – with a maximum of 37% and 23%, respectively.

In conscious rats, intrathecal (i.t.) administration of fadolmidine and clonidine led to dose-dependent reductions in heart rate (HR) and initial increases in mean arterial pressure (MAP) when assessed within the familiar home cage environment. At the highest doses of fadolmidine and clonidine, HR did not return to the baseline level even after 90 min. However, in the open-field settings, HR and MAP were elevated due to exploratory behaviour. Both fadolmidine and clonidine decreased BT dose-dependently throughout the series of experiments, compared to the control.

5.1.5 Spontaneous motility (original publication I)

Effects of s.c. doses of fadolmidine and dexmedetomidine on spontaneous motility in conscious NMRI mice were monitored as an indicator of sedative effects. Fadolmidine reduced both rearing and ambulation movements, but this effect was noticeable only at doses of 300 µg/kg and higher. In contrast, dexmedetomidine displayed sedative effects at a much lower dose, starting at 10 µg/ kg (Table 4).

When studied in the open-field settings by telemetry, fadolmidine decreased the motor activity at a dose of 30 µg in both motor activity measurement sessions. For comparison, clonidine decreased the motor activity at all tested doses in the first open-field session, and at a dose of 100 µg in the second session.

Table 4. The effects of subcutaneous administration of dexmedetomidine and fadolmidine on motor activity in NMRI mice

TREATMENT	N	AMBULATIONS/30 MIN	REARINGS/30 MIN
CONROL	10	120.6 ± 14.9	22.0 ± 2.9
DEXMEDETOMIDINE 3 µg/kg	6	102.0 ± 16.9	20.6 ± 9.4
DEXMEDETOMIDINE 10 µg/kg	6	8.0 ± 4.5[a]	1.3 ± 1.3[a]
FADOLMIDINE 10 µg/kg	10	103.7 ± 20.3	23.1 ± 0.19
FADOLMIDINE 30 µg/kg	10	145.5 ± 21.8	30.0 ± 5.5
FADOLMIDINE 100 µg/kg	10	85.5 ± 26.1[b]	13.4 ± 3.6[b]
FADOLMIDINE 300 µg/kg	10	10.9 ± 3.2[a]	0.9 ± 0.4[a]
FADOLMIDINE 1000 µg/kg	10	1.8 ± 0.9[a]	0 ± 0[a]
FADOLMIDINE 3000 µg/kg	10	2.3 ± 7.0[a]	0 ± 0[a]

N = number of animals in the group. The results are expressed as mean ± S.E.M. The sedation data was analysed by one-way ANOVA, followed by a Fisher's PLSD test

[a] $p < 0.001$ when comparing with the control group.

[b] $p < 0.05$ when comparing the fadolmidine 100 µg/kg and dexmedetomidine 10 µg/kg groups.

From the original publication I.

5.1.6 Kidney function (original publication III)

The impact of fadolmidine on kidney function was assessed by examining acute effects of i.v. and i.t. administrations on urine output, osmolarity, $[Na^+]$ and $[K^+]$ in rats (Sprague–Dawley) subjected to water-loading. The i.v. doses of fadolmidine slightly increased urine output and $[Na^+]$, while the urine osmolarity and $[K^+]$ remained unaffected. In comparison, i.t. doses of fadolmidine resulted in a dose-dependent increase in urine output, with a statistically significant effect observed at the highest dose (30 µg/rat). Moreover, $[Na^+]$ increased dose-dependently, reaching statistical significance at doses of 3, 10 and 30 µg/rat. The urine osmolarity and $[K^+]$ were lower than control levels at all doses, although these differences did not reach statistical insignificance. In comparison, a diuretic furosemide (20 mg/kg s.c.) increased all the measured parameters when compared to the control.

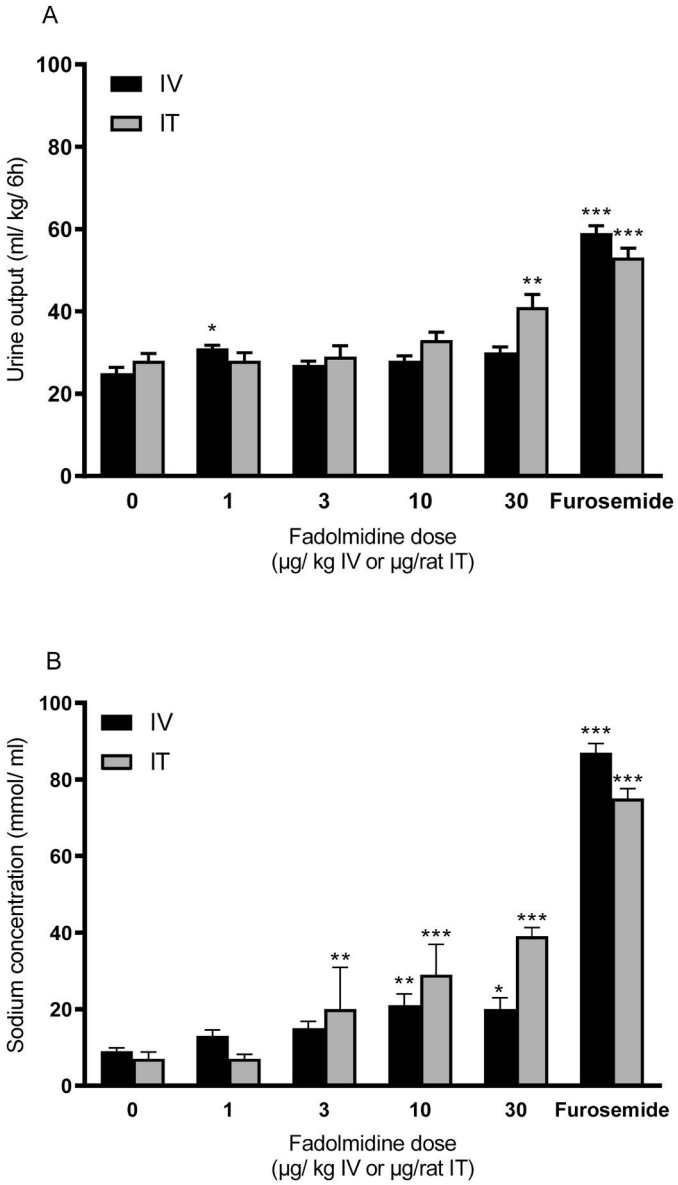


Figure 9. Intrathecal (IT) doses of fadolmidine increased urine output dose-dependently, whereas intravenous (IV) doses had no effects (A) in male Sprague–Dawley rats. Both IV and IT doses of fadolmidine increased urine sodium ion concentration, with IT doses being more potent (B). Furosemide was given subcutaneous (SC) at doses of 20 mg/rat. The data are presented as mean ± S.E.M. For IV dosing, n = 6 except for the control, where n = 5. For IT dosing, n = 8. For the analysis of the statistical significance between treatments and vehicle, a one-way ANOVA was used followed by the Bonferroni corrected t-test. P-values < 0.01 *, <0.002 ** and <0.0002 ***. From the original publication III.

5.1.7 Urodynamics (original publication III)

Effects of i.t. doses of fadolmidine on micturition cycle were studied in anaesthetised male rats (Sprague–Dawley) and compared to equipotent analgesic doses of dexmedetomidine, clonidine and morphine. Continuous infusion of physiological salt solution to the urinary bladder-induced phasic, volume-evoked voiding cycles. All tested compounds disturbed these voiding cycles dose-dependently – starting with minor changes in synchronisation of the cycles and causing the total blockage of the micturition cycles at higher doses, which resulted in overflow incontinence (Figure 10).

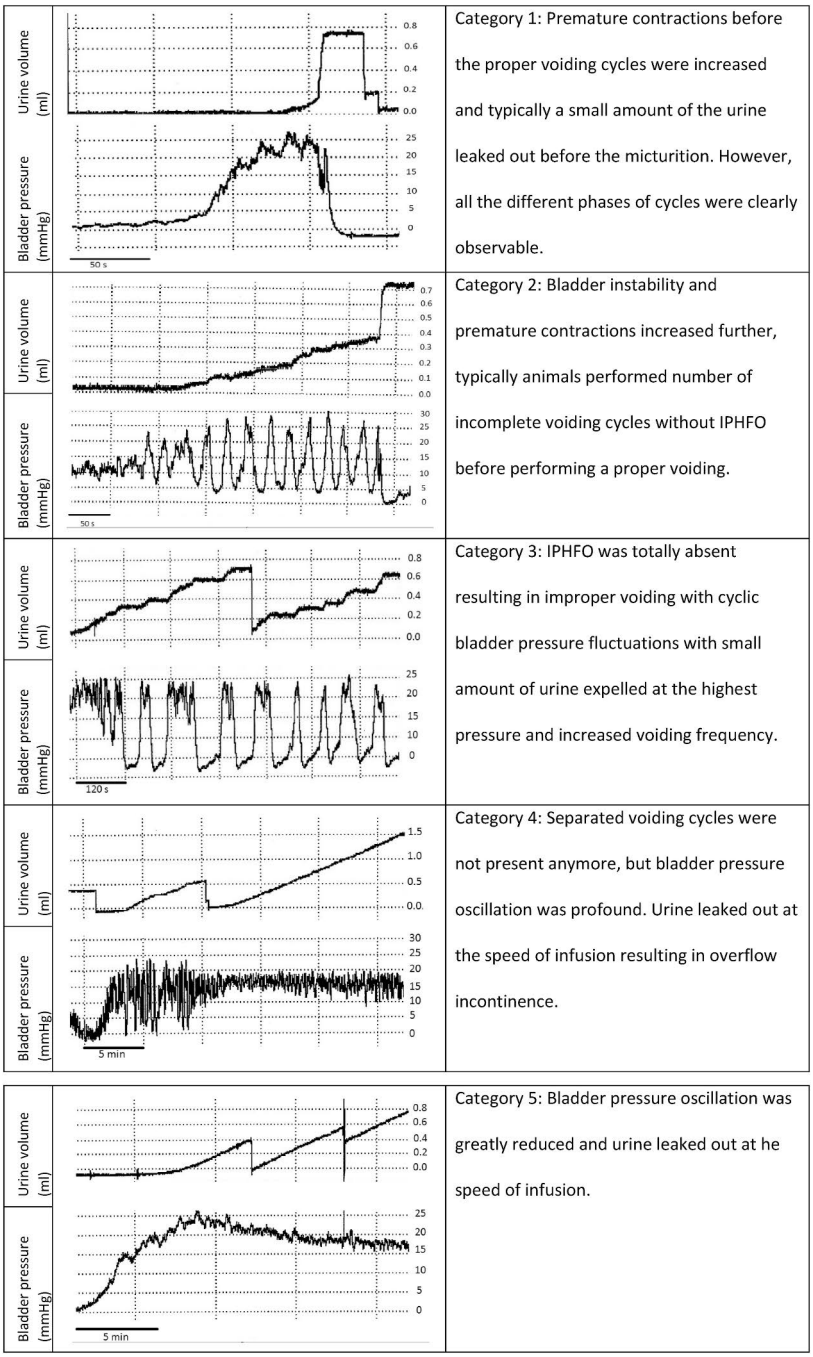


Figure 10. Categories demonstrating the severity of the disrupted voiding cycles measured in anesthetised male Sprague-Dawley rats. For the category graphs, the upper trace is urine output (ml), and the lower trace is the urinary bladder lumen pressure (mmHg). From The original publication III.

To enable comparison of the study compounds, the inhibitory effects were ranked into six categories based on the severity. Table 5 summarises the results of all various drug treatments on the voiding function.

Table 5. Effects of intrathecal drug treatments on voiding functions in urethane anesthetized male Sprague-Dawley rats.

TREATMENT	DOSE µg/ i.t.	CATEGORY						SUBJECTS
		0	1	2	3	4	5	N
CONTROL	0	14	2	1	1		1	19
FADOLMIDINE	1	3	2	1	1		1	8
FADOLMIDINE	3	2	8	4			2	16
FADOLMIDINE	10		1	1	2			4
CLONIDINE	3		1	2				3
CLONIDINE	10			3	1			4
CLONIDINE	30				3	2		5
DEXMEDETOMIDINE	1		1	1		2		4
DEXMEDETOMIDINE	3				3			3
DEXMEDETOMIDINE	10					1	2	3
MORPHINE	0.1		1	2	1			4
MORPHINE	0.3		1	1		1	2	5
MORPHINE	1						5	5

From the original publication III.

5.1.8 Effects on 5-HT$_3$-receptors (original publication III)

Patch clamp study involving transfected cells

Functional effects of fadolmidine on 5-HT$_3$ receptors were measured in the HEK-293 cell lines expressing either recombinant 5-HT$_{3A}$ or 5-HT$_{3AB}$ receptors. Serotonin was used for the assay validation – evoking a rapidly desensitising inward current of 2–20 nA, with EC50 values of 2.8 µM in the 5-HT$_{3A}$ cells and 5.8 µM in the 5-HT$_{3AB}$ cells. Desensitisation was faster in the 5-HT$_{3AB}$ cells. Fadolmidine also induced desensitising inward currents but compared to serotonin, was a weak partial agonist with an EC50 value of 120 nM and IA of 0.09 in 5-HT$_{3A}$ and an EC50 value of 2,010 nM and IA of 0.22 in the 5-HT$_{3AB}$ cells.

Guinea pig ileum ex vivo preparations

In the presence of atipamezole to block the α_2-adrenoceptors, fadolmidine induced dose-dependent ondansetron-sensitive contractions in the guinea pig (Dunklin-Hartley) ileum preparations, with a negative logarithm of an EC50 (pD2) value of 6.50 and an IA value of 0.36, compared to 10 µM acetylcholine. Contractions were considerably diminished when atipamezole was omitted from the incubation medium. The contractions were totally blocked by tetrodotoxin at a concentration of 1 µM.

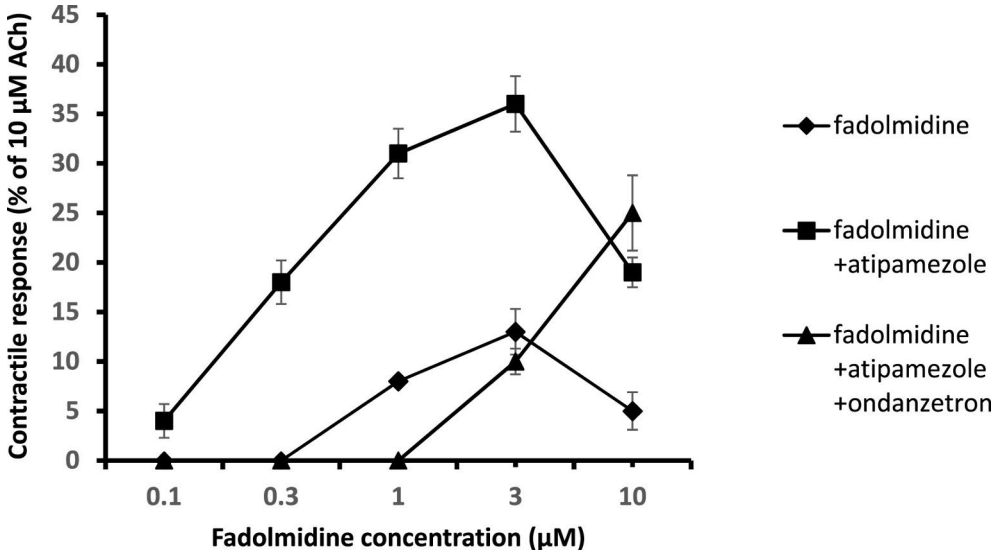


Figure 11. Contractile dose response of fadolmidine in guinea-pig ileum ex vivo preparations without and with atipamezole or atipamezole + ondansetron. Fadolmidine (n = 4) induced contractions were potentiated in the presence of α2-adrenoceptor antagonist atipamezole (1 µM, n = 7) and this effect was inhibited by the 5-HT3 antagonist ondansetron (1 µM, n = 5) being indicative of the 5-HT3 agonistic property of fadolmidine. Data are presented as mean ± S.E.M. From the original publication III.

Bezold–Jarisch reflex

To validate the assay functionality in rats (Sprague-Dawley), a specific 5-HT_3 receptor agonist, 2-methyl-serotonin, was utilized. It elicited the characteristic Bezold–Jarisch reflex, resulting in transient bradycardia and a decrease in mean arterial pressure (MAP). These effects were reversed by a specific 5-HT_3 receptor antagonist, ondansetron (30 µg/rat i.v.), but not with a known α_2-adrenoceptor antagonist, atipamezole (600 µg/rat i.v.). For comparison, fadolmidine (3.0 µg/rat i.v.) induced a transient increase in MAP, followed by reflectory bradycardia. This effect was partially inhibited by i.v. doses of atipamezole (600 µg/rat) or prazosin

(30 µg/rat) and was nearly completely abolished when both compounds were coadministered (Figure 12). However, ondansetron (30 µg/rat) did not produce any discernible effects on fadolmidine's impact on MAP or HR. Like fadolmidine, dexmedetomidine also increased the MAP, followed by decreasing HR. Atipamezole antagonised these effects, while ondansetron or prazosin did not exert any influence.

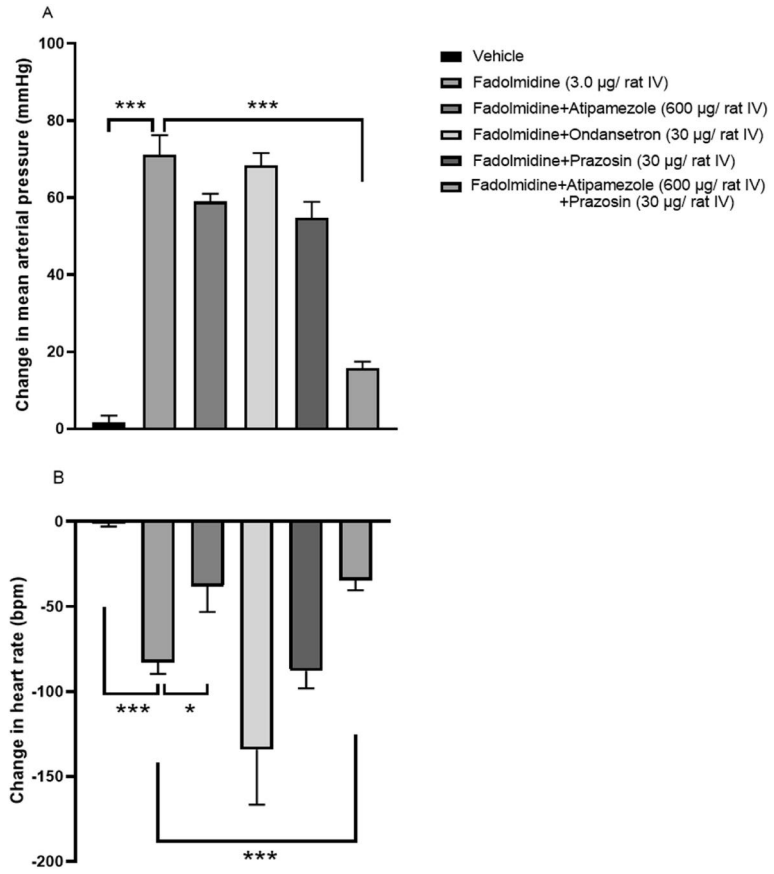


Figure 12. (A) Intravenous doses of fadolmidine induced a transient atipamezole and prazosin sensitive increase in mean arterial pressure in anesthetised Sprague-Dawley rats. (B) The reflectory decrease in heart rate was inhibited by atipamezole and atipamezole + prazosin. The data is presented as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001. From the original publication III.

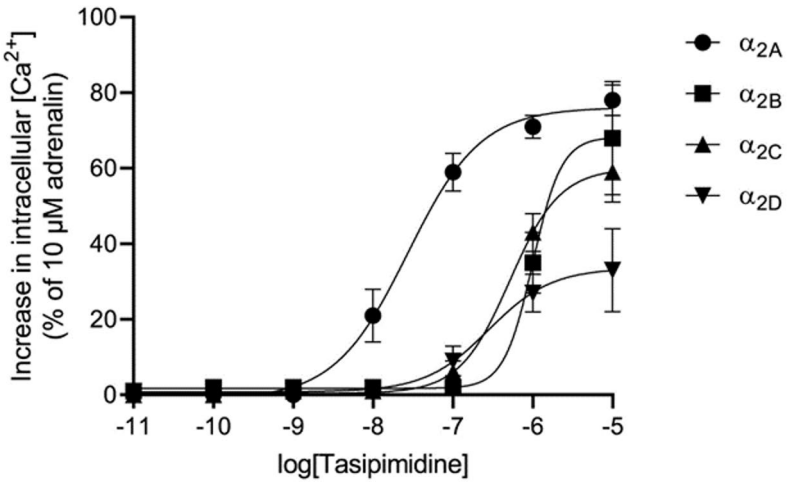
5.2 Pharmacological characterization of tasipimidine (original publication V)

5.2.1 Binding affinity for human α_1- and α_2-adrenoceptors

In the membrane fractions of recombinant CHO cells, tasipimidine demonstrated binding affinity for the human α_{2A}-adrenoceptor subtype, with a Ki value of 8.8 nM. In comparison, affinity for the human α_{2B} and α_{2C} subtypes was weaker, with inhibition of specific binding by 48% and 39% at 1 µM, respectively. Thus, the resulting Ki values were above 1 µM. For the human α_1-adrenoceptor subtypes tasipimidine had low affinity, with Ki values of 3.1 µM (α_{1A}), 0.5 µM (α_{1B}) and 3.1 µM (α_{1D}).

5.2.2 Functional cell-based assay on human and rodent α_2- and α_1-adrenoceptors

Tasipimidine was almost a full agonist on all human α_2-adrenoceptor subtypes in the recombinant CHO cells, whereas on the rodent α_{2D}-adrenoceptors, partial agonist response was observed. Tasipimidine showed subtype selectivity towards the α_{2A}-adrenoceptor over the α_{2B}-, α_{2C}- and α_{2D}-adrenoceptors (Figure 13). α_{2A}-adrenoceptor selective agonist effect was also observed in the GTPγS binding assay – with EC50 values of 44 nM, 380 nM and > 1 µM for the α_{2A}-, α_{2B}- and α_{2C}-adrenoreceptor subtypes, respectively.



	α_{2A}	α_{2B}	α_{2C}	α_{2D}
LogEC50	-7.57	-6.00	-6,29	-6.56
IA	0.76	0.68	0.60	0.34
95% CI LogEC50	-7.79 to -7.33	-6.02 to -5.97	-6.34 to -6.24	-6.65 to -6.46

Figure 13. Functional effects of tasipimidine on the Chinese hamster ovary cells expressing the human α_2-adrenoceptors subtypes and rat α_{2D}-adrenoceptors. N refers to independent assay plates, with each concentration measured as four replicates on a plate. The log(agonist) vs response–variable slope (four parameters) equation was used. Y = Bottom + (Top-Bottom) / (1 + 10^((LogEC50-X)*HillSlope)), using GraphPad Prism 8.0. From the original publication IV.

Effects of fadolmidine on the human α_1-adrenoceptors varied in different assay setups. Table 6 presents functional results for the human and rodent α_{1A}- and α_{1B}-adrenoceptors.

Table 6. Functional effects of tasipimidine on the human and rodent α_1-adrenoceptors

RECEPTOR	METHOD	AGONIST EFFECT
Human α_{1A}-adrenoceptor	Intracellular [Ca2+] assay LnCAP cells endogenously expressing human α_{1A}-adrenoceptor	No agonism
	Inositol phosphate-1 assay AequoZen frozen cells expressing human α_{1A}-adrenoceptor	pEC_{50} < 5.00 IA ND
	Intracellular [Ca2+] assay Chem-1 cell line stably expressing human α_{1A}-adrenoceptor	pEC_{50} 6.79 IA 0.82
	Intracellular [Ca2+] assay with CHO cell line stably expressing human α_{1A}-adrenoceptor	pEC_{50} 7.42 IA 0.85
Human α_{1B}-adrenoceptor	Intracellular [Ca2+] assay HEK-293 cells transiently transfected with human α_{1B}-adrenoceptor	No agonism
Rat α_{1A}-adrenoceptor	Intracellular [Ca2+] assay HEK-293 cells transiently transfected with rat α_{1A}-adrenoceptor	pEC50 6.59 IA 0.57
Rat α_{1B}-adrenoceptor	Intracellular [Ca2+] assay HEK-293 cells transiently transfected with rat α_{1B}-adrenoceptor	EC50 5.22 IA 0.21

pEC50 = -log(half maximal effective concentration), IA = Intrinsic Activity: maximum response relative to adrenaline, ND = no detected. From the original publication V.

5.2.3 Functional agonism on rat α_1- and α_2-adrenoceptors ex vivo

Tasipimidine demonstrated full agonist effect on the rat α_{2D}-adrenoceptor in the electrically stimulated rat prostate ex vivo preparation, with an EC50 value of 5.7 nM. For comparison, tasipimidine was a full but weak agonist on the rat α_1-adrenoceptors – with EC50 values of 8.6 μM and 2.0 μM in the vas deferens and prostate preparations, respectively.

5.2.4 Selectivity profile

In the selectivity profiling assay of 121 receptors, ion channels and enzymes, tasipimidine at 10 µM concentration had significant binding affinity (at least 50% inhibition of specific binding) for eight targets (Table 7).

Table 7. Inhibition of the specific binding by 10 µM tasipimidine on the identified target in the receptor profile study.

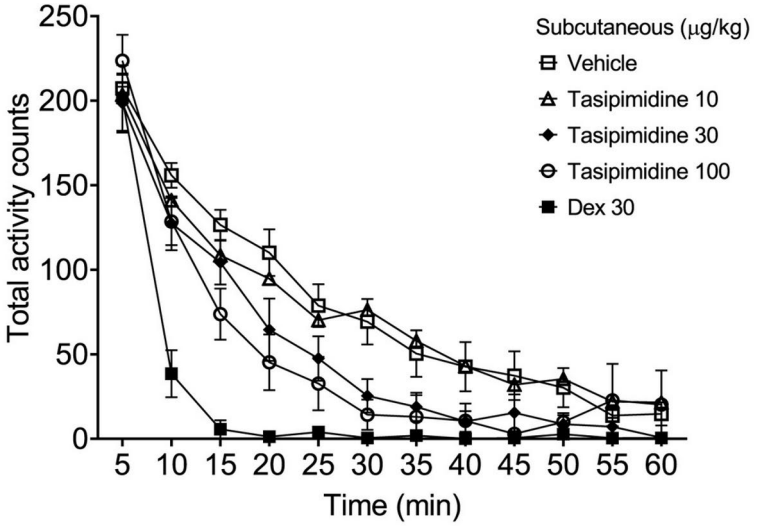
TARGET	% INHIBITION OF SPECIFIC BINDING
NON-SELECTIVE RODENT α_1	68
NON-SELECTIVE RODENT α_2	99
PERIPHERAL BENZODIAZEPINE (RAT)	51
IMIDAZOLE I1 (BOVINE)	102
IMIDAZOLE I2 (RAT)	73
SEROTONIN 5-HT_{1A} (HUMAN)	89
SEROTONIN 5-HT_{1D} (RAT)	53
SEROTONIN 5-HT_7 (HUMAN)	76

From the original publication V.

In the functional cell-based follow-up studies, tasipimidine was a weak partial agonist on the human 5-HT_{1A} and rodent 5-HT_{1D} receptors – with EC50 values of 1.7 µM and > 10 µM, respectively. On the 5-HT_7 receptors, tasipimidine was devoid of any functional effects.

5.2.5 Spontaneous locomotor activity in mice

Inhibition of spontaneous locomotor activity was measured as an indication of tasipimidine's centrally mediated sedative effects in Hsd:ICR mice. Compared to the vehicle group, s.c. doses of 30 and 100 µg/kg of tasipimidine decreased the total activity counts during the first 30 min of measurement. The lowest tested dose of 10 µg/kg did not differ from the vehicle (Figure 14).



		Time (min)							
Treatment	Dose (µg/kg)	10	15	20	25	30	35	40	45
Tasipimidine	30			**		**			
	100		**	***	**	***	*		
Dexmedetomidine	30	***	***	***	***	***	**	*	*

Figure 14. The effect of subcutaneous doses of tasipimidine and dexmedetomidine on locomotor activity in nonhabituated mice. The group means (n = 8–12) ± S.E.M. are presented. The statistical significance values are indicated in the table insert: *p < 0.05, **p < 0.01, ***p < 0.001 vs vehicle group. The lowest dose of tasipimidine was without statistically significant effect. From the original publication IV.

When the oral dosing route was tested, 3 mg/kg dose of tasipimidine significantly decreased activity counts statistically at the five-minute measurement point, and the effect persisted until the 25-minute time point. A lower dose of 0.9 mg/kg produced no effect when compared to the vehicle-treated mice (Figure 15).

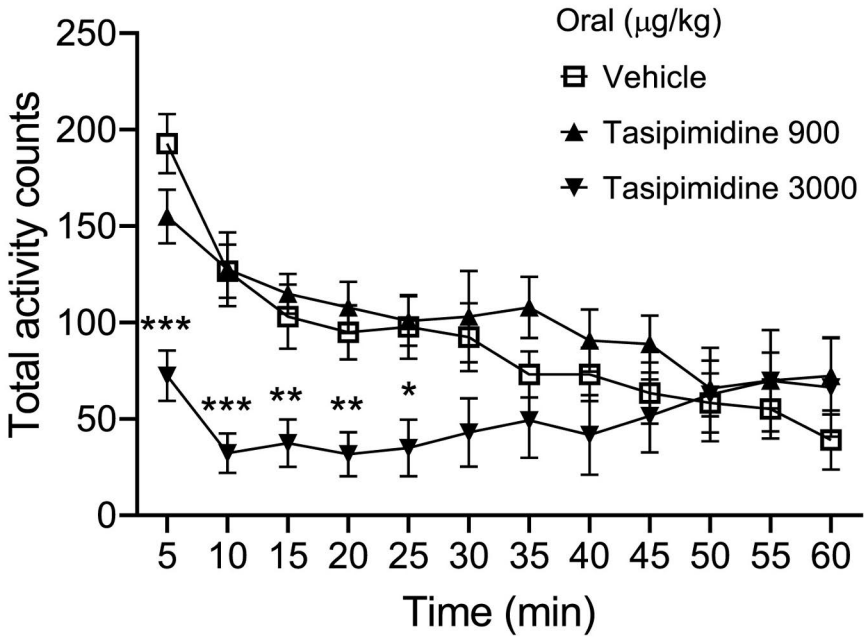
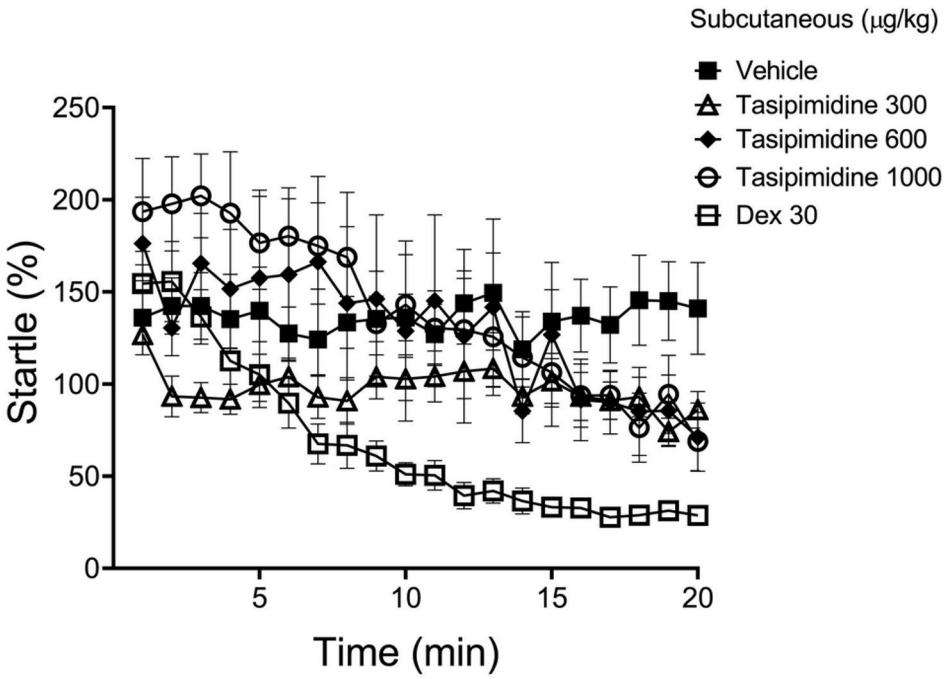


Figure 15. The effect of oral doses of tasipimidine on the locomotor activity counts of nonhabituated male Hsd:ICR mice. Symbols represent group means (n = 8–12) ± S.E.M. of total locomotor activity counts during the measurement period of 120 min with 5 min intervals in male CD-1 mice. *p < 0.05, **p < 0.01, ***p < 0.001 vs vehicle group. From the original publication IV.

5.2.6 Acoustic startle reflex in rats

Inhibition of the acoustic startle reflex by tasipimidine was studied as an indication of centrally mediated effects in HsdRCCHanWist male rats. At doses of 0.6 and 1.0 mg/kg s.c., tasipimidine significantly reduced the magnitude of the acoustic startle reflex statistically, starting 20 min from the injection and reaching the peak effect within 1 h (Figure 16). Sedation lasted up to two hours and was alleviated in four hours.



		Time (min)												
Treatment	Dose (µg/kg)	8	9	10	11	12	13	14	15	16	17	18	19	20
Tasipimidine	600											*	*	**
	1000											**		***
Dexmedetomidine	30	*	**	***	***	***	***	***	***	***	***	***	***	***

Figure 16. The onset of sedation induced by s.c. tasipimidine in male HsdRCCHanWist rats, measured by the startle response analysis. The group means of startle % (n = 10) ± S.E.M are presented. The statistical significance values are indicated in the table insert: *p < 0.05, **p < 0.01, ***p < 0.001 vs vehicle group. The lowest dose of tasipimidine was without statistically significant effect. From the original publication IV.

5.3 Effects of dexmedetomidine, tasipimidine and fadolmidine on sexual incentive motivation and sexual behaviour in male rats (original publications II and IV)

5.3.1 Sexual incentive motivation and general activity

All male rats in the test visited more often and spent longer time in the female incentive zone than in the male incentive zone. Dexmedetomidine had no effects on the preference score or on the time spent in the incentive zone in male Wistar rats. Dexmedetomidine neither modified any aspect of ambulatory activity at the doses used nor had any effects on the preference score. However, it reduced the time spent with the female incentive at a dose of 200 µg/kg and the mean duration of visits to the female incentive zone at 100 µg/kg when compared to the control. There were no effects on the duration of visits to the male incentive zone. A dose of 200 µg/kg of tasipimidine reduced ambulatory activity modestly yet statistically significantly in Wistar Han IGS rats, indicating sedation at this dose (Figure 17).

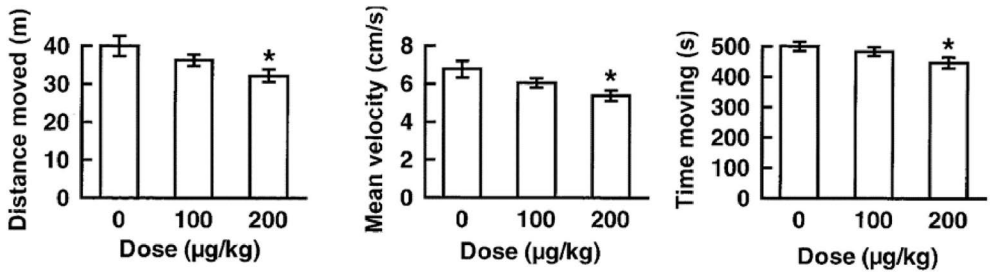


Figure 17. Ambulatory activity in male Wistar Han IGS rats treated with vehicle and tasipimidine at subcutaneous doses of 100 and 200 µg/kg. Data are mean ± SEM. *, different from vehicle, P < .05. N = 12. From the original publication V.

Fadolmidine had no effects on the preference score or the time spent in the incentive zones in male Wistar rats. At the highest dose tested, 100 µg/kg, fadolmidine reduced the number and increased the duration of visits to both incentive zones. At this dose it also clearly reduced ambulatory activity, indicating sedation (Figure 18).

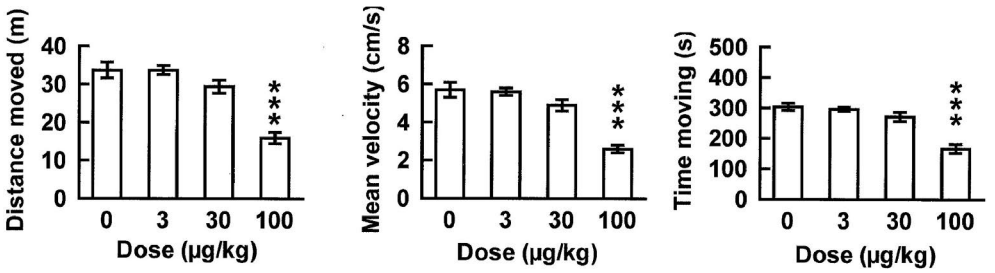


Figure 18. Ambulatory activity in male Wistar rats treated with vehicle and subcutaneous doses of fadolmidine. Data are mean ± SEM. ***, different from vehicle, P < .001. N = 20. From the original publication V.

5.3.2 Copulatory behaviour

Dexmedetomidine had only a few effects on copulatory behaviour. The only feature modified was the first ejaculation latency, which was statistically significantly longer compared to the vehicle at a dose of 1 µg/kg. In the second ejaculatory series, there was no drug effect on any parameter. During the entire test period of 30 min, the number of ejaculations was reduced with the dose of 1 µg/kg (Table 8).

Table 8. Effects of dexmedetomide on copulatory behaviour in male Wistar rats.

PARAMETER	VEHICLE	0.1 µg/kg	1 µg/kg
LATENCY TO 1ST MOUNT (s)	8.5 ± 3.4	4.5 ± 0.72	7.2 ± 1.27
LATENCY TO 1ST INTROMISSION (s)	12.6 ± 5.6	11.8 ± 4.5	10.8 ± 3.0
NUMBER OF MOUNTS I 1ST SERIES	9.5 ± 2.6	7.8 ± 1.6	10.5 ± 2.0
NUMBER OF INTROMISSIONS (1ST SERIES)	13.3 ± 1.4	12.0 ± 1.4	13.9 ± 1.9
EJACULATION LATENCY IN 1ST SERIES (s)	371.9 ± 65.7	344.5 ± 43.9	502.7 ± 87.0**
POSTEJACULATORY INTERVAL IN 1ST SERIES	326.5 ± 17.8	306.6 ± 10.5	327.1 ± 22.3
INTERINTROMISSION INTERVAL IN 1ST SERIES	26.8 ± 3.5	31.6 ± 4.5	41.7 ± 9.8
INTROMISSION RATIO 1ST SERIES	0.67 ± 0.06	0.64 ± 0.06	0.59 ± 0.03
NUMBER OF MOUNTS IN TEST	23.6 ± 5.8	20.3 ± 3.5	21.3 ± 2.9
NUMBER OF INTROMISSIONS IN TEST	26.9 ± 1.9	24.5 ± 1.7	26.1 ± 2.5
NUMBER OF EJACULATIONS IN TEST	3.1 ± 0.2	3.3 ± 0.2	2.6 ± 0.3*
INTROMISSION RATIO IN THE TEST	0.60 ± 0.05	0.57 ± 0.04	0.56 ± 0.03

Different from the vehicle, *p < 0.05, **p < 0.01.
Interintromission interval = (Ejaculation latency in s) / (number of intromissions)
Intromission ratio = (Number of intromissions) / (number of mounts + number of intromissions)
From the original publication IV.

When the effects of tasipimidine were evaluated on the whole study population, only marginal effects on copulatory behaviour were observed in the first ejaculatory series (Table 9), and it was devoid of any effect in the second ejaculatory series. Ejaculation latency tended to increase but did not reach statistical significance. The number of intromissions and ejaculations was reduced at the dose of 200 µg/kg when counted over the entire 30 min study.

Table 9. Effects of tasipimidine on copulatory behaviour in male Wistar Han IGS rats.

PARAMETER	VEHICLE	100 µg/kg	200 µg/kg
LATENCY TO 1ST MOUNT (s)	11.1 ± 2.3	10.0 ± 1.6	68.5 ± 43.6
LATENCY TO 1ST INTROMISSION (s)	17.8 ± 4.7	15.7 ± 2.8	77.8 ± 47.3
NUMBER OF MOUNTS IN 1ST SERIES	10.0 ± 3.3	13.7 ± 4.7	11.6 ± 2.6
NUMBER OF INTROMISSIONS IN 1ST SERIES	12.7 ± 2.3	11.3 ±1.1	11.2 ± 1.7
EJACULATION LATENCY IN 1ST SERIES (s)	323 ± 74	492 ± 144	538 ± 127
POSTEJACULATORY INTERVAL IN 1ST SERIES	255 ± 26	277 ± 15	276 ± 13
INTERINTROMISSION INTERVAL IN 1ST SERIES	29.6 ± 8.7	44.9± 15.2*	53-5 ± 14.7
INTROMISSION RATIO IN 1ST SERIES	0.64 ± 0.06	0.58 ± 0.06	0.51 ± 0.04*
NUMBER OF MOUNTS IN TEST	29.3 ± 3.6	32.4 ± 7.1	22.0 ± 4.5
NUMBER OF INTROMISSIONS IN TEST	28.6 ± 3.2	21.3 ± 2.1	20.5 ± 3.4*
NUMBER OF EJACULATIONS IN TEST	3.6 ± 0.3	3.1 ± 0.3	2.5 ± 0.4*

Different from the vehicle, * $p < 0.05$
Interintromission interval = (Ejaculation latency in s) / (number of intromissions)
Intromission ratio = (Number of intromissions) / (number of mounts + number of intromissions)
Modified from the original publication IV.

The effects of tasipimidine were further studied in fast-ejaculating rats with an ejaculation latency below the 75th percentile after the vehicle (Table 10). In these rats the ejaculation latency was extended in the first series at the dose of 200 µg/kg. No treatment-related effects were observed in the second series. The number of intromissions and ejaculations reduced at the dose of 200 µg/kg.

Table 10. Effects of tasipimidine on copulatory behaviour in fast-ejaculating male Wistar Han IGS rats (below the 75th percentile of the first postejaculatory interval)

PARAMETER	VEHICLE	100 µg/kg	200 µg/kg
NUMBER OF INTROMISSIONS IN 1ST SERIES	10.0 ± 1.3	11.1 ± 0.9	9.6 ± 1.8
EJACULATION LATENCY IN 1ST SERIES (s)	201.1 ± 44.9	285.8 ± 51.0	535.1 ± 165.8*
INTERINTROMISSION INTERVAL IN 1ST SERIES	20.9 ± 3.7	24.7± 3.2*	59.1 ± 19.2*
INTROMISSION RATIO IN 1ST SERIES	0.70 ± 0.1	0.63 ± 0.1	0.52 ± 0.1
NUMBER OF MOUNTS IN TEST	26.9 ± 4.1	32.5 ± 9.9	20.2 ± 5.5
NUMBER OF INTROMISSIONS IN TEST	27.4 ± 3.0	23.0 ± 2.1	18.2 ± 3.9*
NUMBER OF EJACULATIONS IN TEST	4.0 ± 0.3	3.7 ± 0.2	2.6 ± 0.5**

Different from the vehicle, *p < 0.05, **p < 0.01
Interintromission interval = (Ejaculation latency in s) / (number of intromissions)
Intromission ratio = (Number of intromissions) / (number of mounts + number of intromissions)
Modified from the original publication IV.

When the whole study population was included, fadolmidine had hardly any effects on copulatory behaviour, the postejaculatory latency being the only parameter affected (Table 11).

Table 11. Effects of fadolmidine on copulatory behaviour in male Wistar rats.

PARAMETER	VEHICLE	3 µg/kg	30 µg/kg	100 µg/kg
LATENCY TO 1ST MOUNT (s)	5.1 ± 0.9	8.8 ± 3.6	7.6 ± 4.0	11.3 ± 3.8
LATENCY TO 1ST INTROMISSION (s)	6.6 ± 0.9	27.5 ± 12.0	14.5 ± 5.1	15.2 ± 5.5
NUMBER OF MOUNTS IN 1ST SERIES	16.9 ± 1.7	23.0 ± 4.1	22.9 ± 2.9	17.7 ± 1.7
NUMBER OF INTROMISSIONS IN 1ST SERIES	11.5 ± 0.8	12.2 ± 1.0	13.1 ± 0.9	10.7 ± 0.8
EJACULATION LATENCY IN 1ST SERIES (s)	280.9 ± 30.2	296.5 ± 33.8	344.3 ± 59.3	323.3 ± 35.3
POSTEJACULATORY INTERVAL IN 1ST SERIES	366.9 ± 9.3	386.5 ± 16.1	385.9 ± 15.1	454.8 ± 13.9***
INTERINTROMISSION INTERVAL IN 1ST SERIES	24.4 ± 1.6	24.9 ± 2.7	27.2 ± 4.5	29.8 ± 2.8
INTROMISSION RATIO IN 1ST SERIES	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
NUMBER OF MOUNTS IN TEST	36.9 ± 2.3	43.8 ± 4.2	44.8 ± 3.6	37.3 ± 2.7
NUMBER OF INTROMISSIONS IN TEST	25.2 ± 1.3	24.6 ± 1.9	26.5 ± 2.0	20.8 ± 0.1
NUMBER OF EJACULATIONS IN TEST	3.1 ± 0.1	2.7 ± 0.2	3.0 ± 0.2	2.7 ± 0.1

Different from the vehicle, ***p < 0.001
Interintromission interval = (Ejaculation latency in s) / (number of intromissions)
Intromission ratio = Number of intromissions / (number of mounts + number of intromissions)
Modified from the original publication IV.

The post hoc analysis of the rapidly ejaculating rats with an ejaculation latency below the 75th percentile after the vehicle showed that the effects of fadolmidine were also marginal in this subgroup. The results of the first series are listed in Table 12. There were no changes in the second series.

Table 12. Effects of fadolmidine on copulatory behaviour in fast-ejaculating male Wistar rats (below the 75th percentile of the first postejaculatory interval).

PARAMETER	VEHICLE	3 µg/kg	30 µg/kg	100 µg/kg
NUMBER OF MOUNTS IN 1ST SERIES	13.2 ± 1.0	21.0 ± 3.7	22.5 ± 3.0 *	16.9 ± 1.7
NUMBER OF INTROMISSIONS IN 1ST SERIES	9.9 ± 0.6	12.7 ± 1.1	13.1 ± 1.0	10.3 ± 0.8
EJACULATION LATENCY IN 1ST SERIES (s)	217.6 ± 17.9	314.9 ± 40.3	302.9 ± 44.5	321.5 ± 39.1
POSTEJACULATORY INTERVAL IN 1ST SERIES	368.1 ± 11.0	390.0 ± 20.4	383.4 ± 18.2	465.9 ± 16.4 **
INTERINTROMISSION INTERVAL IN 1ST SERIES	22.8 ± 1.9	26.4 ± 3.3	24.9 ± 4.5	31.2 ± 3.4
INTROMISSION RATIO IN 1ST SERIES	0.8 ± 0.3	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.0
NUMBER OF MOUNTS IN TEST	32.6 ± 1.5	43.4 ± 4.3	45.7 ± 4.6 *	36.8 ± 3.5
NUMBER OF INTROMISSIONS IN TEST	24.1 ± 1.6	26.1 ± 2.0	27.3 ± 2.4	20.2 ± 1.4
NUMBER OF EJACULATIONS IN TEST	3.3 ± 0.1	2.8 ± 0.2	3.1 ± 0.2	2.7 ± 0.2 *

Different from the vehicle, *p < 0.05, **p < 0.01.
Interintromission interval = (Ejaculation latency in s) / (number of intromissions)
Intromission ratio = (Number of intromissions) / (number of mounts + number of intromissions)
Modified from the original publication IV.

To further explore the potential effects of fadolmidine in very fast-ejaculating rats, the data of five rats having an ejaculation latency below the 25th percentile after vehicle were reanalysed (Table 13). In the first ejaculatory series, there were no significant treatment effects. The latency tended to increase, but the effect was not statistically significant. In the second series, the interintromission interval increased and intromission ratio consequently decreased at the dose of 100 µg/kg.

Table 13. Effects of fadolmidine on copulatory behaviour in fast-ejaculating male Wistar rats (below the 75th percentile of the first postejaculatory interval).

PARAMETER	VEHICLE	3 µg/kg	30 µg/kg	100 µg/kg
NUMBER OF INTROMISSIONS IN 1ST SERIES	8.8 ± 0.9	16.6 ± 2.3	12.2 ± 1.4	10.4 ± 1.5
EJACULATION LATENCY IN 1ST SERIES (s)	159.4 ± 14.4	354.0 ± 61.3	258.6 ± 56.9	363.4 ± 74.7
INTERINTROMISSION INTERVAL 1ST SERIES	19.2 ± 3.1	26.7 ± 4.1	21.7 ± 4.7	33.0 ± 4.71
INTROMISSION RATIO IN 1ST SERIES	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
NUMBER OF MOUNTS IN TEST	29.8 ± 2.5	47.4 ± 6.5	50.6 ± 8.6	34.2 ± 2.9
NUMBER OF INTROMISSIONS IN TEST	23.6 ± 2.7	26.6 ± 3.7	28.4 ± 4.0	18.8 ± 0.6
NUMBER OF EJACULATIONS IN TEST	3.4 ± 0.2	2.8 ± 0.4	3.2 ± 0.2	± 0.4

* Interintromission interval = (ejaculation latency in s) / (number of intromissions)
Intromission ratio = (number of intromissions) / (number of mounts + number of intromissions)
Modified f
rom the original publication IV.

6 Discussion

6.1 Pharmacological characterisation of fadolmidine

Our studies show that fadolmidine is a potent full agonist on the human α_2 and α_{1A}-adrenoceptors as well as on the rat α_{2D}-adrenoceptors. These effects were validated in functional ex vivo and in vivo models in rodents.

In the pithed rats when CNS control of vasoactive tone is eliminated, i.v. administration of fadolmidine led to significant vasoconstriction. This is line with the studies by Gavin & Docherty (1996), Gericke et al. (2007), and Kanagy (2005) showing that the activation of the peripheral postsynaptic α_{2B}-, α_{2D}- and α_1-adrenoceptors results in vasoconstriction. Accordingly, slow i.v. infusion of fadolmidine in anaesthetised rats transiently increased MAP, followed by a sustained moderate hypotension suggesting slow penetration of fadolmidine over the BBB. The sustained and potent decrease in HR which is considered a compensatory effect to the initial vasoconstriction. This aligns with the understanding that HR is controlled centrally and influenced by reflex responses to the vasoactive tone, as outlined by Solomon et al. (1989). In comparison. dexmedetomidine did not exhibit an initial increase in BP but caused immediate hypotension. Dexmedetomidine crosses BBB easily and consequently induces rapid onset of the central sympatholytic decrease in BP, which overrides the direct vasoconstriction (Detweiler et al. 1993; Eisenach et al. 1994; Kallio et al. 1989). Fadolmidine is a potent full agonist at human α_{1A}- and α_{1B}-adrenoceptors raising questions about the physiological effects of α_1-adrenoceptor agonism in humans as α_1-adrenoceptors control the vasomotor tone of resistance blood vessels in the periphery (Guimarães & Moura 2001).

Fadolmidine was developed as i.t. anaesthetic with local mode of action. This hypothesis was tested in using experimental model assessing the BBB crossing capacity of fadolmidine. Pupillary response assessment is an established research method to study effects of α_2-adrenoceptor agonists at supraspinal level. Stimulation of the inhibitory presynaptic α2-adrenoceptors located on the Edinger–Westphal nucleus in the midbrain leads to a decrease in the parasympathetic input to the muscles that constrict the iris resulting in mydriasis (Heal et al. 1995; Yu & Koss

2005). Fadolmidine caused mydriasis only at high i.v. and s.c., doses compared to dexmedetomidine, suggesting limited penetrance of the BBB. This finding is further supported by the low sedative potency of s.c. administered fadolmidine in the spontaneous motility assay. In this research model sedation is mediated by activation of the α_2-adrenoceptors in the LC in the brainstem (De Sarro et al. 1987). Altogether, these in vivo studies suggest limited capacity of fadolmidine crossing the BBB and spread in the CNS after i.t. dosing consequently suggesting a local mode of action. This observation is also supported by several other studies in rats (Eisenach 1999; Leino et al. 2009; Onttonen et al. 2000; Talke et al. 2003; Xu et al. 2000).

Fadolmidine's binding affinity on the rat serotonin 5-HT_3 receptor observer in the receptor profiling study warranted further functional studies. The 5-HT_3 receptors are ligand-gated ion channels expressed in the periphery and in the CNS. They regulate the release of neurotransmitters such as serotonin, acetylcholine and NA and potentially affect pain perception and/or cardiovascular functions. In the patch clamp assay fadolmidine was a weak partial agonist in the human 5-HT_{3A} and 5-HT_{3AB} receptor subtypes. Also in the guinea pig ileum ex vivo preparations, fadolmidine demonstrated 5-HT_3 agonism by inducing ondansetron and tetrodotoxin-sensitive contractions. However, when compared to the binding affinity at the 5-HT_3 receptors, fadolmidine's functional potency was considerably lower. Whether this is due to species and/or tissue differences in the 5-HT_3 receptors reported by others (Barnes et al. 2009; Bonhaus et al. 1993; Boyd et al. 2002) remains somewhat unresolved. It is also possible that at high concentrations fadolmidine's α_2-agonistic effects dominate the physiological response despite the presence of atipamezole, consequently counteracting the effects on the 5-HT_3 receptors. The responsiveness of contractions to tetrodotoxin implies the release of neurotransmitters (like acetylcholine) or tachykinins (such as substance P), as suggested by studies conducted by Briejer & Schuurkes (1996), Ramirez et al. (1994), and Yamano & Miyata (1996). In vivo studies on 5-HT_3 receptors by using the Bezold–Jarisch model did not support the functional 5-HT_3 receptor agonism in rats. In this model fadolmidine induced MAP, followed by reflectory bradycardia. Both atipamezole and prazosin inhibited this effect, but a selective 5-HT_3 receptor antagonist, ondansetron, had no effects.

In summary, when administered peripherally, fadolmidine does not cause any noticeable 5-HT_3-mediated effects in rats but the functional effects observed are typical for α-adrenoceptor agonists. The modest functional agonism on the human 5-HT_3 receptors in the specific cell assays suggests that 5-HT_3 receptors play a minimal, if any, role in the pharmacological profile of fadolmidine in humans.

α_2-adrenoreceptor agonists have been reported to increase urine output, total osmolarity, and sodium and potassium excretion in rats and dogs (Miller et al. 2001; Sinclair 2003; Strandhoy 1985). The increase in urine output is thought to be caused

by CNS-mediated decrease in vasopressin secretion, whereas increase in sodium excretion in the kidney is considered a peripheral effect (Cabral et al. 1998). Our findings support these previous studies. The i.v. doses of fadolmidine had minor effects on kidney function; only the highest dose increased urine [Na^+] significantly. However, when given i.t. fadolmidine increased urine output and urine [Na^+] dose-dependently, suggesting the dominance of centrally mediated effects. Yang et al. (2022) reported that in mice dexmedetomidine decreased water intake and promoted urine excretion by decreasing the expression of arginine vasopressin in the PVN and in serum. Dexmedetomidine also decreased aldosterone, [Na^+] and [Cl^-] in serum, as well as plasma osmolality. However, it is noticeable that in clinical settings, such as during coronary artery bypass surgery, i.v. dexmedetomidine does not alter renal function and has no effect on urine osmolarity or [Na^+] or [K^+], but it increases urinary output (Leino et al. 2011). Due to its local mode of action, it can be assumed that the effects of fadolmidine on kidney function in clinical settings would be marginal. Moreover, the agonistic effect on α_2-adrenoceptors might be even beneficial for kidney function in clinical use. Preoperative use of clonidine has been reported to prevent renal function deterioration after cardiac surgery (Kulka et al. 1996). Accordingly, dexmedetomidine has been shown to have preventive effect on renal dysfunction during surgical procedures, such as in patients with jaundice (Wu et al. 2019) and those undergoing cardiac valve replacement surgery (Zhai et al. 2017).

In clinical settings the known α_2-adrenoceptor agonists dexmedetomidine, clonidine and morphine have shown to increase the time for first urination after surgery when used as an i.t. adjuvant (Davis & Kopacz 2005; Sudheesh et al. 2015; Tomaszewski et al. 2014; van Tuijl et al. 2008). Accordingly, in experimental rodent models of volume-evoked voiding cycles in conscious and anaesthetised mice and rats (Conte et al. 1991; Lehtimäki et al. 1996; Maggi et al. 1986; Mersdorf et al. 1993) dexmedetomidine and morphine has been shown to inhibit micturition (Aro et al. 2015; Durant & Yaksh 1988; Harada & Constantinou 1993; Horváth et al. 1994; Ishizuka et al. 1996; Streng et al. 2010). We compare effects of i.t. doses of fadolmidine on the volume-evoked micturition in anaesthetised male rats to those of dexmedetomidine, clonidine and morphine and observed that voiding cycles were disrupted dose-dependently by all tested compounds. However, at the expected analgesic dose, fadolmidine was only slightly or moderately inhibitory compared to dexmedetomidine and especially morphine. Ishizuka et al. (1996) proposed that dexmedetomidine's inhibitory impact on urodynamics involves a combination of peripheral and central mechanisms. In contrast, Aro et al. (2015) demonstrated that atipamezole almost completely reversed dexmedetomidine's suppression of the micturition reflex, while a peripheral antagonist, MK-467, had no effect. This observation indicates that dexmedetomidine primarily exerts its influence through

central mechanisms. Our results with i.t. doses of fadolmidine align with the notion that inhibition of the micturition cycle by α_2-adrenoceptor agonists is predominantly central. α_2-adrenoceptor agonists and morphine are widely used in clinical settings, and although they tend to cause urinary retention to some extent, this is not considered the major drawback in their use.

Despite its favourable pharmacological profile, fadolmidine is not currently in clinical use. However, multitude of studies by other research groups have documented antinociceptive efficacy of fadolmidine in diverse pain models in sheep, rats and dogs (Ansah & Pertovaara 2007; Eisenach et al. 1999; Leino et al. 2020; Leino et al. 2021; Onttonen et al. 2000; Onttonen & Pertovaara 2000; Pertovaara & Kalmari 2003; Pertovaara & Wei 2000; Xu et al. 2000; Wei & Pertovaara 2013). Moreover, our results show that at anticipated analgesic i.t. doses, fadolmidine is without significant adverse effects on sedation, MAP or HR and with only modest effects on kidney function and urodynamics – indicating a favourable adverse effect profile in clinical practise. Moreover, fadolmidine has been shown to reduce intraocular pressure in normotensive rabbits (Niemi et al. 2005; Savolainen et al. 2005). Systematic studies on other potential peripheral indications for fadolmidine have not been published.

6.2 Pharmacological characterisation of tasipimidine

Our studies show that tasipimidine is a full agonist on the human α_2-adrenoceptors with subtype selectivity for α_{2A}-adrenoceptor subtype. Tasipimidine demonstrated agonism also on the rat α_{2D}-adrenoceptors but with lower potency. These effects were validated in various functional ex vivo and in vivo models in rodents.

In comparison for the α_2-adrenoceptors, tasipimidine's effects on the human α_1-adrenoceptors were more complex; it unambiguously exhibited low binding affinity for the human α_{1A}-adrenoceptor but demonstrated variable functional effects in vitro. In the LNCaP cells expressing endogenously the human α_{1A}-adrenoceptors (Thebault et al. 2003) tasipidine had no agonistic effects. Accordingly, tasipimidine was only a weak agonist in the human recombinant cells when measured by the intracellular inositol monophosphate accumulation assay. Interestingly, tasipimidine exhibited a full agonistic response in when assessed using the intracellular [Ca^{2+}] assay. Tasipimidine also acted as a full agonist when tested in cells coexpressed the Gα15 protein and the human α_{1A}-adrenoceptors. These discrepancies might be related to sensitivity of various test setups. Liu et al. (2008) compared the inositol monophosphate accumulation and the intracellular [Ca^{2+}] assays and reported good correlation for antagonists, whereas the correlation was more variable for agonist compounds. Similarly, Zhu et al. (2008) observed that the intracellular [Ca2+] assay

displayed nearly 100 times more sensitivity for an α_{1A}-adrenoceptor agonist, phenylephrine, compared to the values reported with the inositol monophosphate assay. The multifunctional Gα15 protein connects various GPCRs to the PLC-β pathway, resulting in a substantial increase in phospholipase C activity for GPCRs naturally linked to Gi or Gs proteins, as explained by Offermanns & Simon (1995). Consequently, the presence of Gα15 might prompt a more intense association with intracellular calcium signalling, thereby amplifying α_{1A}-adrenoceptor signalling. The absence of any signals related to α1A-adrenoceptor agonism by tasipimidine in LNCaP cells could be due to the receptors' low expression levels, as suggested by Jensen et al. (2009) and Thebault et al. (2003).

To assess the specificity of fadolmidine, the in vitro diversity profile was performed comprising of 120 GPCRs, ion channels and enzymes. The results suggested a few potential off-targets, which were subsequently verified in functional pharmacological assays. Tasipimidine had minimal agonist activity on the human 5-HT_{1A} and 5-HT_{1D} receptors and had no functional effects on 5-HT_7 receptors, thus verifying the high specificity of tasipimidine for α_2-adrenoceptors. However, functional studies of tasipimidine were not conducted on the imidazoline receptors. The concept of imidazoline receptors remains a subject of debate within the biomedical community and their molecular identities are still elusive (Bousquet et al. 2020). Imidazoline receptors are not currently listed by the International Union of Basic and Clinical Pharmacology, but the imidazoline binding site is recognised. The peripheral benzodiazepine receptor is the 18 kDa translocator protein with five transmembrane domains and is mainly localised in the outer mitochondrial membrane. Recent research has highlighted alterations in the translocator protein and its ligands in various chronic pain models (Liu et al. 2022). In this regard it might provide an interesting insight on pharmacology for tasipimidine. Nevertheless, the binding affinity at the 10 µM concentration resulted in only 51% inhibition of specific binding, suggesting low affinity that did not warrant further functional studies.

Tasipimidine is developed for CNS indications in veterinary medicine and for humans and accordingly its effects on CNS were studied by assessing spontaneous locomotor activity in mice and acoustic startle reflex in rats in comparison to the known α_2-adrenoceptor agonist, dexmedetomidine. Tasipimidine decreased spontaneous locomotor activity and reduced startle reactivity dose-dependently, indicating effective penetration of the BBB in mice and rats after peripheral administration. However, when administered orally in the spontaneous motility assay tasipimidine was 100 times less potent compared to s.c. dosing indicating low oral bioavailability. In line with this, Lensen et al. (2021) reported oral bioavailability of 2.2% in rats, whereas in dogs the oral bioavailability reached 60%. Compared to tasipimidine, dexmedetomidine exhibited a faster onset of action and

greater potency in both above-mentioned in vivo assessments. This is in line with dexmedetomidine's higher affinity for and more potent agonism on the rodent α_{2D}-adrenoceptors, compared to tasipimidine.

In summary, our results show that tasipimidine is a specific, orally active α_2-adrenoceptor agonist with selectivity towards the α_{2A}-adrenoceptor subtype. These findings are further supported by the in vivo studies in rats and dogs by Lensen et al. (2021). According to their results, tasipimidine demonstrated typical α_2-adrenoceptor-mediated physiological effects: centrally mediated hypotension, peripheral postsynaptic vasoconstriction, bradycardia and reduction in rectal temperature. These findings suggest the predominant α_{2A}-adrenoceptor agonistic effects of tasipimidine in a physiological context. No other studies of basic pharmacology and pharmacokinetics of tasipimidine have been published. However, tasipimidine has been developed further as a veterinary medicine, and it has been approved by the European Medicines Agency for situational anxiety and fear indication in dogs. It has achieved marketing authorisation for this indication in the European Union. It is also currently under clinical evaluation for insomnia by the inventor, Orion Corporation. Considering the clean pharmacological profile of tasipimidine as an α_2-adrenoceptor agonist with modest selectivity for the α_{2A}-adrenoceptor subtype, it provides an interesting addition to clinically relevant α_2-adrenoceptor modulators.

6.3 Effects of dexmedetomidine, tasipimidine and fadolmidine on sexual incentive motivation and sexual behaviour in male rats

Three α_2-adrenoceptor agonists, dexmedetomidine, tasipimidine and fadolmidine, were studied for sexual incentive motivation and copulatory behaviour in rats to evaluate potentially beneficial effects of these drugs on the symptomatic treatment of premature ejaculation. We hypothesised that sympatholytic activity by the activation of CNS and/or peripherally expressed α_2-adrenoceptors could increase ejaculation latency without affecting sexual incentive motivation.

Dexmedetomidine and fadolmidine had no direct effects on sexual motivation, but fadolmidine clearly reduced ambulatory activity at the highest tested dose (100 µg/kg), indicating sedation. Although fadolmidine has limited penetrance across the BBB, it is known that penetration occurs at high peripheral doses (Lehtimäki et al. 2008), causing typical CNS-related α_2-adrenergic pharmacological effects. Tasipimidine decreased the preference score (100 µg/kg) and reduced the time spent in the female incentive zone (200 µg/kg). However, since the parameters were not affected simultaneously, it is unlikely that tasipimidine does alter

motivation in any functionally relevant way. At a dose of 200 µg/kg, tasipimidine decreased ambulatory activity, being indicative of mild sedation.

It is known that α_2-adrenoceptor agonists decrease sexual motivation. Viitamaa et al. (2006) reported modest reduction of sexual incentive motivation at a dose of 4 µg/kg of dexmedetomidine and mildly reduced locomotor activity at 8 µg/kg. Clark et al. (1985) showed that activation of α_2-adrenoceptors by clonidine decreases sexual motivation. Consequently, increased adrenergic activity by the inhibition of the α_2-adrenoceptors increases sexual arousal without stimulation of copulatory behaviour (Chu & Ågmo 2016; Clark et al. 1985). Although Chu and Ågmo (2016) did not find any subtype specific role of α_2-adrenoceptors by specific pharmacological tools, Shishkina et al. (2001) reported that inhibition of α_{2A}-adrenoceptor subtype expression in the LC by antisense oligonucleotides resulted in increased sexual motivation in male rats. Moreover, acute administration of mirtazapine stimulates sexual motivation in male rats by antagonist effects on the brain α_2-adrenoceptors (Benelli et al. 2003).

Dexmedetomidine statistically significantly prolonged the time to the first ejaculation, accompanied by a reduction in the number of ejaculations achieved during the test. No other parameters of copulatory behaviour or general activity changed.

Tasipimidine had only minimal effects on sexual behaviour when the whole study population was analysed. However, at the highest tested dose (200 µg/kg), there was a decrease in the number of mounts and intromissions during the test, possibly due to its motor-slowing effects. The post hoc analysis of data from the rats with short ejaculation latencies (falling below 75th percentile of the first postejaculatory interval) demonstrated that the interintromission interval in the first ejaculatory series was prolonged and ejaculation latency was enhanced at the dose of 200 µg/kg.

Fadolmidine increased the postejaculatory interval at the dose of 100 µg/kg, which most likely attributed to reduced general activity. In the rats with short ejaculation latency (below the 75th percentile of the first postejaculatory interval) fadolmidine increased the postejaculatory interval after first ejaculation and decreased the number of ejaculations throughout the test. In the very fast-ejaculating rats (below the 25th percentile of the first postejaculatory interval) the interintromission interval increased and the intromission ratio decreased in the second series. These effects on sexual behaviour were likely consequences of sedation, manifested as slowing of the pace of sexual interaction and reduced recovery of responsivity to the female.

Ejaculation is controlled by the lumbar spinothalamic neurons in the spinal cord, several supraspinal sites in the brain and the peripheral sympathetic and parasympathetic nerves and motoneurons (Truitt et al. 2003; Truitt & Coolen 2002;

Veening & Coolen 2014). Accordingly, α_2-adrenoceptor agonists inhibit sexual behaviour and ejaculation when administered directly into the brain (Clark et al. 1991; Clark & Smith 1990; Yonezawa et al. 1986, 1991, 2005) or the spinal cord (Carro-Juárez & Rodríguez-Manzo 2006). These effects can be antagonised by centrally acting α_2-adrenoceptor antagonists. Accordingly, Chu and Ågmo (2016) showed that vatinoxan, a peripheral α_2-adrenoceptor antagonist, had no effect on sexual behaviour in male rats. Altogether, these observations suggest the dominance of central α_2-adrenoceptors on the regulation of sexual behaviour over peripheral α_2-adrenoceptors. Accordingly, our results showed that at doses having no or little effect on the CNS, fadolmidine had no effects on any parameters of male rat sexual behaviour. This suggests that the effects of tasipimidine and dexmedetomidine are most likely related to actions within the CNS and that the peripheral mode of action for the α_2-adrenoceptor agonist is not adequate to increase the ejaculation latency, but the central mechanism is needed. Whether similar specificity of action can be obtained in men remains to be studied.

Erectile dysfunction is a common comorbidity with premature ejaculation (Cilio et al. 2022), and thus, any premature ejaculation treatments should not interfere with erectile function. It was noticed that occasionally high doses of tasipimidine and fadolmidine decreased the intromission ratio. This ratio measures the ease of achieving vaginal penetration and is dependent on contraction of the ischiocavernosus muscle (Sachs 1982) and increased intracavernous pressure (Giuliano et al. 1994), the latter possibly being affected by α_2-adrenoceptors. Studies on human cavernous tissue have shown that α_2-adrenoceptors are expressed on smooth muscle cells and that they may control intracavernous tone (Traish et al. 1997). Accordingly, Tejada et al. (1999) showed that α_2-adrenoceptor antagonists enhance nitric oxide release and facilitate erection, whereas clonidine, an α_2-adrenoceptor agonist, has been reported to suppress copulatory behaviour and erectile reflexes in male rats and dogs (Clark 1991; Clark & Smith 1990). However, Yonezeva et al. (1986, 1992, 2005) reported that intracerebroventricular and systemic doses of clonidine had quite a specific effect in delaying ejaculations but did not affect erection in dogs. Lin et al.'s (1988) studies suggest local effects of clonidine on penile vasoconstriction, resulting in suppression of erection either by direct α_2-adrenoceptor activation on the vascular smooth muscle or by inhibition of the vasodilator nerve. Our results with rats do not indicate any effects on the erectile function in rats by the test compounds at the concentrations used.

Yajima (1989) reported that postsynaptic α_1- and α_2-adrenoceptors are expressed in the rabbit corpus cavernosum. Studies on isolated rabbit corpus cavernosum suggest that a flaccid state is maintained mainly by alpha α_1-adrenoceptor activation. This is also true for humans; NA contracts both corpus cavernous and penile vessels via stimulation of α_1-adrenoceptors in men (Andersson 2011). Fadolmidine is only a

weak partial agonist on rat α_1-adrenoceptors; therefore, it was unlikely to have any major physiological effects in this study setup. However, fadolmidine is a full agonist on human α_{1A}-adrenoceptors, and therefore, potential effects on the cavernous tissue cannot be totally ruled out but should be further evaluated. Furthermore, fadolmidine might have direct contractile effects on the vas deferens, epidymis and prostate by the activation of α_1-adrenoceptors, which could potentially counteract the α_2-adrenoceptor agonistic effects on ejaculation function.

Rats are considered a relevant model for testing the effect of drugs on male ejaculation function since many drugs working on this indication in humans also do so in rat models. SSRIs (Chan et al. 2008; Waldinger 2007a,b, 2007b), sildenafil (Giulani et al. 2002; Ottani et al. 2002; Steidle et al. 2007) and tramadol (Olivier et al. 2016; Zhang et al. 2020) have been previously studied in rats. Especially short ejaculation latency in male rats is considered a valid model of premature ejaculation (Chan et al. 2008; Olayo-Lortia et al. 2014; Pattij et al. 2005). The post hoc analyses of the fast-ejaculating rats revealed that tasipimidine extended ejaculation in this subgroup, although it had no statistically significant effect on the whole study population. These results indicate that tasipimidine might have potential for symptomatic treatment of premature ejaculation. On the contrary, fadolmidine did not increase the ejaculation latency in either normally ejaculating rats or fast-ejaculating or very fast-ejaculating rats, even at the sedative doses.

One of the aims of the study was to determine whether there is a therapeutic window for the α_2-adrenoceptor agonists on premature ejaculation indication without considerable side effects. Sedation is a typical physiological response for the α_2-adrenoceptor agonists. This was observed at the high doses of tasipimidine and fadolmidine, whereas the doses of dexmedetomidine were below the sedative level. Thus, obviously, there was a safety window for dexmedetomidine. Whether there is enough safety margin for tasipimidine should be further studied. One can speculate that probably mild sedation is not a major challenge, especially when considering sympathetic overactivity as a cause of premature ejaculation. Fadolmidine was used in this study as a tool to evaluate CNS vs peripheral effects of α_2-adrenoceptor agonists on premature ejaculation. Fadolmidine is intended for spinal use and not peripheral dosing. Carro-Juárez and Rodríguez-Manzo (2006) demonstrated that α_2-adrenoceptor agonists inhibit ejaculations when dosed at the spinal spinal generator of ejaculation Accordingly, fadolmidine might work at the spinal level, but central dosing of therapeutics for premature ejaculation is not warranted.

7 Summary/Conclusions

In this study we assessed pharmacological profiles of two novel α_2-adrenoceptor agonists, fadolmidine and tasipimidine. Moreover, the effects of α_2-adrenoceptor agonists, dexmedetomidine, fadolmidine and tasipimidine, were evaluated on sexual incentive motivation and copulatory behaviour in male rats to test the hypothesis of beneficial symptomatic effects of the α_2-adrenoceptor stimulation on premature ejaculation.

Fadolmidine was a full agonist on all three human α_2-adrenoceptor subtypes and on the rodent presynaptic α_{2D}-adrenoceptors, with binding affinity and functional potency at nanomolar concentrations. It demonstrated full agonist efficacy also on the human α_{1A}- and α_{1B}-adrenoreceptors and on the rat α_1-adrenoceptor, even though with lower potency. Fadolmidine was also a full agonist in various in vivo models measuring cardiovascular parameters, sedation and BT in anaesthetised and conscious rats. Fadolmidine had limited capacity to cross the BBB, demonstrated by minute CNS-mediated effects (mydriasis and sedation) after the systemic dosing. Pharmacological profiling of fadolmidine, in the context of known α_2-adrenoceptor-related physiological effects, suggested a favourable safety profile in clinical use. Effects on urodynamic parameters and kidney function were mild at the expected therapeutic dose range. The binding affinity for the 5-HT_3 receptor did not translate to functional efficacy.

Tasipimidine exhibited ligand binding affinity and acted as full agonist at nanomolar concentrations on all human α_2-adrenoceptors, with sub-type selectivity towards α_{2A}-adrenoceptors over the human α_{2B}- and α_{2C} and the rodent α_{2D}-adrenoceptors. Tasipimidine displayed only low binding affinity on the human α_1-adrenoceptors and, accordingly, had no functional effects on the LNCaP cells endogenously expressing the human α_{1A}-adrenoceptors. Notably, agonist effect on the human α_{1A}-adrenoceptors was observed in the transfected cell models, but no functional effects were noted on the human α_{1B}-adrenoceptors.

Tasipimidine's receptor profile showed a high degree of specificity. In vivo experiments highlighted centrally mediated effects of tasipimidine, reduction in the acoustic startle reflex in rats following the s.c. doses and decreased spontaneous locomotor activity in mice with s.c. and higher oral doses. In conclusion,

tasipimidine is an orally active selective α_{2A}-adrenoceptor agonist. Tasipimidine is used for situational anxiety and fear in dogs in veterinary medicine and is under development for an insomnia indication in humans.

Premature ejaculation is the most common sexual disorder in men, with a prevalence of 20%–30% in all age groups and only limited medical treatment options available. The aetiology of lifelong premature ejaculation is not fully known, but the experimental animal models and clinical evidence suggest sympathetic overactivity as one potential cause for lifelong premature ejaculation. It is well known that α_2-adrenoceptors play a key role in regulating sympathetic tone in both the periphery and the CNS and consequently it was hypothesised that α_2-adrenoceptor activation might be beneficial for the symptomatic treatment of premature ejaculation. The hypothesis was tested by assessing effects of α_2-adrenoceptor agonists, dexmedetomidine, fadolmidine and tasipimidine, on sexual incentive motivation and copulatory behaviour in male rats.

Dexmedetomidine functioned according to the hypothesis and increased the ejaculation latency to the first ejaculation and had no effect on sexual incentive motivation or locomotor activity. Dexmedetomidine is widely used in human and veterinary medicine. It is a potent and efficacious compound, but in certain indications its use is limited due to poor oral bioavailability.

Unlike dexmedetomidine, tasipimidine has a good oral bioavailability consequently enabling oral dosing. Tasipimidine did not modulate the ejaculation latency when the whole study population was analysed but prolonged ejaculation latency and the interintromission interval in the subpopulation of the fast-ejaculating rats while having no significant effects on sexual incentive motivation.

Fadolmidine has limited capacity to cross the BBB, consequently having a local mode of action. In this study, peripheral dosing of fadolmidine was used to study whether the activation of the peripheral α_2-adrenoceptors might be sufficient to prolongate the ejaculation latency. However, fadolmidine had no systematic effect on copulatory behaviour, not even in fast-ejaculating and very fast-ejaculating males at nonsedative doses. Neither had fadolmidine any effects on sexual incentive motivation at any of the doses used. At the largest dose studied, fadolmidine was clearly sedative, demonstrating penetrance over the BBB and central effects.

In summary, our observations suggest that centrally acting α_2-adrenoceptor agonists might provide symptomatic relief for premature ejaculation by prolonging ejaculation latency without affecting any other parameter of sexual behaviour or sexual incentive motivation. Whether the peripheral mode of action provide additional value is still open. However, purely the peripheral mode of action for α_2-adrenoceptor agonists appears to be insufficient for this indication. The study procedures used are standard in the field and provides reliable data. Whether the

effects observed in male rats are directly transferrable to men can only be determined through clinical studies.

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