Fadolmidine – Favourable adverse effects profile for spinal analgesia suggested by *in vitro* and *in vivo* models

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ABSTRACT

Fadolmidine is an α_2-adrenoceptor full agonist developed for spinal analgesia with a local mode of action. The purpose of this study was to demonstrate the safety of fadolmidine on known α_2-adrenoceptor-related effects: kidney function, urodynamics and cardiovascular variables. Furthermore, the binding affinity of fadolmidine for the 5-HT_3 receptor prompted functional studies on 5-HT_3. According to the binding affinity data, fadolmidine demonstrated partial agonism on the 5-HT_3 receptor in transfected cells and in guinea pig ileum preparation. However, intravenous (IV) fadolmidine did not produce any 5-HT_3-related hemodynamic effects in anaesthetised rats. In urodynamic studies, intrathecal (IT) fadolmidine interrupted volume-evoked voiding cycles and induced overflow incontinence at high concentrations in anaesthetised rats; however, at the analgesic dose range, the effects were mild. The effects of fadolmidine on kidney function were studied in conscious rats after IV and IT dosing. While IT fadolmidine increased dose-dependent urine output, sodium ion concentration, IV doses increased only sodium ion concentrationThe effects of IT fadolmidine on heart rate (HR), mean arterial pressure (MAP) and sedation were evaluated in the home cage and in the open field using a telemetry system. In resting conditions, fadolmidine decreased HR dose-dependently and increased initial MAP, whereas in actively moving rats, there were no effects at analgesic doses. The results suggest that at anticipated analgesic clinical doses, IT fadolmidine provides analgesia without significant adverse effects on sedation, MAP or HR and with only modest effects on kidney function and urodynamics.

Keywords: 5-HT_3, α_2-Adrenoceptor, Agonist, Fadolmidine, Kidney function, Urodynamics

1. Introduction

The α_2-adrenoceptors are G_i-protein coupled receptors that mediate many central physiological responses such as arousal, hypotension, bradycardia and nociception. Accordingly α_2-adrenergic agonists clonidine and dexmedetomidine are used as sedatives and analgesics in humans (Malinovsky, 2003; Nguyen et al., 2017). However, at high intrathecal (IT) doses, these lipophilic compounds cause typical α_2-adrenoceptor-related adverse effects, which limit their use (Giovannoni et al., 2009). Opiates are commonly used for pain relief, but their potentially serious adverse effects and development of tolerance are considered a safety risk in chronic use (Baldini et al., 2012; Berger and Whistler, 2010). α_2-Adrenergic agonists potentiate opiate efficacy in anaesthesia and in acute and chronic pain management, thus potentially providing an opiate-sparing effect (Tonner, 2017).

Fadolmidine (3-(1H-Imidazol-4-ylmethyl)-indan-5-ol hydrochloride) is a potent α_2-adrenoceptor agonist developed as a spinal analgesic with a local mode of action (Lehtimäki et al., 2008). Earlier studies have shown fadolmidine to be analgesic in various animal models of inflammatory, postoperative and neuropathic pain (Leino et al., 2009; Pertovaara, 2004). This study assessed the potential adverse effects of fadolmidine in various pharmacological assays.

Adrenergic α_2-agonists induce a centrally mediated increase in urine output, by lowering the antidiuretic hormone and vasopressin secretion, and a peripherally mediated increase in sodium excretion in the kidney (Cabral et al., 1998; Sinclair, 2003). In humans, clonidine increases urine output and electrolyte excretion associated with a decrease in plasma renin activity (Vahabi and Kazemi, 2011). α_2-adrenergic agonists have also shown to interfere with the micturition reflex in conscious mice (Aro et al., 2015) and rats (Kontani et al., 2000) and in anesthetised male rats (Streng et al., 2010).

The binding profile of fadolmidine demonstrates a binding affinity for the human serotonin 5-HT_3 receptor (IC_{50} 6.3 nM) – a ligand gated ion channel expressed in the peripheral and central nervous systems which regulates the release of various neurotransmitters (Cortes-Altamirano et al., 2018; Hayashida et al., 2012; Ramage and Villalon, 2008) – thus warranting further functional studies.

The aim of this study was to demonstrate the safety of fadolmidine regarding the potential α_2-adrenoceptor-related side effects and 5-HT_3 receptor functions. The effects of fadolmidine on kidney function were studied in conscious rats after both IT and intravenous (IV) dosing. Furthermore, IT doses of fadolmidine were studied on micturition parameters in anaesthetised rats and compared to equipotent analgesic doses of dexmedetomidine, clonidine and morphine known to inhibit the micturition and possibly cause urine retention (Kim et al., 2013; Kleinmann and Wolter, 2017). The observed binding affinity of fadolmidine for the 5-HT_3 receptor warranted functional studies of fadolmidine using cell assays, guinea pig ileum preparations and Bezold-Jarisch reflex measurements on heart rate (HR) and mean arterial pressure (MAP). Additionally, the hemodynamic effects of fadolmidine were measured at analgesic IT doses in conscious animals using a minimally invasive telemetry transmitter system in the home cage and in the open field to examine the impact of physical activity, environmental stimuli and stress on HR and MAP.

2. Materials and Methods

2.1. Drugs

Fadolmidine and dexmedetomidine (α_2-adrenoceptor agonists) and atipamezole (α_2-adrenoceptot antagonist) were synthesised by Orion Corporation (Finland). Clonidine (α_2-adrenoceptor agonist), 2-methylserotonin (5-HT_3 agonist) and tetrodotoxin (sodium channel

blocker, neurotoxin) were purchased from Research Biochemicals International (USA); ondansetron (5-HT_3 antagonist, Zofran 2mg/ ml IV inject) was obtained from Glaxo UK Ltd. (England); and prazosin (α_1-adrenoceptor agonist) from Pfizer (USA). Acetylcholine (Ach receptor agonist) was purchased from Sigma (USA). Furosemide (Furesis 10 mg/ ml) was purchased from Orion Corporation (Finland) and morphine (opioid receptor agonist) from Leiras (Finland).

2.2. Animals

Experiments were performed with male Sprague-Dawley rats (B&K, Sweden) and male Dunklin-Hartley guinea pigs (B&K, Sweden) housed in groups of five and 10 animals, respectively, in a temperature controlled room (22 ± 2°C), at a relative humidity of 50 ± 10% and on a 12 h light/dark cycle (lights on at 06.00 a.m.) 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.

2.3. IT catheterisation

IT catheters were implanted for male rats under a midazolam (5 mg/kg, Dormicum® 5 mg/ml, Roche, Switzerland) and fentanyl (0.25 mg/kg)-fluanisone (8 mg/kg) (Hypnorm®, Janssen, UK) subcutaneous (SC) combination anaesthesia according to the method of Yaksh and Rudy (1976), with minor modifications. Briefly, the atlanto-occipital membrane was incised right below the skull and the polyethylene catheter (PE10, Intramedic, USA) filled with sterile saline was introduced 8 cm into the spinal cavity so that the catheter tip extended to the rostral

edge of the lumbar enlargement. The externalised end of the catheter was closed by melting. The location of the catheter tip was confirmed by giving 0.5 mg lidocaine (Lidocain pond® 50 mg/ml, Medipolar, Finland) IT on the 3rd day after catheterisation. Transient paralysis of both hind limbs was the indication of successful catheterisation. After the surgery, the animals were housed individually in standard polypropylene cages and were given a recovery period of at least one week. Animals with visually observed normal neurological function were selected for the experiment.

2.4. *In vitro* patch clamp measurements for serotonin 5-HT_3 activity

Stably transfected 5-HT_{3A} and 5-HT_{3AB} cell lines were created in Molecular Biology of Orion Pharma at Viikki campus. Cells were kept for 1-3 days in six well plates in an incubator at 37°C in a 5% CO_2 and 95% O_2 atmosphere in Dulbeccos modified Eagle's medium with 10% foetal calf serum (heat inactivated), 10 IU penicillin, 10 µg streptomycin, 10 mM HEPES, and 0.2 mg/ml geneticin. For the 5-HT_{3AB} cells, the medium also contained 0.2 mg/ml hygromycin. The medium was changed after 2-3 days and, about once a week, the cells were replaced using 0.25% trypsin solution to remove adhered cells. After inoculation, the cells that adhered to a glass coverslip at the bottom of the well were used for the experiments.

All experiments were performed at room temperature with a tight-seal, whole cell voltage-clamp configuration, using the patch clamp technique (Hamill et al., 1981) and an Axopatch 200B amplifier (Axon Instruments, USA). The coverslip with the adhered cells was placed on an extracellular solution consisting of (in mM) 150 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 2.5 glucose, 10 HEPES (pH 7.4 adjusted with NaOH); the osmolarity was adjusted to 305 mOsm using the Osmostat OM-6020 osmometer (DIC Kyoto Daiihi Kagagu Co. Ltd, Japan). The chamber was perfused with an extracellular solution at 2 ml/min. All drugs were dissolved in the extracellular solution and were locally applied with RSC-200 rapid solution changer (Bio-

Logic, France). The intracellular solution consisted of (in mM) 120 KCl, 5 BABTA (tetrapotassium salt), 0.5 $CaCl_2$, 1 $MgCl_2$, 2 adenosine triphosphate (disodium salt) and 10 HEPES (pH 7.2 adjusted with KOH) and the osmolarity was adjusted to 290 mOsm. The calculated free Ca^{2+} concentration was 23 nM (with BAD4 program). Pipettes were pulled with a P-2000 puller (Sutter Instruments, Co. USA) from borosilicate glass (Clark Electromedical, England) and had a resistance of 1-1.5 MOhm. In all cells, the cell capacitance was compensated by the circuitry in the amplifier. No series resistance compensation was used. In all recordings, the cell voltage was clamped to -70 mV except during voltage ramps. All recordings were digitised using a Digidata 1200 interface (Axon Instruments, USA) and were sampled at 2 kHz, recorded with Clampex 8.0 and analysed using Clampfit 8.0 software (Axon Instruments, USA). The dose response results were fitted using the free Hill equation in SigmaPlot 4.01.

Endogenous 5-HT_3 agonist serotonin was used as a reference compound. The test items were perfused for 20 s with increasing concentrations and a 90-100 s washout in between. When studying the agonist effects of fadolmidine, 3 µM serotonin (for the 5-HT_{3A} cells) or 6 µM serotonin (for the 5-HT_{3AB} cells) was first applied for 20 s. After a washout of 90-100 s, increasing concentrations of fadolmidine were applied.

2.5. 5-HT_3 activity measurements in guinea pig ileum ex vivo preparation

2.5.1. Equipment

Studies were performed using a four-position Schuler organ bath with a tissue chamber volume of 10 ml (Hugo Sachs Elektronik, Germany). The temperature of the chambers was kept constant (37˚C) using a circulated water bath (Hetofrig, Heto, Denmark). The isometric contractions of the preparations were measured using a Grass force-displacement transducer,

model FT03 (Grass Instruments, Quincy, Mass., USA), connected to a Grass D.C. Low-Level Pre-Amplifier and a Grass driver amplifier type 7DAG. The amplified signals were plotted onto paper with a Grass ink writer oscillograph, type 7WU 16F.

2.5.2. Preparation of ileal segments

The animals were killed by CO_2-suffocation. Segments of the ileum valve approximately 20 cm in length were used for electrical and chemical stimulation. The ileal segments were divided into 8-12 preparations, each 2 cm long, on a petri dish containing the physiological solution with the following composition (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. Four ileal segments were studied in parallel and the remaining preparations were kept waiting in a carbogen aerated physiological solution at room temperature. The preparations were allowed to stabilise under the resting tension of 1.0 g for 30 min. During the stabilisation period, the ileal segments were washed three times by overflow.

2.5.3. Chemical stimulation

To determine the individual maximal contractile capability of the segments, two boluses of 1 μM acetylcholine were given with six min intervals separated by washes. The test substances were given in a non-cumulative fashion separated by washes with 15 min intervals using half logarithmic increments and two min contact time. Atipamezole and prazosin were routinely added into the incubation medium 16-17 min before the agonist to prevent possible effects on presynaptic α_2-adrenergic and postsynaptic α_1-adrenergic receptors, respectively. In some studies, ondansetron was added 15 min before the test substance to prevent the effects on 5-HT_3. Tetrodotoxin, a sodium channel blocker, was used to evaluate the characteristics of

agonist-mediated contractions. Isometric contractions of the ileal segments were measured as a response to 5-HT_3 agonists.

2.6. Bezold-Jarisch reflex measurements in rats

2.6.1. Equipment

Pressure transducers (type MP-15, Micron Instruments, USA) connected to two channel bridge amplifier (type 301, Hugo Sachs Elektronik, Germany) were used to measure MAP. Voltage signals were delivered from the amplifiers through A/D-converter to the computer-based data acquisition system MP100WS (Biopac Systems Inc., USA) and saved for further analyses.

2.6.2. Experimental procedures

The rats were anaesthetised by an IV injection of 1.25 mg/kg of sodium barbital (Mebunat 60 mg/ml, Lääkefarmos, Orion Corporation, Finland) and fixed in a supine position on a thermostat-regulated, water-filled heating block (37˚C). The left femoral artery was cannulated with a polyethylene tube and connected to a pressure transducer to measure the MAP. The right femoral vein was cannulated for the IV dosing. Three boluses of 0.2 ml were given at five min intervals. First, saline was injected to evaluate possible volume effects on the HR and/or MAP. A second injection was given to introduce the antagonist of the 5-HT_3 receptor (ondansetron), α_1-adrenoceptors (prazosin), α_2-adrenoceptors (atipamezole) or both α-adrenoceptor antagonists. When no antagonist was used, saline was injected to compensate for the possible volume effect of the antagonist. In the third injection, a bolus of either fadolmidine, 2-Methyl-5-HT (5-HT_3 agonist) or dexmedetomidine was given. MAP and HR were continuously monitored and registered electronically.

2.7. Kidney function measurements

2.7.1. Animals

This study used IV dosing for 70 male rats weighing 209-274 g and IT dosing for 48 rats weighing 250-300 g. The rats were randomised in six weight-matched groups of 12 (for IV dosing) or eight (for IT dosing) animals a day before the experiment. Before the experiment, the rats were fasted for approximately 15 h, with tap water given *ad libitum*.

2.7.2. Experimental procedures

The rats received tap water (30 ml/kg) perorally and were treated immediately either IV (1 ml /kg b.wt.) or IT (10 µl/ rat) with fadolmidine or saline. Furosemide (Furesis, Orion Corporation, Finland) was given SC (2.0 mg/ kg). The treated rats were placed in metabolic cages (Tecniplast, Cod.170022, Italy) either two animals (IV dosing) or one animal (IT dosing) per cage, for urine collection. During the collection phase, the rats were deprived of food and water. After six h, urine output was measured with 0.1 ml accuracy and the rats were killed. The urine was centrifuged (Jouan Plasma 1000, France) at 600 g for seven min followed by determination of urine osmolarity using Osmostat OM-6020 osmometer (Kyoto, Japan). The urine samples were stored in a freezer (-20 °C) in closed falcon tubes and sent in dry ice for the analysis of Na+ and K+ concentrations (Yhtyneet laboratoriot Oy, Finland).

2.8. Urodynamic measurements

The rats were cannulated for IT dosing. Urodynamic parameters were measured within one week after the lidocaine test. The rat was anaesthetised with urethane 1.0-1.4 g/kg intraperitoneally and fixed in a supine position on a thermostat-regulated, water-filled heating block (37˚C). The abdomen was opened, the urinary bladder was exposed and a 22G IV cannula was introduced into the bladder dome. Physiological, room temperature saline was infused continuously through the cannula to the bladder dome at a constant speed of 0.1 ml/min using a perfusion pump (Perfusor ED 2, B. Braun melsungen AG, Germany). A pressure transducer (type MP-15, Micron Instruments, USA) was connected through a T-tube to the cannula to measure the intravesical pressure. To measure the voiding volume, a plastic tube (length 110 mm, inner diameter 1.0 mm) was connected to the penile urethra to direct the urine into a beaker connected to a force transducer (type K30 Hugo Sachs Elektronik, Germany) which was used as a scale. Changes in intravesical pressure and the voiding volume of the volume-evoked micturition were monitored simultaneously. Pressure and volume signals were amplified using a two channel bridge amplifier (type 301, Hugo Sachs Elektronik, Germany), delivered through an A/D-converter to a computer-based data acquisition system MP100WS (Biopac Systems Inc., USA) and saved for further analyses. As soon as the volume-evoked micturition cycles were stabilised and at least three reproducible cycles were detected, the test compound was introduced through the IT cannula at a volume of 10 µl. Rats incapable of performing reproducible voiding cycles were not used. The test compounds were tested in separate experiments, but fadolmidine and saline groups were included in all studies for comparisons.

2.9. Hemodynamic measurements in *in vivo* open field and home cage tests

2.9.1. Transmitter implantation

Male rats weighing 300-500 g (8-17 weeks old) were habituated to an open field test system for two weeks' training twice (3 min/one training session) a week before (altogether four times) telemetry implantation. The rats were anaesthetised by SC injection with a combination of midazolam (5 mg/kg, Dormicum® 5 mg/ml, Roche, Switzerland) and fentanyl (0.25 mg/kg)-fluanisone (8 mg/kg) (Hypnorm®, Janssen, UK) and operated according to instructions provided by Data Sciences. Briefly, a midline abdominal incision was made and the descending aorta was carefully isolated. While the aorta was clamped, the catheter tip of the telemetry transmitter was inserted upwards just above the iliac bifurcation. The cannula was then fixed with cyanoacrylate adhesive glue and a 5 x 5 mm cellulose fibre patch. The body of the telemetry transmitter was inserted in the peritoneal cavity and sutured to the inside of the muscle wall. The skin incision was closed using non-absorbable sutures. After the surgery, the rats were housed individually in standard polypropylene cages and allowed to recover for at least one week before IT catheterisation.

2.9.2. MAP and body temperature (BT) measurements by telemetry system

HR, MAP and BT were recorded and analysed using a Dataquest IV system (Data Sciences, St. Paul, MN) in conscious freely moving rats. The telemetry system consists of BP and BT monitoring transmitters (model TL11M2-C50-PTX), receivers (model RLA2000) that telemetered data from the implant, a consolidation matrix (BCM100) that powers and multiplexes the data, and a computer with the Dataquest LabPRO™ software that collects, displays and stores the data.

2.9.3. Motor activity measurement by open field system

Exploratory activity was investigated in the open field test using an open arena with walls. The square open field apparatus was made from non-reflecting black plastic and consisted of

an arena (70 x 70 cm) surrounded by walls (38 cm high). Ambulation of an animal was monitored by a video camera (mounted 220 cm above the arena) linked to a computer through an image analyser (Poly-Track video tracking system, San Diego Instruments, USA). The arena was divided into nine equal squares (23 x 23 cm) by the computer software (Chromotrack, Prototype Systems Ltd. USA) and the amount of ambulation was counted manually as the number of squares visited. At the beginning of the measurement, the rat was placed at the centre of the open field. The trial was started by the experimenter and ended by the computer. All behavioural experiments were performed in a calm and noiseless room. Two days before testing, eight rats (fadolmidine group n = 4 and clonidine group n = 4) were habituated for handling (immobilisator) and to open field system (1 x 3 min) twice daily.

On the morning of each testing day, the animals were moved to individual solid bottom polypropylene cages with stainless steel mesh lids (subsequently termed ‘home cage’) and transferred to the experimental room. The animals were allowed to habituate to the surroundings at least for one h before the start of the experiment. The rat was given the test compound or sterile water as a control. The test compounds were dissolved in sterile water (Aquasteril®, Orion Corporation, Finland). Drugs were administered IT using a Hamilton syringe in a volume of 10 µl. The injection of each drug was followed by an additional sterile water injection of 15 µl to flush the drug left in the catheter lumen. The following drug doses were used: fadolmidine: 0 (control), 1, 3, 10 and 30 µg/rat; and clonidine: 0 (control), 10, 30 and 100 µg/rat. The animals received all drug doses (cross-over, once a day) and randomisation was performed by choosing the treatment dose order randomly. The washout period between the different doses was 24 h in the fadolmidine group and at least 48 h in the clonidine group. Immediately after administration, the rat was placed back into the home cage. BP and BT recordings at the respective sampling rates of 500 Hz and 250 Hz were taken every five min for 10 s during the whole study synchronously with the motor activity measurements. The value 15 min after the injection was taken as the value for home cage 1

(Fig. 1). Then, at the time point 19 min (open field 1) and again at 59 min (open field 2) after the injection, the rat was placed gently in the centre of the arena of the open-field apparatus and allowed to explore freely. Spontaneous motor activity was measured for 2 x 3 min and telemetry measurements at time points 20 and 25 min and 60 and 65 min after the injection were taken as the values for open field 1 and open field 2, respectively. After taking the measurements, the rat was returned to the home cage. The telemetry values at 55 min and 95 min after the injection were taken as the value for home cages 2 and 3, respectively.

2.10. Statistical analysis

For the Bezold-Jarisch measurements the data is presented as mean ± S.E.M. and for the statistical analysis, one-way ANOVA was used followed by the t-test by using the GraphPad Prism 8 software (GraphPad Software, USA).
P-values <0.05 *, <0.01 ** and <0.001 ***.

In the kidney function studies the results are presented as mean ± S.E.M. Urine output was measured and concentrations of sodium and potassium ions in the urine were determined and the amounts of excreted ions were calculated as mmol/kg b.wt. The osmolarity of the urine was measured and expressed as mOsm/kg H2O. The statistical analyses were done using the one-way analysis of variance (ANOVA), followed by the Bonferroni corrected (by the number of comparisons made) t-test as a post hoc test, when appropriate. The Bonferroni P-value < 0.01 (five comparisons) was considered statistically significant. Statistical analysis was done using Statview software (Abacus Concepts Inc., Canada).

The open field HR, MAP and BT results are expressed as an average of 2 x 10 s values and the open field motility results as a sum of 2 x 3 min measurement values. The results are presented as mean ± S.E.M. The data were analysed using repeated measures analysis of

variance (ANOVA) for multiple comparisons, and the significance of the differences between doses was evaluated using Tukey's post hoc test. *P*-values < 0.05 were considered statistically significant (SPSS 14.0 and 15.0 for Windows, SPSS, USA).

3. Results

3.1. Effects of 5-HT_3 receptors in the cell assays

The binding profile of fadolmidine (data in file, Orion Corporation) demonstrates a binding affinity for the human serotonin 5-HT_3 receptor with an IC_{50} value of 6.3 nM against ^{3}H-BRL 43696 (0.4 nM) in the rat brain cortex preparation (Study report 1188/ORI/97, Neurotech S.A., Geneva, Switzerland), a ligand gated ion channel expressed in the peripheral and central nervous systems. The 5-HT_3 receptor regulates the release of various neurotransmitters such as serotonin, acetylcholine and noradrenaline, thus acting as a neuromodulator in various tissues and organs. The potential effects of the 5-HT_3 receptors on nociception and/or cardiovascular effects warrant further functional studies *in vitro* and *in vivo*.

The agonist effects of serotonin on 5-HT_3 receptors were measured in two HEK-293 cell lines expressing either recombinant 5-HT_{3A} or 5-HT_{3AB} receptors. In both cell lines, an endogenous 5-HT_3 agonist serotonin evoked a rapidly desensitising inward current of 2-20 nA resulting in an EC50 value of 2.8 μM and a Hill coefficient of 2.1 in the 5-HT_{3A} cells and an EC50 value of 5.8 μM and a Hill coefficient of 1.4 in the 5-HT_{3AB} cells. Desensitisation was markedly faster in the 5-HT_{3AB} cells compared to the 5-HT_{3A} cells. Similarly to serotonin, fadolmidine evoked desensitising inward currents but with only a partial agonistic response. In the 5-HT_{3A} cells, fadolmidine had an EC50 value of 120 nM and an intrinsic activity of 0.09 (Hill coefficient 2.0) and in the 5-HT_{3AB} cells, an EC50 value of 2010 nM and an intrinsic activity 0.22 (Hill coefficient 0.92) was observed.

3.2. Functional studies of 5-HT_3 effects on the guinea pig ileum, *ex vivo* preparation

In the guinea pig ileum preparations, fadolmidine induced dose-dependent ondansetron sensitive contractions in the presence of 1 μM atipamezole and 1 μM prazosin (Fig. 2) resulting in a pD2 value of 6.50 and an IA value of 0.36 compared to 10 μM acetylcholine. Contractile effects were profoundly diminished when atipamezole was omitted from the incubation medium. Contractile effects of fadolmidine were totally blocked by 1 μM tetrodotoxin.

3.3. Bezold-Jarisch measurements in anaesthetised rats

The 5-HT_3 agonistic effects of fadolmidine were further studied in anesthetised rats using the Bezold-Jarisch reflex model that measures the 5-HT_3 receptors mediated effects of on the MAP and HR. A selective 5-HT_3 receptor agonist, 2-methyl-serotonin (30 μg/rat IV), induced transient but profound bradycardia and decreased MAP concomitantly. These effects were reversed by a specific 5-HT_3 antagonist, ondansetron (30 μg/rat IV), but not with an α_2-adrenoceptor antagonist, atipamezole (600 μg/rat IV). Saline, used as vehicle, had no effects on either HR or MAP.

Fadolmidine (3.0 μg/rat IV) induced a transient increase in MAP followed by reflectory bradycardia. These effects were partly inhibited by atipamezole (600 μg/rat IV) and prazosin (30 μg/rat IV) and almost abolished when both compounds were co-administered (Figure 3A and B). The specific 5-HT_3 receptor antagonist, ondansetron (30 μg/rat), was devoid of any effects on the MAP or HR and it did not change the effects of fadolmidine on the MAP or HR

(Fig. 3 A and B). IV dose of dexmedetomidine (3.0 μg/rat) increased the MAP followed by a decrease in HR. Atipamezole antagonised these effects whereas ondansetron or prazosin were without any effects. Atipamezole induced a transient increase in MAP but of a lower magnitude compared to fadolmidine. Prazosin (30 μg/rat) decreased the MAP and HR.

3.4. Kidney function

Adrenergic α_2-agonists are known to induce a centrally mediated increase in urine output, by lowering the antidiuretic hormone and vasopressin secretion, and a peripherally mediated increase in sodium excretion in the kidney. In humans, clonidine increases urine output and electrolyte excretion is associated with a decrease in plasma renin activity. This information warranted further studies of fadolmidine effects on kidney function to evaluate the potential risk in analgesic use.

Acute effects of IV and IT doses of fadolmidine on urine output, osmolarity and sodium and potassium concentration were studied in water-loaded male rats. Furosemide was included as a positive control agent.

IV doses of fadolmidine slightly increased urine output, sodium ion concentration (Fig. 4 A and B). The urine osmolarity and potassium ion concentrations were not affected.

IT doses of fadolmidine increased urine output dose-dependently; the highest dose (30 μg/rat) having a statistically significant effect. The concentration of sodium ions was considerably and dose-dependently increased, resulting in statistically significant increase at doses of 3, 10 and 30 μg/ rat. The urine osmolarity and potassium ion concentrations were below the control values at all doses but the difference was not statistically insignificant.

The positive control substance furosemide (20 mg/kg SC) increased all the measured parameters statistically significantly when compared to saline used as a control (Fig. 4 A and B).

3.5. Urodynamic studies in anaesthetised rats

Adrenergic α_2-agonists have shown to interfere with the micturition reflex in studies of anesthetised and conscious mice and rats. It is also known that in clinical use, morphine inhibits the micturition reflex and can cause urine retention. Thus, it was considered relevant to evaluate the potential risk of urine retention for IT doses of fadolmidine in anaesthetised rats and compare it to equipotent analgesic doses of the following clinically used analgesics: dexmedetomidine, clonidine and morphine.

In anesthetised male rats the continuous infusion of saline induced phasic, volume-evoked voiding cycles (Fig. 5). At increasing doses, all tested compounds appeared to disturb the voiding cycles resulting in an improper voiding function, starting with minor changes in synchronisation of the micturition cycles and leading to the total blockage of the micturition cycles and overflow incontinence at higher doses. To compare the effects of different compounds, the inhibitory effects were divided into six categories according to the severity (Fig. 6). Table 1 summarises the results of all the separate studies of various drug treatments on the voiding function.

3.6. Open field test

α_2-Adrenergic agonists may evoke hypotension and bradycardia, thus limiting their therapeutic use. The effects of fadolmidine and α_2-adrenoceptor agonist clonidine were evaluated in conscious rats as a comparison, following IT administration on the spontaneous

locomotor activity (sedation), HR, MAP and BT. Exploratory activity was investigated in the open field and in the home cage setup by measuring the motor activity and HR, MAP and BT. Figure 7 illustrates the time course of the HR measurements. Table 2 presents the effects of fadolmidine and clonidine on spontaneous motor activity in the open field and simultaneously on cardiovascular responses in conscious, resting and moving rats. In the home cage, IT fadolmidine and clonidine induced a dose-dependent decrease in HR and an increase in initial MAP. A slight decrease in MAP was seen at a low dose of clonidine (10 µg) and after fadolmidine at a dose of 10 µg in the home cage 3 period. HRs did not return to the baseline level at 90 min (in home cage 3) after administration of the highest doses of fadolmidine and clonidine. In the open field, the animals explored their surroundings and this was reflected as an increase in their HR and MAP. In the control groups, the motor activity was lower during the second open field period than during the first one. Fadolmidine decreased the motor activity (a sedative effect) at a dose of 30 µg in both motor activity measurement periods (in open fields 1 and 2). Clonidine decreased the motor activity at all tested doses in the open field 1 period and at a dose of 100 µg in the open field 2 period. BT decreased dose-dependently after fadolmidine and clonidine administration during the series of experiments compared to the control.

4. Discussion

The binding affinity of fadolmidine on human 5-HT_3 receptors prompted additional functional *in vitro* and *in vivo* studies. In cell assays, fadolmidine functioned as a partial agonist in both human 5-HT_{3A} and 5-HT_{3AB} channels. Accordingly, in the guinea pig ileum preparations, fadolmidine induced ondansetron and tetrodotoxin sensitive contractions which were markedly enhanced in the presence of selective α_2-adrenoreceptor antagonist atipamezole. However, compared to the binding affinity for the 5-HT_3 receptor, the functional potency was

much lower. Whether this is due to the hydrophilic properties of fadolmidine or to species and tissue differences in the 5-HT_3 receptors as suggested by Bonhaus et al. (1993) Zifa and Fillion (1992) needs to be studied. It is possible that at high concentrations fadolmidine provokes α_2-adrenergic responses despite the presence of atipamezole. A blockade of the contractile effects by tetrodotoxin indicates a neuronally mediated response by the release of neurotransmitters like acetylcholine and tachykinins, such as substance P (Briejer and Schuurkes, 1996; Ramirez et al., 1994; Yamano and Miyata, 1996).

In anaesthetised rats, IV doses of fadolmidine induced an atipamezole and prazosin sensitive increase in MAP followed by reflectory bradycardia mirroring *in vitro* binding and functional responses of fadolmidine via α_2- and α_1-adrenergic receptors, respectively. Ondansetron had no effects on the hemodynamic effects of fadolmidine. The observed functional effects of fadolmidine on the measured hemodynamic parameters are typical for α_2-adrenoceptor agonists as demonstrated by the experiments with dexmedetomidine in this and previous studies (Lehtimäki et al., 2008). However, the *in vivo* effects of fadolmidine on the 5-HT_3 receptors might be masked by the opposite effects on α_2-adrenoceptor agonists. Alternatively, the dose might be too low to induce any effects on the 5-HT_3 receptors since fadolmidine had no bradycardic effects even when atipamezole and prazosin were introduced to inhibit the effects on α_2- and α_1-adrenoceptors. Additionally, species and tissue variation may have a role in the binding affinity of various ligands on the 5-HT_3 receptors (Bonhaus et al., 1993; Zifa and Fillion, 1992).

The effects of fadolmidine on kidney function in conscious freely moving rats were studied after IT and IV dosing. The effects of the IV doses were marginal and only the highest dose achieved statistical significance in the increase of Na^+ ion concentrations. In comparison, IT doses of fadolmidine induced a dose-dependent and statistically significant increase in Na+ ion concentration and excretion. Altogether, these results suggest the central mode of action.

The α_2-adrenoreceptor agonists are known to increase urine output and total osmolarity as well as sodium and potassium ion excretion in rats and dogs (Miller et al., 2001; Sinclair, 2003). It is also reported that α_2-adrenergic agonists induce a centrally mediated increase in urine output, caused by lowering vasopressin secretion, and a peripherally mediated increase in sodium excretion in the kidney (Cabral et al., 1998). However, the results of this study suggest that the increase in sodium excretion is at least partially mediated by the central modulation of α_2- adrenergic agonists since IT doses of fadolmidine were more potent compared to IV doses.

In anaesthetised male rats and mice, the infusion of saline into the bladder dome induced the phasic volume-evoked voiding cycles described in earlier studies (Conte et al., 1991; Lehtimäki et al., 1996; Maggi et al., 1986; Mersdorf et al., 1993). This cyclicity was sensitive for all tested compounds: fadolmidine, dexmedetomidine, clonidine and morphine. At the analgesic dose range, fadolmidine and clonidine appeared to be only slightly or moderately inhibitory, whereas the effects of dexmedetomidine were more profound. Morphine inhibited the micturition cycles completely at the dose of just 1 μg. It is previously reported that morphine and α_2-adrenoreceptor agonist dexmedetomidine inhibits volume-evoked micturition cycles in conscious and urethane anaesthetised male rats (Aro et al., 2010; Durant and Yaksh, 1988; Harada and Constantinou, 1993; Ishizuka et al., 1996; Streng et al., 2010). Ishizuka et al. (1996) speculated that the inhibitory effects of dexmedetomidine are mediated partly by a peripheral and partly by a central mechanism. However, Aro et al. (2015) suggested that this inhibition of the micturition reflex is due to the central mode of action by demonstrating that α_2-adrenergic antagonist atipamezole reversed almost totally the effects of dexmedetomidine, whereas treatment with a peripheral antagonist, MK-467, showed no inhibition of dexmedetomidine on voiding functions. The results of the fadolmidine support the hypothesis of the central mode of action.

It is known that anaesthetics relaxing the striated muscles or blocking the impulse trafficking in the nervous systems that control the activity of the lower urinary tract are not suitable for cystometric studies since they block the micturition cycles (Maggi and Meli, 1986; Mersdorf et al., 1993). In this study, the ability of the rats to produce reproducible voiding cycles was considered an indication of a steady state anaesthesia suitable for urodynamic evaluation. A more precise evaluation of the depth of the anaesthesia could improve standardisation of the test system. Alternatively, *in vivo* studies could be performed by using telemetric instrumentation in conscious animals.

The effects of α_2-adrenoceptor agonists, fadolmidine and clonidine on the spontaneous locomotor activity (sedation), HR, MAP and BT were evaluated at analgesic IT doses in conscious rats using telemetry monitoring in an open field test system.

To evaluate the effects on spontaneous motor activity, MAP and HR were measured at the analgesic and sedative doses. During the experiment (120 min), the dose range of 1-30 µg IT of fadolmidine and 10-100 µg IT of clonidine (Post et al., 1987; Solomon et al., 1989) were selected. Antinociceptive ED_{50} dose range of IT fadolmidine in the rat tail-flick tests was reported to be from 0.73 to 1 µg (Leino et al., 2009; Pertovaara and Wei 2000; Xu et al., 2000) and the duration of antinociception at the dose of 2.5 µg was up to 1.5 h (Xu et al., 2000). After IT doses of clonidine, the corresponding values were from 0.26 to 6.4 µg (Leino et al., 2009; Nishiyama and Hanaoka, 2001; Ouyang et al., 2012) and the duration at the dose of 3 µg was up to five h (Nishiyama and Hanaoka, 2001). In addition, effects on sedation (a motor activity) were reported after IT doses of fadolmidine and clonidine at doses of 30 µg (Leino et al., 2009) and 10 µg (Hao et al., 1996; Lee and Yaksh, 1995; Leino et al., 2009), respectively. Furthermore, IV doses of fadolmidine increased BP dose-dependently and inhibited electrically induced tachycardia by peripheral sympathetic mechanism in pithed rats (Lehtimäki et al., 2008). In anaesthetised rats, IT doses of fadolmidine first increased and then

decreased BP, and simultaneously decreased HR (Leino et al., 2009) presumably by activating the α_2-adrenergic receptors in the blood vessels (vasoconstriction) and in the brain, thus reducing the sympathetic outflow from the central nervous system (Nguyen et al., 2017).

In the stressless home cage conditions, where the locomotor activity of the animals was low, both IT fadolmidine and clonidine decreased HR dose-dependently. The bradycardic effect of clonidine is due to the direct inhibition of the central sympathetic tonus and increased vagal tone (Kawamata et al., 2003; Nguyen et al., 2017). In addition, in this study, clonidine evoked a well-known biphasic effect on the MAP; central α_2-adrenoceptors mediated a depressor effect at low to moderate doses and vascular α-adrenoceptors mediated a pressor response at higher doses (Horvath et al., 2002; Solomon et al., 1989). Clonidine as a lipophilic compound crosses the blood-brain barrier and is rapidly absorbed systemically from the spinal space (Eisenach et al., 1996; Kawamata et al., 2003; Post et al., 1987; Solomon et al., 1989). In comparison, the main effect of fadolmidine on MAP was an initial increase at higher doses. Together with the earlier findings (Lehtimäki et al., 2008; Leino et al., 2009), these data suggest that fadolmidine does not cross the blood-brain barrier easily but instead redistributes to some extent from the injection site to the periphery. However, a decrease in BT and delayed hypotension indicate that fadolmidine induces central nervous system effects (Eisenach et al., 1999; Leino et al., 2009). Further studies are needed to verify the concentration of fadolmidine in the brain and plasma after IT fadolmidine administration.

In the open field conditions in a new environment, the animals showed high motor activity by exploring the surroundings, which was reflected as an increase in HR and MAP. Furthermore, during the first open field period, the animals were more active than in the second period, reflecting their habituation in the new environment during the measurements (Ericson et al., 1991). In moving the animals with no sedation (a decrease in locomotor activity), a decrease

in BT and changes in HR and MAP were observed at the analgesic dose ranges (1-3 µg) of fadolmidine. Clonidine decreased the motor activity and BT in the first open field period.

These *in vitro* and *in vivo* studies of kidney, urodynamic and cardiovascular functions indicate no major safety concerns at the expected analgesic IT dose of fadolmidine suggesting a favourable adverse effects profile for spinal analgesia.

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Conflict of interest

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References

Aro, E., Bastman, S., Andersson, K.E., Streng, T., 2015. Is there a peripheral site of action contributing to the voiding effects of α_2-adrenoceptor agonists and antagonists? World J. Urol. 33, 433–440. https://doi.org/10.1007/s00345-014-1336-z.

Baldini, A., Von Korff, M., Lin, E.H., 2012. A review of potential adverse effects of long-term opioid therapy: A practitioner's guide. Prim. Care Companion CNS Disord. 14. https://doi.org/10.4088/PCC.11m01326.

Berger, A.C., Whistler, J.L., 2010. How to design an opioid drug that causes reduced tolerance and dependence. Ann. Neurol. 67, 559–569. https://doi.org/10.1002/ana.22002.

Bonhaus, D., Wong, E., Stefanich, E., Kunysz, E., Eglen, R., 1993. Pharmacological characterization of 5-hydroxytryptamine3 receptors in murine brain and ileum using the novel radioligand [3H]RS-42358-197: Evidence for receptor heterogeneity. J. Neurochem. 61, 1927–1932. https://doi.org/10.1111/j.1471-4159.1993.tb09835.x.

Briejer, M., Schuurkes, J., 1996. 5-HT3 and 5-HT4 receptors and cholinergic and tachykininergic neurotransmission in the guinea-pig proximal colon. Eur. J. Pharmacol. 18, 308, 173–180. https://doi.org/10.1016/0014-2999(96)00297-X.

Cabral, A., Kapusta, D., Kenings, V., Varner, K., 1998. Central alpha2-receptor mechanisms contribute to enhanced renal responses during ketamine-xylazine anesthesia. Am. J. Physiol. 275, 1867–1874. https://doi.org/10.1152/ajpregu.1998.275.6.R1867.

Conte, B., Maggi, C., Parlani, M., Lopez, G., Manzini, S., Giachetti, A., 1991. Simultaneous recording of vesical and urethral pressure in urethane-anesthetized rats: Effect of neuromuscular blocking agents on the activity of the external urethral sphincter. J. Pharmacol. Methods 26, 161–171.

Corters-Altamirano, J.L., Olmos-Hernandez, A., Jaime, H.B., Carrillo-Mora P., Bandala C., Reyes-Long S., Alfaro-Rodríguez, A., 2018. Review: 5-HT1, 5-HT2, 5-HT3 and 5-HT7 receptors and their role in the modulation of pain response in the central nervous system. Curr. Neuropharmacol. 16, 210–221. https://doi.org/10.2174/1570159X15666170911121027.

Durant, P., Yaksh, T., 1988. Drug effects on urinary bladder tone during spinal morphine-induced inhibition of the micturition reflex in unanesthetized rats. Anesthesiology. 68, 325–334.

Eisenach, J.C., De Kock, M., Klimscha, W., 1996. α_2-Adrenergic agonists for regional anesthesia: A clinical review of clonidine (1984-1995). Anesthesiology. 85, 655–674. https://doi.org/10.1097/00000542-199609000-00026.

Eisenach, J.C., Lavand'homme, P., Tong, C., Cheng, J.K., Pan, H.L., Virtanen, R., Nikkanen, H., James, R., 1999. Antinociceptive and hemodynamic effects of a novel α_2-adrenergic agonist, MPV-2426, in sheep. Anesthesiology. 91, 1425–1436. https://doi.org/10.1097/00000542-199911000-00036.

Ericson, E., Samuelsson, J., Ahlenius, S., 1991. Photocell measurements of rat motor activity. A contribution to sensitivity and variation in behavioral observations. J. Pharmacol. Methods. 25, 111–122. https://doi.org/10.1016/0160-5402(91)90002-M.

Giovannoni, M.P., Ghelardini, C., Vergelli C., Dal Piaz, V., 2009. α_2-Agonists as analgesic agents. Med. Res. Rev. 29, 339–368. https://doi.org/10.1002/med.20134.

Hao, J.X., Yu, W., Xu, X.J., Wiesenfeld-Hallin Z., 1996. Effects on intrathecal vs. systemic clonidine in treating chronic allodynia-like response in spinally injured rats. Brain Res. 736, 28–34. https://doi.org/10.1016/0006-8993(96)00703-2.

Hayashida, K., Kimura, M., Yoshizumi, M., Hobo, S., Obata, H., Eisenach, J.C., 2012. Ondansetron reverses antihypersensitivity from clonidine in rats after peripheral nerve injury: Role of γ-aminobutyric acid in α2-adrenoceptor and 5-HT3 serotonin receptor analgesia. Anesthesiology. 117, 389-398. https://doi.org/10.1097/ALN.0b013e318260d381.

Harada, T., Constantinou, C., 1993. The effect of alpha 2 agonists and antagonists on the lower urinary tract of the rat. J. Urol. 149, 159–164. https://doi.org/10.1016/S0022-5347(17)36030-5.

Horvath, G., Brodacz, B., Holzer-Petsche, U., 2002. Blood pressure changes after intrathecal co-administration of calcium channel blockers with morphine or clonidine at the spinal level. Naunyn Schmiedebergs Arch. Pharmacol. 366, 270–275. https://doi.org/10.1007/s00210-002-0591-5.

Ishizuka, O., Mattiasson, A., Andersson, K., 1996. Role of spinal and peripheral alpha 2 adrenoceptors in micturition in normal conscious rats. J. Urol. 156, 1853–1857.

Kawamata, T., Omote, K., Yamamoto, H., Toriyabe, M., Wada, K., Namiki, A., 2003. Antihyperalgesic and side effects of intrathecal clonidine and tizanidine in a rat model of

neuropathic pain. Anesthesiology. 98, 1480–1483. https://doi.org/10.1097/00000542-200306000-00027.

Kim, J., Jung, J., Cho, M., 2013. Continuous intrathecal morphine administration for cancer pain management using an intrathecal catheter connected to a subcutaneous injection port: A retrospective analysis of 22 terminal cancer patients in korean population. Korean J. Pain. 26, 32–38. https://doi.org/10.3344/kjp.2013.26.1.32.

Kleinmann, B., Wolter, T., 2017. Intrathecal opioid therapy for non-malignant chronic pain: A long-term perspective. Neuromodulation. 20, 719–726. https://doi.org/10.1111/ner.12617.

Kontani, H., Tsuji, T., Kimura, S., 2000. Effects of adrenergic α_2-adrenoceptor agonists on urinary bladder contraction in conscious rats. Jpn. J. Pharmacol. 84, 381–390. https://doi.org/10.1254/jjp.84.381.

Lee, Y.W., Yaksh, T.L., 1995. Analysis of drug interaction between intrathecal clonidine and MK-801 in peripheral neuropathic pain rat model. Anesthesiology. 82, 741–748. https://doi.org/ 10.1097/00000542-199503000-00016.

Lehtimäki, J., Mäkelä, S., Viljamaa, J., Yagi, A., Paranko, J., Santti, R., 1996. Neonatal estrogenization of the male mouse results in urethral dysfunction. J. Urol. 156, 2098–2103.

Lehtimäki, J., Leino, T., Koivisto, A.,Viitamaa, T., Lehtimäki, T., Haapalinna, A., Kuokkanen, K., Virtanen, R., 2008. In vitro and in vivo profiling of fadolmidine, a novel potent and α_2-adrenoceptor agonist with local mode of action. Eur. J. Pharmacol. 599, 65–71. https://doi.org/10.1016/j.ejphar.2008.10.003.

Leino, T., Viitamaa, T., Haapalinna, A., Lehtimäki, J., Virtanen, R., 2009. Pharmacological profile of intrathecal fadolmidine, a α_2-adrenoceptor agonist, in rodent models. Naunyn Schmiedebergs Arch. Pharmacol. 380, 539–550. https://doi.org/10.1007/s00210-009-0460-6.

Maggi, C., Meli, A., 1986. Suitability of urethane anesthesia for physiopharmacological investigations. Part 3: Other systems and conclusions. Experientia. 42, 531–537.

Maggi, C., Santicoli, P., Meli, A., 1986. The nonstop transvesical cystometrogram in urethane-anesthetized rats: A simple procedure for quantitative studies on the various phases of urinary bladder voiding cycle. J. Pharmacol. Methods. 15, 157–167.

Malinovsky, J.M., Malinge, M., Lepage, J.Y., Pinaud, M., 2003. Sedation caused by clonidine in patients with spinal cord injury. Br. J. Anaesth. 90, 742–745. https://doi.org/10.1093/bja/aeg134.

Mersdorf, A., Schmidt, R., Tanagho, E., 1993. Urodynamic evaluation and electrical and pharmacologic neurostimulation. The rat model. Urol. Res. 21, 199–209. https://doi.org/10.1007/bf00590037.

Miller, J., McCoy, K., Coleman, A., 2001. Renal actions of the alpha2-adrenoceptor agonist, xylazine, in the anaesthetised rat. N. Z. Vet. J. 49, 173–180. https://doi.org/10.1080/00480169.2001.36229.

Nishiyama, T., Hanaoka, K., 2001. The synergistic interaction between midazolam and clonidine in spinally-mediated analgesia in two different pain models of rats. Anesth. Analg. 93, 1025–1031. https://doi.org/10.1097/00000539-200110000-00045.

Nguyen, V., Tiemann, D., Park, E., Salehi, A., 2017. Alpha-2 agonists. Anesthesiol. Clin. 35, 233–245. https://doi.org/10.1016/j.anclin.2017.01.009.

Ouyang, H., Bai, X., Huang, W., Chen, D., Dohi, S., Zeng, W., 2012. The antinociceptive activity of intrathecally administered amiloride and its interactions with morphine and clonidine in rats. J. Pain. 13, 41–48. https://doi.org/10.1016/j.jpain.2011.09.008.

Pertovaara, A., 2004. Antinociceptive properties of fadolmidine (MPV-2426), a novel α_2-adrenoceptor agonist. CNS Drug Rev. 10, 117–126. https://doi.org/10.1111/j.1527-3458.2004.tb00008.x.

Pertovaara, A., Wei, H., 2000. Attenuation of ascending nociceptive signals to the rostroventromedial medulla induced by a novel α_2-adrenoceptor agonist, MPV-2426, following intrathecal application in neuropathic rats. Anesthesiology. 92, 1082–1092. https://doi.org/10.1097/00000542-200004000-00027.

Post, C., Gordh Jr, T., Minor, B.G., Archer, T., Freedman, J., 1987. Antinociceptive effects and spinal cord tissue concentrations after intrathecal injection of guanfacine or clonidine into rats. Anesth. Analg. 66, 317–324.

Ramage, A,G. Villalón, C.M., 2008. 5-hydroxytryptamine and cardiovascular regulation. Trends Pharmacol. Sci. 29, 472–281. https://doi.org/10.1016/j.tips.2008.06.009.

Ramirez, M., Cenarruzabeitia, E., Del Rio, J., Lasheras, B., 1994. Involvement of neurokinins in the non-cholinergic response to activation of 5-HT3 and 5-HT4 receptors in guinea-pig ileum. Br. J. Pharmacol. 111, 419–424. https://doi.org/10.1111/j.1476-5381.1994.tb14751.x.

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

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

Streng, T., Santti, R., Andersson, K.E., 2010. Voiding effects mediated by α2-adrenoceptors in the anaesthetized male rat. BJU Int. 106, 1546–1549. https://doi.org/10.1111/j.1464-410X.2010.09228.x.

Tonner, P.H., 2017. Additives used to reduce perioperative opioid consumption 1: Alpha2-agonists. Best Pract. Res. Clin. Anaesthesiol. 31, 505–512. https://doi.org/10.1016/j.bpa.2017.10.004.

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

van den Buuse, M., Van Acker, S.A., Fluttert, M., De Kloet, E.R., 2001. Blood pressure, heart rate and behavioral responses to psychological "novelty" stress in freely moving rats. Psychophysiology. 38, 490–499. https://doi.org/10.1017/s0048577201990687.

Xu, M., Kontinen, V.K., Kalso, E., 2000. Effects of radolmidine, a novel α_2-adrenergic agonist compared with dexmedetomidine in different pain models in the rat. Anesthesiology. 93, 473–481. https://doi.org/10.1097/00000542-200008000-00027.

Yaksh, T.L., Rudy, T.A., 1976. Chronic catheterization of the spinal subarachnoid space. Physiol. Behav. 17, 1031–1036. https://doi.org/10.1016/0031-9384(76)90029-9.

Yamano, M., Miyata, K., 1996. Investigation of 5-HT3 receptor-mediated contraction in guinea-pig distal colon. Eur. J. Pharmacol. 317, 353–359. https://doi.org/10.1016/s0014-2999(96)00754-6.

Zifa, E., Fillion, G., 1992. 5-Hydroxytryptamine receptors. Pharmacol. Rev. 44, 401–458.

Figure captions

Fig. 1. Open field timeline in min.

Fig. 2. 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 α_2-adrenoceptor antagonist atipamezole (1 µM, n = 7) and this effect was inhibited by the 5-HT_3 antagonist ondansetron (1 µM, n=5) being indicative of the 5-HT_3 agonistic property of fadolmidine. Data are presented as mean ± S.E.M.

Fig. 3. Intravenous (IV) doses of fadolmidine (3.0 µg/rat) induced a transient atipamezole and prazosin sensitive increase in mean arterial pressure (MAP) in anesthetised rats (A). The reflectory drop in heart rate (HR) was inhibited by atipamezole (B). The data are presented as mean ± S.E.M. For the statistical analysis, one-way ANOVA was used followed by the t-test. *P*-values <0.05 *, <0.01 ** and <0.001 ***.

Fig. 4. Intrathecal (IT) doses of fadolmidine increased urine output dose-dependently, whereas intravenous (IV) doses had no effects (A). Both IV and IT doses of fadolmidine increased urine sodium ion concentration, with IT doses being more potent (B). Furosemide was given subcutanous (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 ***

Fig. 5. Representative recording of a normal micturition cycle in an anesthetised male rat. The upper trace measures urine output (µl) and the lower trace measures the urinary bladder lumen pressure (mmHg). Numbers in the lower trace are as follows:

1. During the initial phase, bladder pressure increases from the resting level to the opening pressure by a tonic contraction of the detrusor smooth muscles of the bladder wall as a response to stretching of the bladder wall caused by the infused volume. The bladder neck and urethra remain closed preventing the leaking of the urine.
2. During the voiding, the bladder neck and urethral smooth muscles relax and the urethral lumen open allowing the urine to flow out of the bladder. This phase is characterised by a high frequency oscillation in the bladder pressure which is thought to be caused by the fast contractions of the striated muscles around the urethra (rhabdosphincter).
3. At the end of the voiding cycle, high frequency oscillation and urine flow cease, bladder neck and urethra close again causing a rapid increase in the contracted bladder.
4. In the final phase, the bladder musculature relaxes and the bladder pressure decreases to the basal level.

Fig. 6. Categories demonstrating the severity of the disrupted voiding cycles measured in anesthetised male rats. For the category graphs, the upper trace is urine output (ml) and the lower trace is the urinary bladder lumen pressure (mmHg).

Fig. 7. Time-course of heart rate (HR) in conscious rats after intrathecal bolus injection (at time point 0 min) of clonidine (A) and fadolmidine (B). Clonidine and fadolmidine induced a dose-dependent decrease in HR, and HR changes reflected the rats' motor activity (measured in open field 1 and 2). Values are represented as a mean, n = 4/dose.

Figure1

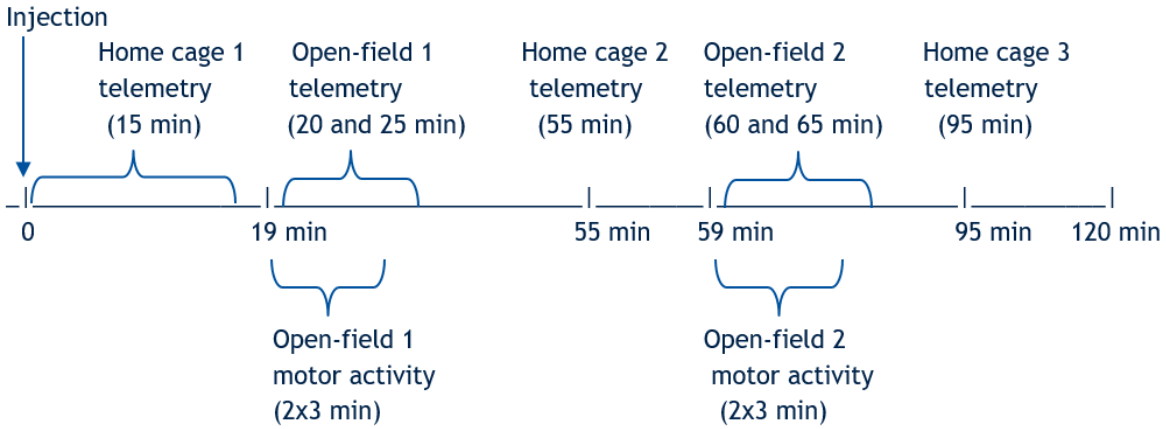


Fig.1.

Figure2

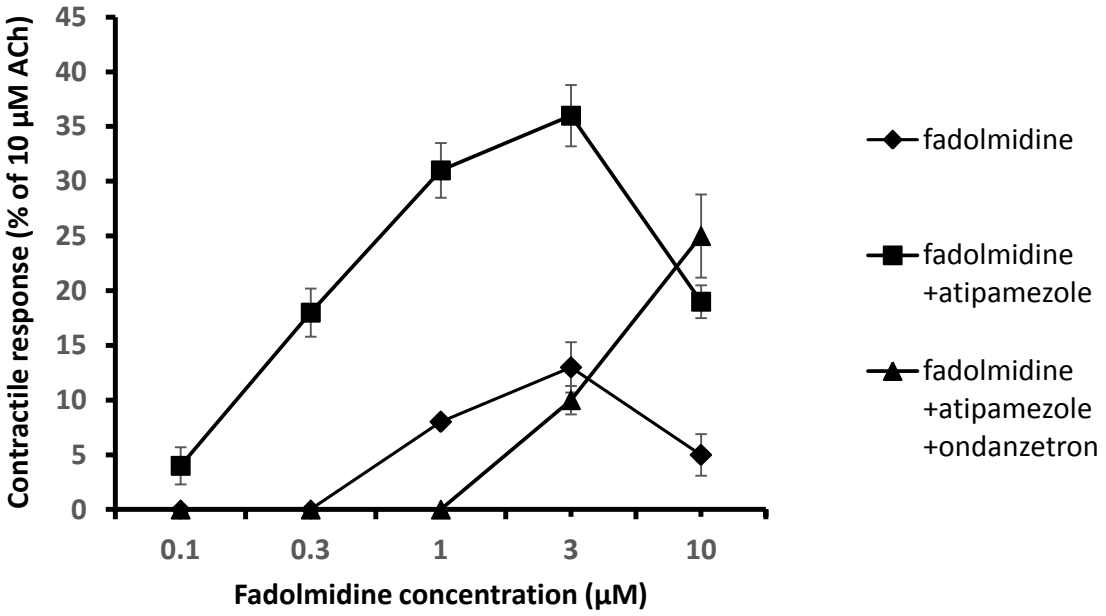


Fig. 2.

Figure3

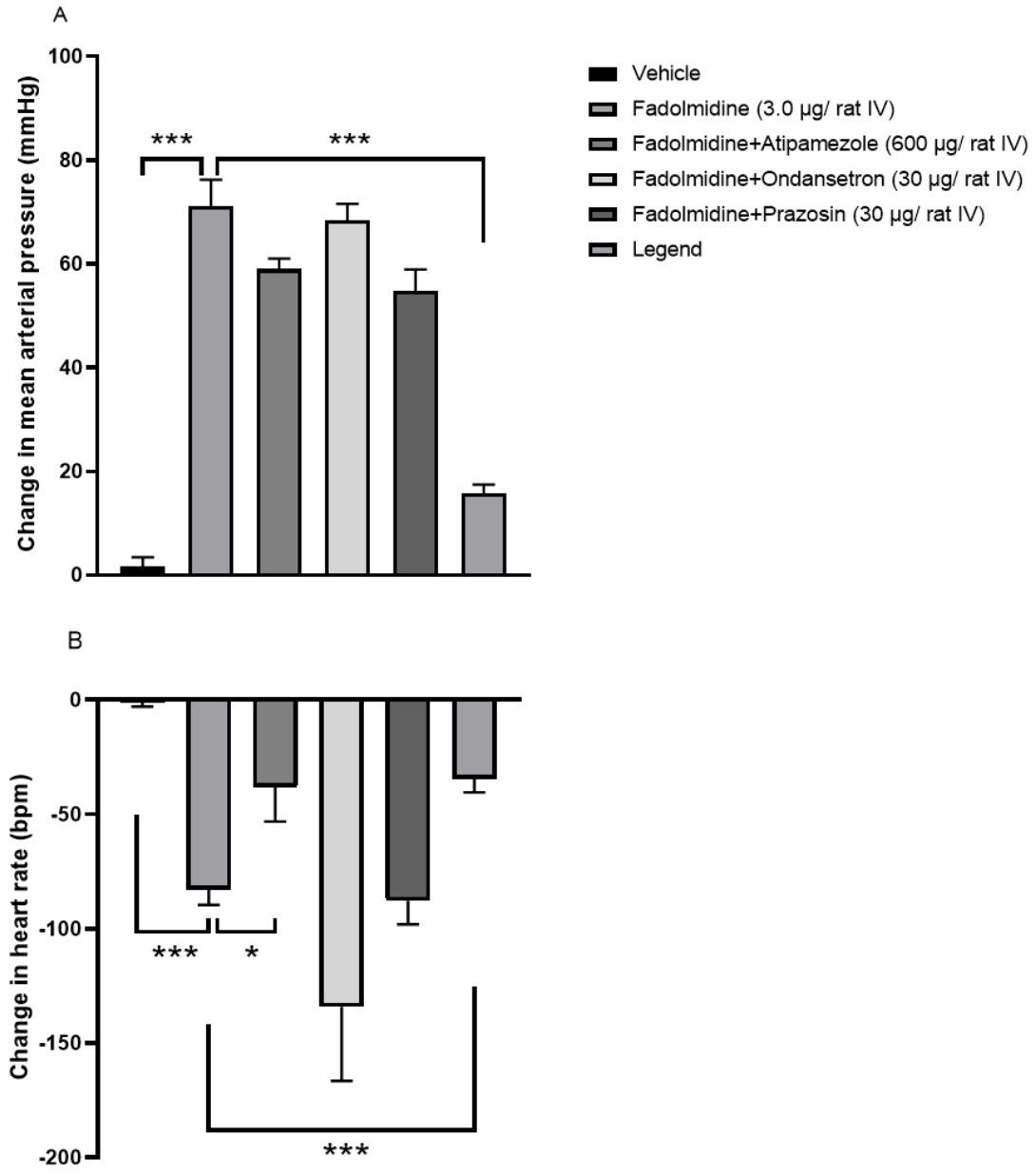


Fig. 3

Figure4

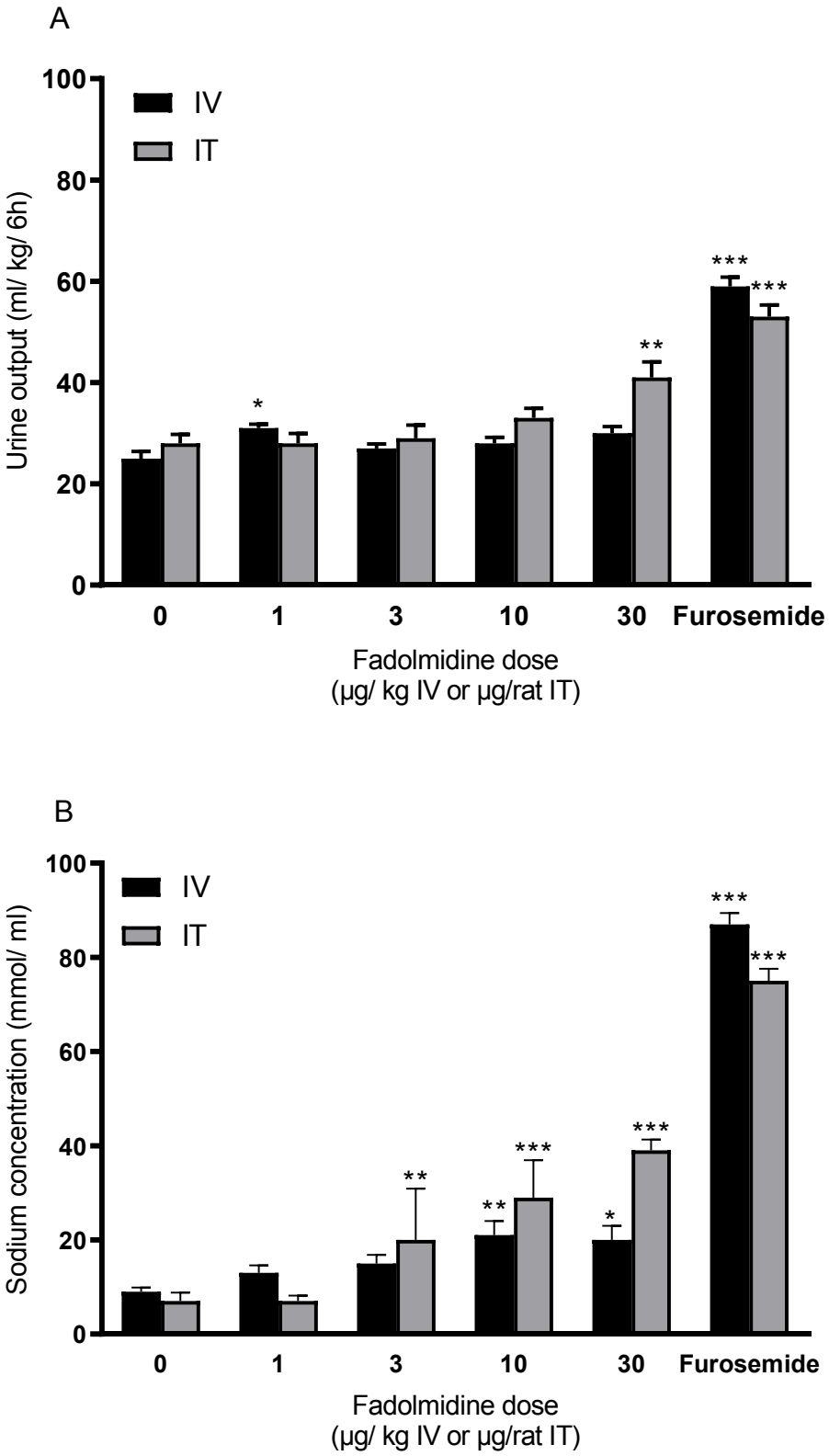


Fig. 4.

Figure5

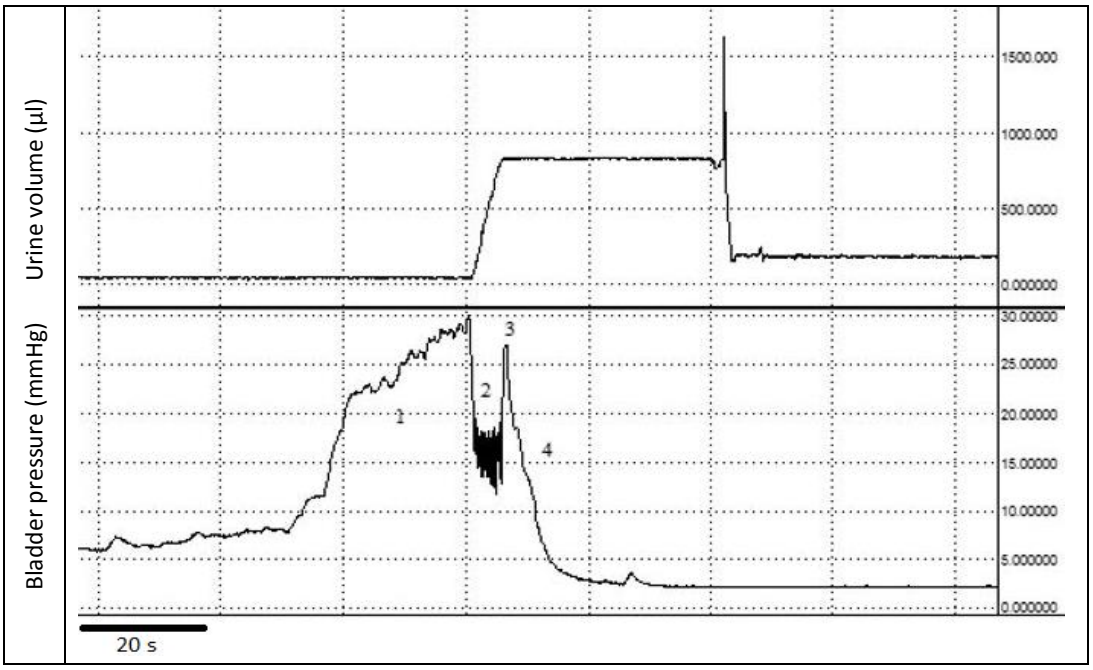
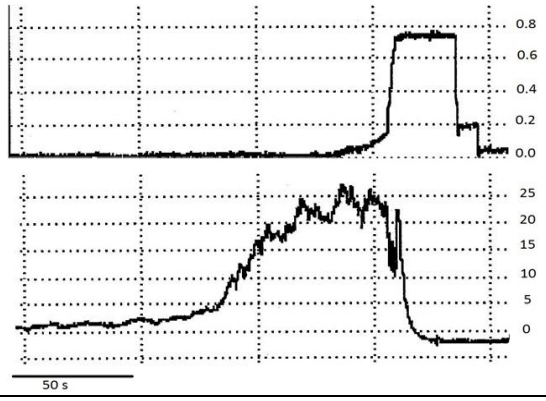
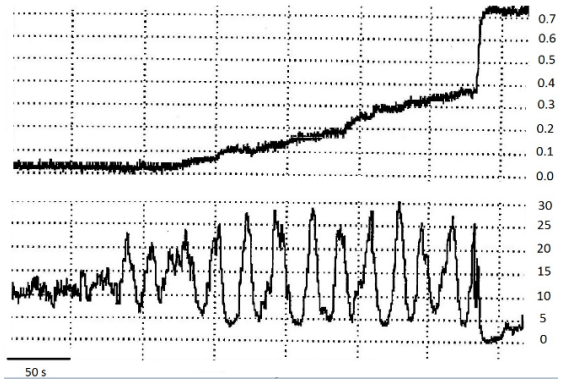
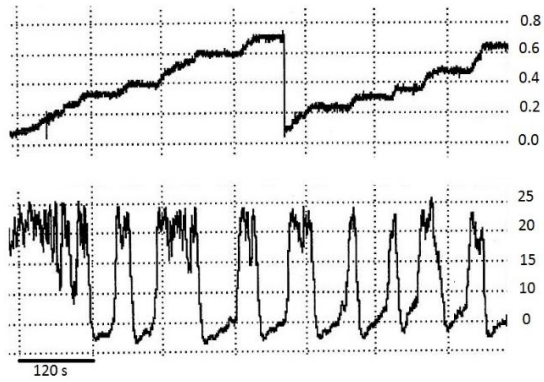
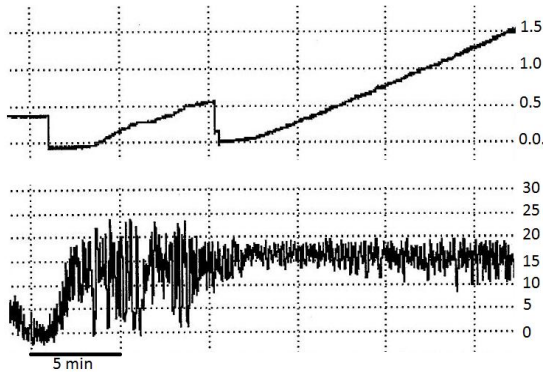


Fig. 5.

Figure6

Urine volume (ml) Bladder pressure (mmHg)		Category 1: Premature contractions before the proper voiding cycles were increased and typically a small amount of the urine leaked out before the micturition. However, all the different phases of cycles were clearly observable.
Urine volume (ml) Bladder pressure (mmHg)		Category 2: Bladder instability and premature contractions increased further, typically animals performed number of incomplete voiding cycles without IPHFO before performing a proper voiding.
Urine volume (ml) Bladder pressure (mmHg)		Category 3: IPHFO was totally absent resulting in improper voiding with cyclic bladder pressure fluctuations with small amount of urine expelled at the highest pressure and increased voiding frequency.
Urine volume (ml) Bladder pressure (mmHg)		Category 4: Separated voiding cycles were not present anymore, but bladder pressure oscillation was profound. Urine leaked out at the speed of infusion resulting in overflow incontinence.

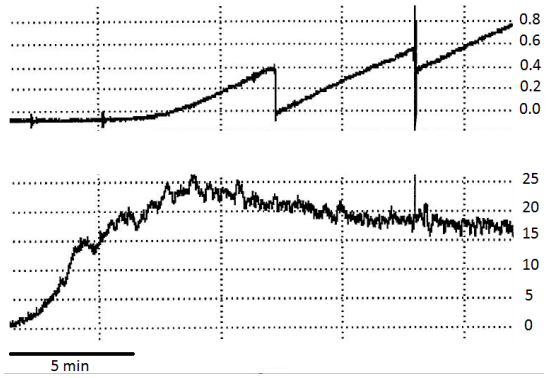
Urine volume (ml) Bladder pressure (mmHg)		Category 5: Bladder pressure oscillation was greatly reduced and urine leaked out at he speed of infusion.

Fig. 6.

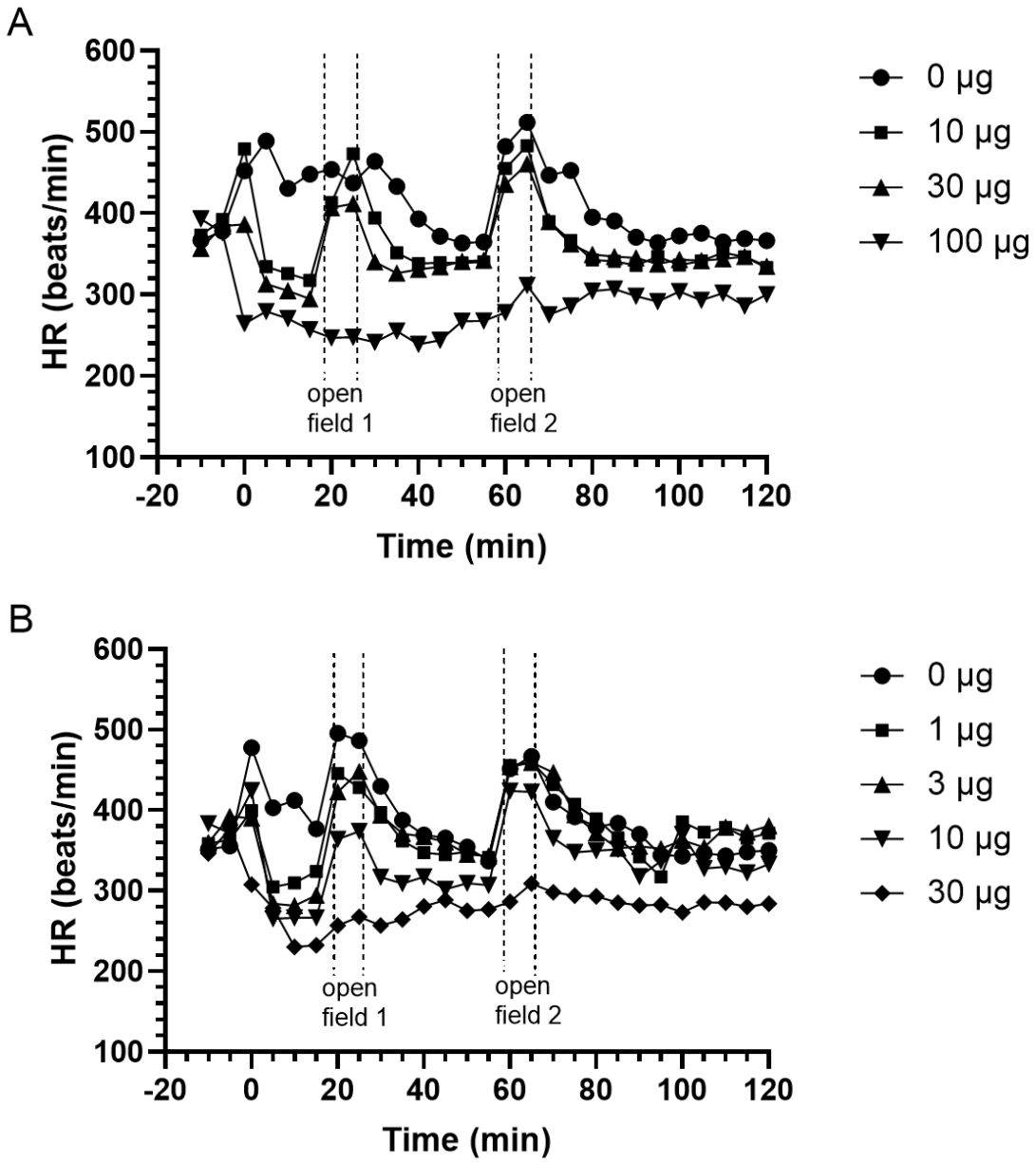


Fig. 7.

Table 1

Effects of intrathecal (i.t.) drug treatments on voiding functions in urethane anesthetized male rats

	Dose	Category						Animals
Treatment	µg i.t.	0	1	2	3	4	5	n
Control	0	14	2	1	1		1	19
Fadolmidine	1	3	2	1	1		1	8
	3	2	8	4			2	16
	10		1	1	2			4
Clonidine	3		1	2				3
	10			3	1			4
	30				3	2		5
Dexmedetomidine	1		1	1		2		4
	3				3			3
	10					1	2	3
Morphine	0.1	1	2	1				4
	0.3	1	1		1		2	5
	1						5	5

The rats were ranked to six categories (0-5) according to severity of drug effect on the voiding function. The categorisation is described in the figure 6. Numbers in the category columns indicates the number of rats in that specific category.

Table 2

The effects of the α_2-adrenoceptor agonists clonidine and fadolmidine on heart rate (HR), mean arterial pressure (MAP), body temperature (BT) and spontaneous motor activity (visits / 6 min) after intrathecal administration in conscious rats by using telemetry monitoring in a home cage and in an open field setup

Environment and parameters	Dose (μg/rat)								
	Clonidine				Fadolmidine				
	0 μg	10 μg	30 μg	100 μg	0 μg	1 μg	3 μg	10 μg	30 μg
Home cage 1									
HR (b/min)	448 ± 14	317 ± 4[c]	295 ± 9[c]	257 ± 12[c]	377 ± 32	324 ± 12	294 ± 8	267 ± 13[b]	232 ± 21[b]
MAP (mmHg)	128 ± 5	109 ± 6	142 ± 5	172 ± 8[b]	114 ± 8	111 ± 3	122 ± 7	134 ± 7	156 ± 5
BT (°C)	38.0 ± 0.1	36.9 ± 0.2[a]	36.7 ± 0.2[a]	36.6 ± 0.1[b]	38.0 ± 0.1	37.6 ± 0.0	37.2 ± 0.3[a]	37.2 ± 0.2	37.0 ± 0.2[b]
Open field 1									
Motor activity	35 ± 6	12 ± 6[a]	8 ± 2[b]	5 ± 2[b]	45 ± 23	32 ± 14	43 ± 16	41 ± 13	19 ± 5

HR (b/min)	446 ± 30	447 ± 20	409 ± 33	247± 6[a]	491 ± 9	437 ± 21	435 ± 20	369 ± 26[a]	262 ± 18[c]
MAP (mmHg)	140 ± 4	143 ± 4	147 ± 5	154 ± 4	134 ± 7	130 ± 5	128 ± 7	126 ± 8	146 ± 5
BT (°C)	38.6 ± 0.3	36.9 ± 0.3	36.7 ± 0.2[a]	36.2 ± 0.1[a]	38.2 ± 0.1	37.7 ± 0.1	37.2 ± 0.2	37.0 ± 0.3[a]	36.7 ± 0.2[b]
Home cage 2									
HR (b/min)	365 ± 17	340 ± 10	342 ± 7	268 ± 6[b]	337 ± 13	341 ± 6	343 ± 15	307 ± 11	277 ± 19[a]
MAP (mmHg)	110 ± 3	98 ± 4	113 ± 8	133 ± 7	99 ± 5	92 ± 5	96 ± 4	95 ± 7	103 ± 4
BT (°C)	38.0 ± 0.1	37.2 ± 0.3	36.8 ± 0.3	35.7 ± 0.1[b]	38.2 ± 0.2	38.1 ± 0.2	37.9 ± 0.1	37.0 ± 0.3[a]	36.2 ± 0.4[b]
Open field 2									
Motor activity	14 ± 3	6 ± 2	12 ± 2	4 ± 2[a]	31 ± 13	21 ± 5	25 ± 9	26 ± 8	11 ± 4
HR (b/min)	497 ± 11	469 ± 11	448 ± 11	295 ± 22[c]	459 ± 18	458 ± 14	457 ± 20	423 ± 21	298 ± 16[b]
MAP (mmHg)	142 ± 2	129 ± 2	131 ± 5	133 ± 6	127 ± 7	124 ± 7	118 ± 6	119 ± 6	118 ± 7
BT (°C)	38.1 ± 0.1	36.9 ± 0.2	37.0 ± 0.2	35.5 ± 0.1[c]	38.1 ± 0.1	37.9 ± 0.2	37.8 ± 0.0	36.9 ± 0.2[a]	36.0 ± 0.2[c]
Home cage 3									
HR (b/min)	364 ± 6	347 ± 10	338 ± 6	291 ± 14[b]	345 ± 5	317 ± 36	352 ± 24	337 ± 19	283 ± 29
MAP (mmHg)	111 ± 3	97 ± 5	113 ± 4	119 ± 9	100 ± 4	94 ± 8	93 ± 2	89 ± 6	94 ± 5

BT (°C)	38.0 ± 0.1	37.4 ± 0.4	37.1 ± 0.2	35.8 ± 0.3[b]	38.1 ± 0.2	38.2 ± 0.3	37.6 ± 0.3	37.4 ± 0.3	36.6 ± 0.5[a]

Values are presented as mean ± S.E.M., n=4/dose. HR, MAP, BT and motor activity were analysed by ANOVA for multiple comparisons and followed by Tukey's post hoc tests. [a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$, when compared with the corresponding control (0 μg) response.