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ORIGINAL ARTICLE

Effects of fadolmidine, an α_2-adrenoceptor agonist, as an adjuvant to spinal bupivacaine on antinociception and motor function in rats and dogs

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Abstract

α_2-Adrenoceptor agonists such as clonidine and dexmedetomidine are used as adjuvants to local anesthetics in regional anesthesia. Fadolmidine is an α_2-adrenoceptor agonist developed especially as a spinal analgesic. The current studies investigate the effects of intrathecally administered fadolmidine with a local anesthetic, bupivacaine, on antinociception and motor block in conscious rats and dogs. The antinociceptive effects of intrathecal fadolmidine and bupivacaine alone or in combination were tested in the rat tail-flick and the dog's skin twitch models. The durations of motor block in rats and in dogs were also assessed. In addition, the effects on sedation, mean arterial blood pressure, heart rate, respiratory rate and body temperature were evaluated in telemetrized dogs. Concentrations of fadolmidine in plasma and spinal cord were determined after intrathecal and intravenous administration in rats. Co-administration of intrathecal fadolmidine with bupivacaine increased the magnitude and duration of the antinociceptive effects and prolonged motor block without hypotension. The interaction of the antinociceptive effect was synergistic in its nature in rats. Concentration of fadolmidine in plasma was very low after intrathecal dosing. Taken together, these studies show that fadolmidine as an adjuvant to intrathecal bupivacaine provides enhanced sensory-motor block and enables a reduction of the doses of both drugs. The results indicate that co-administration of fadolmidine with intrathecal bupivacaine was able to achieve an enhanced antinociceptive effect without hypotension and could thus represent a suitable combination for spinal anesthesia.

KEYWORDS

α_2-adrenoceptor agonist, analgesia, blood pressure, heart rate, intrathecal, motor function

Abbreviations: %MPE, percent maximum possible effect; ED, effective dose; HR, heart rate; MAP, mean arterial blood pressure.

1 | INTRODUCTION

The intrathecal injection of variety α_2-adrenoceptor agonist, such as clonidine and dexmedetomidine, has been shown to produce significant analgesia and is extensively used for anesthesia and intensive care medicine.[24,40] However, these drugs tend to induce hypotension, bradycardia and sedation which means that their role is limited to adjuvants as analgesics.[18,21,67] One local anesthetic, bupivacaine, is capable of achieving adequate pain relief and therefore it is commonly used in spinal anesthesia. However, the short duration of action and dose-dependent cardiovascular adverse effects, such as hypotension, tend to limit the use of bupivacaine.[45,52] In the clinic, the administration of vasoconstrictors is required to maintain blood pressure, although the use of vasoconstrictors offers another benefit, that is, decreasing the systemic absorption of local anesthetics.[8,18,56] α_2-Adrenoceptor agonists, like clonidine and dexmedetomidine, when combined with local anesthetics, have been shown to enhance the analgesic effect by prolonging the duration of sensory-motor block of local anesthetics.[10,58,67] The combination allows a reduction of the doses of both drugs and furthermore, causes less side effects in perioperative anesthesia.[8,15,57]

Fadolmidine, 3-(1H-imidazol-4-ylmethyl)-indan-5-ol, is an α_2-adrenoceptor agonist especially developed for spinal analgesia.[31] Fadolmidine has been demonstrated to induce antinociceptive effect after intrathecal administration in rats,[33,43,44,48,49,61] dogs[34] and sheep.[20] Furthermore, due to its pharmacokinetic properties, fadolmidine passes poorly across the blood-brain barrier[20,31,43,49] and does not distribute significantly to the central nervous system.[62] During a 24-h continuous intrathecal infusion of fadolmidine in dogs, a good antinociceptive effect was achieved without any signs of adverse effects such as hypotension, respiratory depression and hypothermia, which were evident during the intrathecal infusion of clonidine.[34]

The effects of intrathecal fadolmidine as an adjuvant to local anesthesia have not been studied previously. The aim of the present study was to evaluate if the combination of intrathecally administered fadolmidine with bupivacaine would exert antinociceptive effects (an increase in the thermal response latency) assessed with a rat tail-flick and a dog skin twitch models. Both the rat tail-flick and dog skin twitch test are well established and validated methods to assess the efficacy of analgesic drugs.[4,9,64] A noxious heat stimulation of the tail and skin of the lower back produces a nociceptive reflex response a flick of the tail away from the heat source[9] and contraction of the trunci cutaneous musculature of the lower back[17] respectively without changes in spontaneous or evoked behavioral responses of animals.[3] The duration of motor block of bupivacaine was assessed by measuring motor scores and rotarod performance tests in rats and by defining the duration of hind limb paralysis in dogs. In addition to rats, dogs were used in this study because the duration of subarachnoid conduction motor blockade in dogs has been shown to be qualitatively similar as the values for spinal anesthesia reported in humans.[22] Furthermore, the dog seems to be a more appropriate species than rodents for the evaluation of the cardiovascular effects of α_2-adrenergic compounds.[19,29,34] Therefore, the effects of bupivacaine and fadolmidine on safety parameters as sedation, mean arterial blood pressure (MAP), heart rate (HR), respiratory rate and body temperature were determined in dogs fitted with telemetry transmitters. Furthermore, the concentrations of ^{3}H-labeled fadolmidine at the dose of 3 μg/kg in plasma and spinal cord were determined after intravenous and intrathecal administration in rats.

2 | MATERIALS AND METHODS

2.1 | Animals

The care and treatment of experimental animals were in accordance with the European Communities Council Directive 86/609/EEC. The study was approved by the Laboratory Animal Care and Use Committee of Orion Corporation (610/712-86), Finland. Male (n = 434) and female (n = 72) Sprague Dawley rats (B&K), mean body weight (weight range) 313 g (193–443 g) and 6–13 weeks old, and male Beagle dogs (Harlan-Winkelman Hundezucht), mean body weight (weight range) 9 kg (8–11 kg) and 10–21 months old, n = 5 were used.

α_2-Adrenoceptor-induce antinociception has been shown to be sex-specific and attenuated by oestrogen in female rats.[39] Thus, to avoid oestrogen-related individual variation in the nociception response the pharmacodynamics part of the study was carried out in male animals. Before intrathecal catheterization, the rats were housed (Makrolon IV cage, 550 × 330 mm (bottom inside) × 200 mm (height) with aspen chips as bedding material) in groups of five and after surgery, individually in solid bottom polypropylene cages (Makrolon III cage, 382 × 220 mm (bottom side) × 150 mm (height) with aspen chips as bedding material) with stainless steel mesh lids in a temperature controlled room at 22 ± 2°C, at a relative humidity 50% ± 10% and on a 12 h/12 h light/dark cycle (lights on at 06.00 a.m.) with free access to tap water and food (Rat/Mouse 1 maintenance Expanded SQC, supplied by Special Diet Service). Rats were acclimatised in the animal housing facility for at least one week before the experiments were carried out. The dogs were housed in groups of 2–3 and after surgery in individual cages (in 2 × 1.25 m [floor]) and fed with a standard certified pelleted dog feed (A (E) SQC, supplied by Special Diet Service). Tap water was available ad libitum. The room was equipped with automatic control of 12 h/12 h light/dark cycle (lights on at 06.00 a.m.), temperature at 18 ± 4°C and a relative humidity 50% ± 20%.

2.2 | Drugs

Fadolmidine hydrochloride (an α_2-adrenoceptor agonist) and bupivacaine hydrochloride (Bicain spinal® 5 mg/ml) (a local anesthetic) were synthesized by Orion Corporation.

2.3 | Test formulations for pharmacodynamics experiments

In rats, fadolmidine and bupivacaine were dissolved and diluted in sterile purified water (Aquasteril®, Orion Corporation) and administered by a Hamilton syringe in a volume of 10 µl. In dogs, fadolmidine and bupivacaine were dissolved and diluted in sterile physiological saline (Natrosteril®, Orion Corporation) and administered by syringe in a volume of 0.5 ml. The intrathecal injections of drugs were followed by an additional saline injection of 10 µl in rats and 0.5 ml in dogs to flush the drug remaining in the catheter lumen.

2.4 | Test formulations for pharmacokinetic experiments

An unlabeled stock solution of the test substance was prepared by dissolving 10 mg of fadolmidine in 50 ml of 0.050 M hydrochloric acid to produce a concentration of 200 µg/ml of the drug and was stored at 0–5°C until used within 10 days of preparation. ^{3}H-labeled fadolmidine (a radiochemical purity of 98.36%, determined by thin layer chromatography [TLC]), ref. no. TRQ8005 (Amersham), specific radioactivity ca. 1850 GBq/mmol, was custom synthesized by Amersham international plc., and stored in the freezer at −20°C for the pharmacokinetic study.

Preparation of a test formulation for intrathecal administration: a measured amount of ^{3}H-labeled fadolmidine in methanol was evaporated to dryness with gentle flow of nitrogen at 30°C. The residue was dissolved in an aliquot of the unlabelled fadolmidine stock solution described above. Then the pH of the solution was adjusted to 6.0 with 0.1 M NaOH and finally its volume was brought to 1.5 ml by adding purified water. The target concentration of the test compound in the solution was 0.100 mg/ml and radioactivity 111 MBq/ml. Preparation of a test formulation for intravenous administration: a measured amount of ^{3}H-labeled fadolmidine in methanol was evaporated to dryness and dissolved in a dilution of the above stock solution. Then the pH of the solution was adjusted to 6.0 with 1 M NaOH and finally its volume was brought to 40 ml by adding purified water. The target concentration of the test compound in the solution was 3 µg/ml and radioactivity 3.7 MBq/ml. The radioactivities from intrathecal (2 samples, 10 µl/sample diluted with 1990 µl of water) and intravenous (2 samples, 40 µl/sample diluted with 1960 µl of water) solutions were counted in a Wallac 1214 RackBeta liquid scintillation counter using six parallel aliquots of each sample. Specific radioactivity was calculated taking into account the dilution factors and sample volumes. The solutions for intrathecal and intravenous administration were stored at 4°C and were used for dosing within 3 days from preparation.

Prior to the drug treatment, the rats were fasted overnight. Food was available to those rats remaining 3 h after dosing. Tap water was available ad libitum except during dosing and sampling. On the dosing day of drug, a single intrathecal bolus dose (10 µl) of ^{3}H-fadolmidine formulation was given via intrathecal catheter to 36 male and 36 female rats. The intrathecal formulation was followed by the same volume of physiologic saline (Natrosteril®, Orion Corporation). For intravenous dosing, a single bolus dose (300 µl) of ^{3}H-Fadolmidine formulation was given via tail vein to 36 male and 36 female rats.

2.5 | Intrathecal catheterization in rats

Intrathecal catheters were implanted under midazolam (5 mg/kg, Dormicum® 5 mg/ml) and fentanyl (0.25 mg/kg)—fluanisone (8 mg/kg) (Hypnorm®) subcutaneous combination anesthesia according to the method of Yaksh and Rudy[66] with minor modifications. Briefly, the atlanto-occipital membrane was incised (with clean, ethanol conditioned and sterile water rinsed instruments) immediately below the skull and a polyethylene catheter (PE10, Intramedic) filled with sterile saline was introduced 8 cm into the spinal cavity such that the catheter tip extended into the rostal edge of the lumbar enlargement. The externalised end of the catheter was closed by melting. The location of the catheter tip was confirmed by administering 10 µl of 2% (0.2 mg) lidocaine (Lidocain pond® 50 mg/ml, Orion Corporation) intrathecally approximately on the third day after catheterization. Transient paralysis of both hind limbs was the indication of successful catheterization. After at least a one-week recovery period, animals with visually observed normal neurological function were used in the nociceptive and a motor function tests. There was a recovery period between experiments of at least 3 days. The animals were randomized within drugs in different groups with the Latin Square principle.

2.6 | Intrathecal catheterization and implantation of telemetry transmitter in dogs

Intrathecal catheterization and implantation of radio-telemetry transmitters were undertaken simultaneously under sterile conditions. Intrathecal catheterization was performed according to the method of Atchison et al.[6] with minor modifications. Briefly, the anesthesia was induced with medetomidine hydrochloride 40 µg/kg, i.m. (Domitor® 1 mg/ml, Orion Corporation, Finland) and maintained with propofol 6.5 mg/kg as bolus intravenous injection and infusion of 0.9 ml/kg/h (Diprivan® 10 mg/ml, Zeneca). Surgical areas were shaved and prepared with Betadine® solution. The dog's head was positioned in a holder. We applied a sterile technique with autoclaved instruments to make a small skin incision between the skull base and C1 and the dura was exposed. An incision in the dura was made and a clear nylon epidural 19G catheter (Portex®, Portex limited) was passed caudally into the intrathecal space. The length of the catheter was approximately 40 cm, but was measured for each animal to ensure that it reached the L2–L3 spinal segment. The catheter was connected with a catheter connector and the connector was fixed subcutaneously on the dorsal surface of the neck. The volume of the dead space of the catheter and connector was about 0.36 ml. The catheter's location and patency were verified post-operatively

by the appearance of clear flowing cerebrospinal fluid from the lumbar intrathecal catheter. The catheter was flushed once a week with physiological saline (0.5 ml/dog) to help maintain catheter patency. The location of the catheter tip was confirmed by administering 6 mg lidocaine (Lidocain® 20 mg/ml, Orion Corporation) intrathecally approximately on the third day after catheterization. Transient paralysis of both hind limbs was the indication of successful catheterization. All animals showed an uneventful postoperative recovery with no sensory or motor deficits or evidence of discomfort.

In the implantation of a telemetry transmitter for MAP and HR measurements, a flank incision was made and the device body of the sterile telemetry transmitter (model TL10M2-D70-PCT, Data Sciences) was tunnelled into a pouch under the skin. Then an incision in the inguinal area over the femoral artery (deep femoral or muscular branch) was made and the tip of the catheter was passed cranially into the artery. The vessel was ligated and subsequently the incisions were closed. After the surgery, the functioning of the telemetry system was confirmed as instructed by Data Sciences.

The animals were allowed to recover with appropriate postoperative care with buprenorphine 0.015 mg/kg, i.m. (Temgesic® 0.3 mg/ml, Reckitt & Colman) twice on the day of surgery and daily with ampicillin 500 mg i.v. (A-pen® 500 mg inject, Orion Corporation) for 5 days starting on the day of surgery for at least 1 week. After recovery, animals with visually observed normal neurological function were used in the tests.

During the measurements (sedation, antinociception, MAP, HR, respiratory rate, and body temperature) the dogs (n = 5) were standing on the operating table.

2.7 | Tail-flick test in rats

The rat tail-flick test was performed with an analgesia meter (Ugo Basile, model DS-20) consisting of an infrared heating spot and an automatic tail-flick detector. The rats were habituated to the plastic immobilization chamber twice a day (2 × 2 min) before the start of the experiment. The tail of an immobilized rat was placed on the photocell of the apparatus allowing the infrared beam to strike the tail. The time in seconds at which the rat withdrew the tail from the heat beam was recorded. Failure to respond in 5 s was the maximum (cut-off time) to prevent tissue damage. During the study no tissue damage was noted. When studying the dose responses and time course of the antinociceptive effect, the rats were administered intrathecally either with bupivacaine (0, 1, 3, 10, 30, 50, 100 and 300 μg, n = 8/dose except at the doses of 0 and 100 μg n = 16/dose), fadolmidine (0, 0.3, 1, 3 and 10 μg, n = 8/dose) and the combination of bupivacaine (10 μg) with fadolmidine (0.3, 1, 3 and 10 μg) and control (saline, 10 μl) (n = 7/dose). In the combination study, the bupivacaine dose and fadolmidine doses were selected based on the antinociceptive effect not causing any sedative effects. The nociceptive observations were made at the following time points after bupivacaine administration: 10, 20, 30, 45, 60, 90 and 120 min. In the experiments with either fadolmidine or the combination of bupivacaine with fadolmidine, the following time points were used; 0.5, 1, 2, 4 and 6 h. The fadolmidine and bupivacaine doses (n = 7/ dose group) used for isobolographic analysis are presented in Table 1. For isobolographic analysis, the observation time point of 30 min after drugs injection were used. At this time point both drugs have their maximum antinociceptive effect.[42,61] The nociceptive observation time points were at 10, 20, 30, 45, 60, 120, 180, 240, 300, 360 and 420 min after dosing. The tests were performed in a blinded manner. Both predrug and postdrug latencies were determined three times to diminish the effects of a possible unspecific reaction, and the mean value was used.

2.8 | Motor score, Rotarod performance and body temperature measurements in rats

The measurements were performed in the same rats: first, the determination of the motor score and immediately after that there was the assessment of Rotarod performance and the measurement of body temperature. The motor function was scored by a slightly modified method of Penning and Yaksh.[46] Motor function was evaluated grading bilaterally with the following parameters (1) sedation (scored 0–2), (2) the placing/stepping reflex of the left (scored 0–2) and right (scored 0–2) hind legs, (3) the muscle tone of the right (scored 0–2) and left (scored 0–2) hind legs by stretching the legs, and (4) the righting reflex (scored 0–2). The scores were 0 = absent, 1 = impaired and 2 = normal, the normal baseline score being thus 12. The duration of action on the motor score is the first measurement time point after drug dosing when the motor score is the normal baseline score being 12. The muscle tone of the fore limbs (right (scored 0–2) and left (scored 0–2) fore limb) was also measured. The animals with a pre-test score of 16 were accepted for the study. The effect on motor co-ordination was evaluated on a Rotarod treadmill for rats (Ugo Basile) consisting of four drums (diameter of 70 mm, 4 r/min) separated by five flanges. After training, only those rats that were able to stay for at least 2 min on the rotating rod were selected for test. The rectal temperature was measured by a digital thermometer (Ellab) at a depth of 2 cm.

TABLE 1 The doses (μg/rat) of intrathecal fadolmidine and bupivacaine in the isobolographic analysis

ED50 ratio/dose (μg/rat)	Fadolmidine	Bupivacaine
2 × ED_{50}	2.4	175
1 × ED_{50}	1.2	87
1/2 × ED_{50}	0.6	45
1/4 × ED_{50}	0.3	22
1/6 × ED_{50}	0.2	15
1/8 × ED_{50}	0.15	11
1/10 × ED_{50}	0.12	9
1/12 × ED_{50}	0.1	7
1/16 × ED_{50}	0.08	5.4

n = 7/dose group.

The rats were administered intrathecally either with bupivacaine at the doses of 0, 1, 3, 10, 30, 50 and 100 in the study 1 (n = 8/dose) and at the doses of 0, 100 and 300 μg in the study 2 (n = 8/dose) and fadolmidine at the doses of 0.3, 1, 3 and 10 μg (n = 8/dose). The effects on the motor score with the bupivacaine doses under 300 μg were minor and the durations of motor impairment were short; therefore, the bupivacaine dose of 300 μg was selected for the combination with fadolmidine at the doses of 0.3, 1, 3 and 10 μg and control (saline, 10 μl) (n = 8/dose). The motor function and body temperature measurements were made at the following time points after bupivacaine administration: 10, 20, 30, 45 and 60 min and after fadolmidine, and combination bupivacaine with fadolmidine at 0.5, 1, 2, 4 and 7 h after dosing. The measurements were performed in a blinded manner.

2.9 | Skin twitch response in dogs

The thermally evoked skin twitch response was measured using a probe with an approximately 1 cm surface area maintained at approximately 62.5 ± 0.5℃. The probe was applied sequentially to the shaven thoracolumbar areas of the animal's back. When a brisk contraction of the local musculature within 1–3 s of probe placement was detected, the probe was removed and the latency recorded. Failure to respond within 6 s (cut-off time to prevent tissue damage) was assigned as the latency. During the study no tissue damage was noted even with 6 s cut-off time. For analytical proposes, the nociceptive response is presented as the mean of the two latencies. Nociceptive observations were made at the following time points: 0 (pre), 0.5, 1, 1.5, 2, 3, 4 and 6 h post intrathecal injection.

2.10 | Motor function measurement in dogs

The time to onset and the duration of motor block were evaluated following the intrathecal injection. Onset of motor blockade was defined as the time between completion of the intrathecal injection and the time when the dog's hind limbs were unable to support its weight. The duration of motor blockade was defined as the time from onset of motor blockade to the time when the animal was again able to support its own weight.

2.11 | MAP and HR measurement in dogs

MAP and HR were recorded and analysed using Dataquest IV system (Data Sciences). The telemetry system consisted of the transmitters, receivers (model RLA2000), consolidation matrix (model BCM100) and software Dataquest LabPRO (version 3.11). Telemetry system recordings were taken every 5-min for 20 s at the sampling rate of 500 Hz during the experiment starting 20 min before drug administration, and continuing up to 60 min thereafter and further at time points 1.5, 2, 3, 4 and 6 h after dosing starting 10 min before each measurement. The MAP and HR values are presented here for the following time points after dosing; 0, 0.17 (10 min), 0.33 (20 min), 0.5 (30 min), 0.67 (40 min), 0.83 (50 min), 1 (60 min), 1.5, 2, 3, 4 and 6 h. The hemodynamic values of all cardiac cycles within these periods were averaged.

2.12 | Sedation, respiratory rate and body temperature measurements in dogs

Drug-induced sedation was monitored simultaneously with the telemetry recording. Sedation was scored (0–4) according to the following criteria: 0 = normal alertness and responsiveness to the investigators, 1 = quiet response, eyes closed, but readily alerted and retaining head tone continuously, 2 = quiet, drowsing, eyes transiently closed, minimal neck tone, but arousable, 3 = significant depression, eyes remain shut, loss of neck tone, difficult to arouse, 4 = not arousable, total loss of neck tone, no overall response to strong stimuli applied to paws. The behavioural assessment points were before drug administration (0) and 0.5, 1, 1.5, 2, 3, 4 and 6 h after drug dosing.

The respiratory rate was measured by observation of chest expansion and contraction. The measurement points were before drug administration (0) and at 0.5, 1, 1.5, 2, 3, 4 and 6 h after drug dosing.

The effect on body temperature (rectally 3–4 cm with a thermometer) was measured before dosing (0) and 1, 2, 3, 4 and 6 h after drug dosing.

2.13 | Study design in dogs

Based on our unpublished pre-study pilot experiments, we evaluated the following approximately 50% effective antinociceptive doses: ED_{50} of fadolmidine 60 μg and bupivacaine 3 mg [evoked maximal antinociceptive effect alone, %MPE = 100%] and saline 0.5 ml (as a control). After a 20 min stabilising period, an intrathecal bolus injection was administered of bupivacaine alone, fadolmidine alone or saline or the combination bupivacaine and fadolmidine. The experiment was designed in a cross-over manner and randomization was performed by choosing the treatment dose order randomly. Each individual was used as its own control. The washout period between the treatments for individual animals was at least 48 h. The dogs were fasted for 24 h before each experiment.

2.14 | Plasma and spinal cord samples in rats

At any predetermined time point, a group of three male and three female rats was quickly euthanized with carbon dioxide. Blood was collected with terminal bleeding by cardio puncture into Li-heparin tubes (Venoject®, Terumo) and a small blood sample (approx. 3 ml) was taken and the sample was centrifuged (10 min, 1600 g, +4℃) to separate plasma. The time points for sampling were 0.083, 0.17,

0.33, 0.5, 1, 2, 3, 5, 7, 24 and 72 h following the drug administration. Additional sample of thoracolumbar spinal cord (2–3 cm) was taken at 0.17, 1, 5, 24 and 72 h following the drug administration. Plasma (collected into storage vial) and spinal cord (collected into Oxidizer vial) samples were weighed, frozen and stored at −20°C.

Analyses of samples total radioactivities: plasma and spinal cord samples were burned in the Oxidizer (Junitek, Kaarina, Finland) to convert tritium into tritiated water. Radioactivity of the tritiated water was counted with a liquid scintillation fluid counter (Wallac RackBeta 1214) using OptiPhase ''Hisafe'' 3 scintillation cocktail. Total radioactivity per mass unit of wet tissue was calculated and converted to mass equivalent of fadolmidine by using a conversion factor derived from the original specific radioactivity of the dosing solutions.

2.15 | Evaluation of the results and statistical analyses

The tail-flick and skin twitch data are expressed as means ± SEM For analysis, the tail-flick and the skin twitch latencies were converted to %MPE according to the formula: %MPE = [(postdrug latency − predrug latency) / (cut-off time − predrug latency)] × 100. To investigate the interaction between bupivacaine and fadolmidine, an isobolographic analysis was used.[59,60] The respective ED_{50} values of bupivacaine and fadolmidine were determined. We applied the ED_{50} value from the dose response curves of bupivacaine at the doses of 1, 3, 10, 30, 50 100 and 300 μg and the ED_{50} value of 1.2 μg for fadolmidine (data in file, Orion Corporation), determined by the Graph Pad Prism (version 1.03) software. A dose-response curve was obtained by co-administration of the two drugs of 2 ED_{50}, 1 ED_{50}, 1/2 ED_{50}, 1/4 ED_{50}, 1/6 ED_{50}, 1/8 ED_{50}, 1/10 ED_{50}, 1/12 ED_{50} and 1/16 ED_{50} doses (Table 1). From the dose-response curve, the ED_{50} values of the combination of bupivacaine and fadolmidine were calculated.

The isobolographic analysis of the analgesia interaction was done graphically by the methods of Tallarida et al.[60] and Tallarida.[59] The type of interaction was calculated by two equations.

Equation 1 ([59] equation 3):

$$\text{Additivity } z_1/z_1* + z_2/z_2* \begin{cases} < 1 \text{ is superadditive} \\ = 1 \textit{ is additive} \\ > 1 \textit{ is sub additive} \end{cases}$$

where z_1 is the ED_{50} dose of fadolmidine in combination and z_1^* is the ED_{50} dose of fadolmidine alone; z_2 is the ED_{50} dose of bupivacaine in combination and z_2^* is the ED_{50} dose of bupivacaine alone.

Equation 2 ([59] equation 4):

$$\text{Potency of a theoretically additive combination } z*_{\text{add}} = z_1 * /(p_1 + \text{R}p_2)$$

where z_1^* and as above, p_1 is the proportion of drug 1, p_2 is the proportion of drug 2, R is the relative potency (z_1^*/z_2^*). The additive point (dose) must be calculated both for fadolmidine and bupivacaine separately.

Motor score and rotarod performance values are expressed as mean ± SD. Magnitude of motor blocking effect and duration of action were analyzed by using dose levels with observed positive motor score effects. Kruskal–Wallis test for ranks were used to evaluate overall treatment differences (studies 1 and 2) and followed by using Dwass, Steel, Critchlow-Fligner pairwise comparisons (study 1). The overall statistical significance of treatments on body temperature in rats was tested with Mauchly's sphericity test and by using Greenhouse-Geisser correction and with 2-Factor repeated-measures analysis of variance (ANOVA) and if the level of probability (p) was <.05 (considered statistically significant), this was followed by a post-hoc two-tailed Dunnett's t-test (SPSS v. 9.0.0). The baseline values of MAP and HR were the average of three consecutive values registered before drug administration. The MAP, HR, respiratory rate, body temperature, and the onset time of motor block and the duration of motor block data are expressed as mean ± SD. Statistical analyses of MAP and HR during the first 1.5 h (during the antinociceptive effect of bupivacaine), and antinociception (skin twitch), respiratory rate and body temperature during the measurement times (6 h, for the nature of the responses) following intrathecal injection were performed by the analysis of covariance model for repeated measurements and for baseline between groups with one-way ANOVA. In the models, there were two within-dog factors, that is, dose (d) and time (t), with the time points before dose administration being used as a covariate. If the level of probability (p) was <.05 (considered statistically significant), then a pair-wise comparison was conducted. Contrasts (pair-wise comparisons) were also made to characterize the differences in more detail. p-values $^*p < .05$, $^{**}p < .01$ and $^{***}p < .001$ were considered statistically significant. Statistical analyses were performed with SAS® software (SAS Institute Inc.). Plasma and spinal cord radioactivity values are presented as mean ± SD.

2.16 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[25] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[2]

3 | RESULTS

3.1 | Antinociception in rats

The antinociceptive effects of intrathecal bupivacaine and fadolmidine alone and in combination were evaluated in the rat tail-flick test. Bupivacaine (Figure 1) and fadolmidine (Figure 2) intrathecally evoked dose-dependent antinociception. The calculated ED_{50} values at the time point of 30 min were 87 μg for bupivacaine and 1.2 μg for fadolmidine. The co-administration of fadolmidine at the doses of 1, 3 and 10 μg (but not at the dose of 0.3 μg) with a low dose of

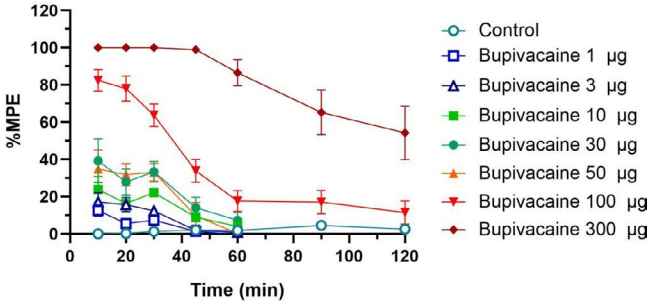

FIGURE 1 Time course of the antinociceptive effects (%MPE; percent maximum possible effect) of intrathecal bupivacaine at the doses of 1, 3, 10, 30, 50, 100 and 300 μg (n = 8/dose except at the dose of 100 μg n = 16/dose) and control (saline, 10 μl, n = 16) in the rat tail-flick test. Each point represent mean ± SEM

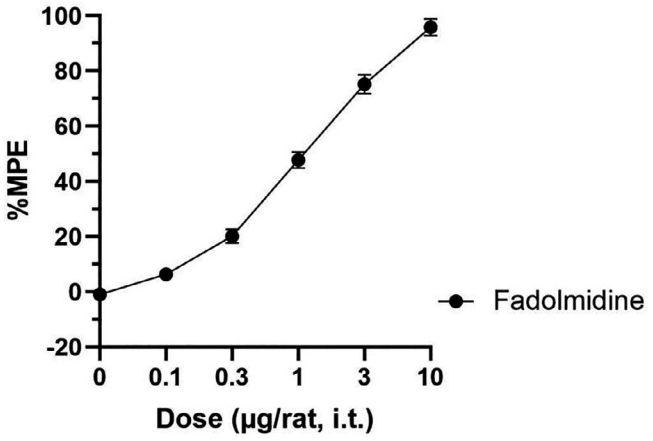

FIGURE 2 Antinociceptive effects (%MPE; percent maximum possible effect) of fadolmidine in the rat tail-flick test when given intrathecally (i.t.) 30 min before testing. Each point represent mean ± SEM., n = 8–13 per dose

bupivacaine (10 μg) was observed to increase both the magnitude and the duration of the antinociceptive effect when compared to either compound alone (Figure 3A–D). In the isobolographic analysis, we used the ED_{50} dose of bupivacaine of 87 μg and 1.2 μg for fadolmidine. The calculated ED_{50} values for the fadolmidine and bupivacaine combination were 0.26 μg (confidence limits; CL 0.21–0.34 μg) and 19.4 μg (CL 15.3–24.5 μg), respectively (Figure 4). The isobolographic analysis of the data showed that the interaction displayed a synergistic (i.e. superadditive) (equation 1 yielded a value of 0.44) nature. The calculated theoretical additive point (equation 2) would have been fadolmidine = 0.62 μg and bupivacaine = 45.1 μg.

3.2 | Motor function and body temperature in rats

The interaction between intrathecal bupivacaine and fadolmidine on motor function was studied by measuring the motor score and rotarod performance tests in rats. Fadolmidine at the doses of 0.3, 1, 3 and 10 μg, bupivacaine at the doses of 1, 3, and 10 μg and control (saline, 10 μl) had no effects on the motor scores. After these doses, the motor score values were 12 in all groups and at all measurement time points. Bupivacaine at the doses of 30, 50, 100 and 300 μg dose-dependently impaired the motor scores. The motor score had returned to normal at 60 min after administration with all doses (Figure 5). The maximum effect and the duration of action on motor score are presented in Table 2. The maximum effect and the duration of action on motor score were statistically significant (Kruskal–Wallis test) across the bupivacaine doses of 30 and 100 μg (p = .0007 and p = .0019, respectively), and 50 and 100 μg (p = .0133 and p = .0062, respectively) in the study 1 and across the bupivacaine doses of 100 and 300 μg (p = .0007 and p = .0009, respectively) in the study 2. There was no impairment of the function of the fore limbs observed in any of the treatment groups. The values of the motor score in fore limbs were 12. Fadolmidine at the doses of 0.3, 1 and 3 μg and control (saline, 10 μl) exerted no effects on the Rotarod performance. The Rotarod performance times were 120 s (cut-off value) at all of those doses. Fadolmidine slightly decreased Rotarod performance at the dose of 10 μg. The poorest Rotarod performance time (mean ± SD.; 91 ± 44 s) was encountered with the dose of 10 μg at the 0.5 h time point. After the combination of the bupivacaine at the dose of 300 μg and fadolmidine at the doses of 0.3, 1, 3 and 10 μg, the motor score values and Rotarod performance times were decreased and the duration of those effects were prolonged in a dose-dependent manner (Table 3). In body temperature there was a significant difference between treatments (2-Factor repeated-measures ANOVA (Greenhouse-Geisser method); Time F = 65.60, p < .001, Time × Group, F = 5.80, p < .001). Both bupivacaine (300 μg) and fadolmidine at a dose of 10 μg decreased body temperature. In the combinations, the decreases in body temperatures were potentiated also by low doses of fadolmidine (Table 3).

3.3 | Antinociception and motor block in dogs

The effects of intrathecal fadolmidine 60 μg added to the spinal bupivacaine 3 mg on antinociception were studied in the skin twitch test and the effect on motor block was evaluated by measuring the duration of the hind limb paralysis. Bupivacaine 3 mg inhibited the skin twitch response maximally for 60 min and the antinociceptive effect had disappeared by 2 h (Figure 6). Fadolmidine 60 μg i.t. inhibited the nociceptive skin twitch response and fadolmidine combined with bupivacaine increased statistically significantly (covariance model for repeated-measures one-way ANOVA, p = .0002) the magnitude and duration of the antinociceptive effects of bupivacaine during the measurement time (6 h). Fadolmidine alone did not exert any effect on motor block nor on the onset time of motor block produced by bupivacaine. The onset times of motor block (mean ± SD.; range of variation) were 5 ± 3 min (2–8 min) after bupivacaine 3 mg and 5 ± 4 min (2–11 min) after the combination bupivacaine and fadolmidine. The duration of the motor block (mean ± SD.; range of variation) of bupivacaine was 1.26 ± 0.68 h (0.70–2.33 h); it was prolonged (2.16 ± 0.55 h; 1.50–2.97 h) when bupivacaine was combined with intrathecal fadolmidine.

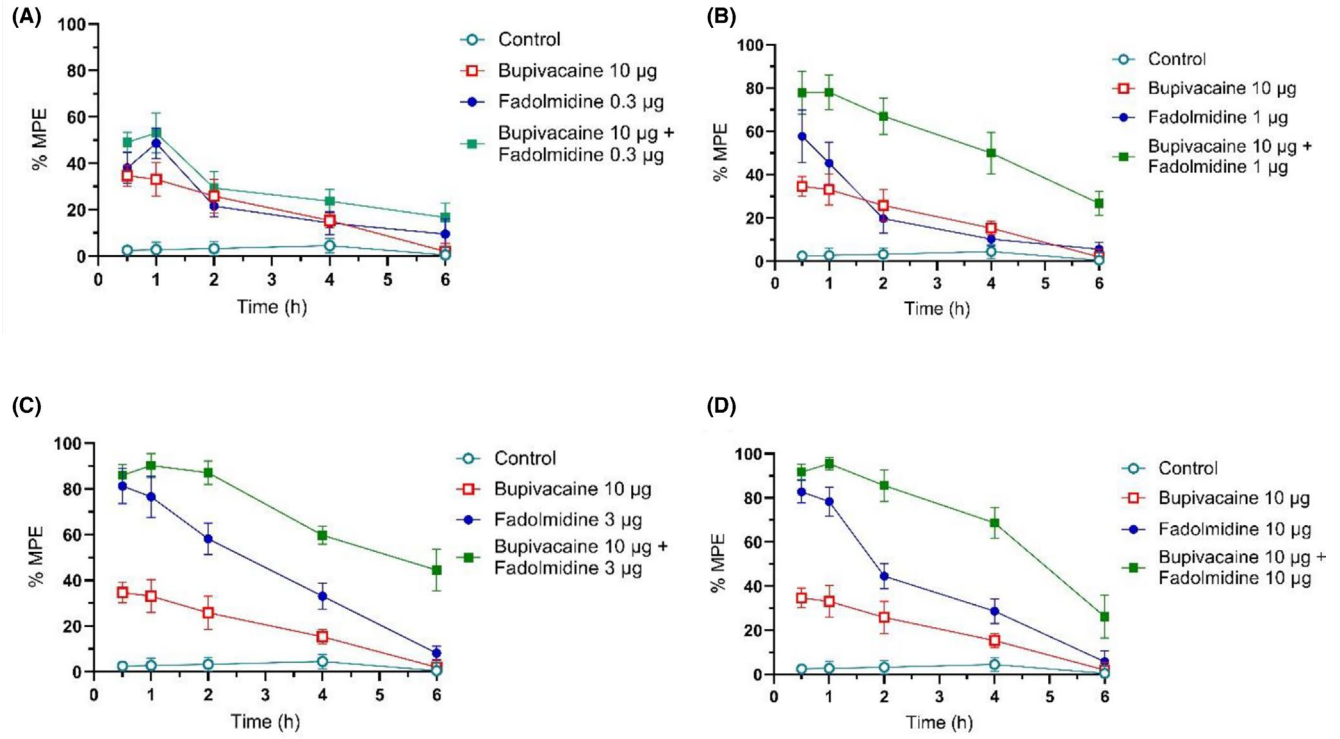

FIGURE 3 Time course of the antinociceptive effects (%MPE; percent maximum possible effect) of intrathecal fadolmidine at the doses of 0.3 (A), 1 (B), 3 (C) and 10 µg (D) (n = 8/dose) and control (n = 7) and the combination bupivacaine 10 µg and fadolmidine at the doses of 0.3, 1, 3 and 10 µg (n = 7/dose group) and control (n = 7) in the rat tail-flick test. Each point represent mean ± SEM

3.4 | MAP and HR in dogs

The effects of intrathecally administered fadolmidine 60 µg, bupivacaine 3 mg and the combination of bupivacaine and fadolmidine on MAP and HR were evaluated in telemetrized dogs. A statistically significant increase in MAP (covariance model for repeated-measures one-way ANOVA, p = .0292) was observed after the combination bupivacaine and fadolmidine in comparison with the saline group (a pair-wise comparison between the combination bupivacaine and fadolmidine and saline, p < .05) during the first 1.5 h after intrathecal injection (Table 4). No significant difference (covariance model for repeated-measures one-way ANOVA, p = .0529) was detected in HR of the four groups (Table 4).

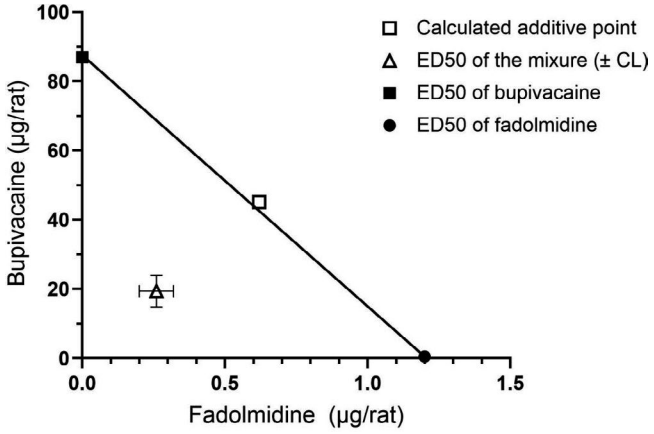

FIGURE 4 Isobolograph for the intrathecal interaction of bupivacaine and fadolmidine in the rat tail-flick test given 30 min before testing. The X and Y axes show the dose (µg/rat) of fadolmidine and bupivacaine, respectively. The calculated ED_{50} values (n = 7/dose group) for fadolmidine and bupivacaine were 0.26 and 19.4 µg, respectively. The line between the X-axis and Y-axis is the theoretical additive line. The point in the middle of the line is the theoretical additive point calculated from separate ED_{50} values. The experimental point (±confidence limits (CL)) below the additive line, indicating synergism

3.5 | Respiratory rate, body temperature and sedation in dogs

The effects of intrathecally administered fadolmidine 60 µg, bupivacaine 3 mg, and the combinations bupivacaine and fadolmidine on respiratory rate, body temperature and sedation were evaluated in dogs. Statistically significant differences were detected in the respiratory rate of the four groups (covariance model for repeated-measures one-way ANOVA, p = .0308). Bupivacaine (a pair-wise comparison between bupivacaine and saline, p < .01) and the combination of bupivacaine and fadolmidine (a pair-wise comparison the combination of bupivacaine and fadolmidine and saline, p < .05) decreased the respiratory rate whereas fadolmidine alone had no effects on the respiratory rate when compared

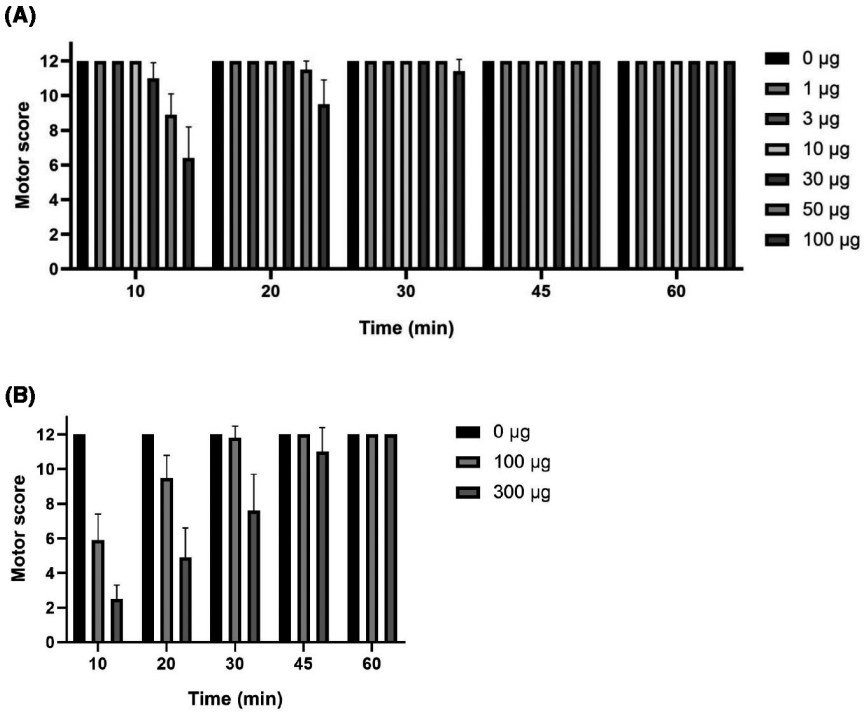

FIGURE 5 Effects on motor score was determined every 10–15 min after drug dosing. The effect of bupivacaine at the doses of 0, 1, 3, 10, 30, 50 and 100 µg (*n* = 8/dose) in the study 1 (A), and 0, 100 and 300 µg (*n* = 8/dose) in the study 2 (B) on motor score (12 = normal muscle tone, 0 = muscle tone absent) over time after intrathecal administration in rats. Values are presented as mean ± SD

TABLE 2 Motor score (the maximum effect and the duration of action) observed after intrathecal administration of bupivacaine in the study1 and the study 2 in rats

	Study 1		Study 2	
Dose (µg)	Maximum effect (motor score)	Duration of action (min)	Maximum effect (motor score)	Duration of action (min)
Bupivacaine 0 (*n* = 8)	12.0 ± 0.0	—	12.0 ± 0.0	—
Bupivacaine 1 (*n* = 8)	12.0 ± 0.0	—	—	—
Bupivacaine 3 (*n* = 8)	12.0 ± 0.0	—	—	—
Bupivacaine 10 (*n* = 8)	12.0 ± 0.0	—	—	—
Bupivacaine 30 (*n* = 8)	11.0 ± 0.9**	20.0 ± 0.0**	—	—
Bupivacaine 50 (*n* = 8)	8.9 ± 1.2*	25.0 ± 5.3*	—	—
Bupivacaine 100 (*n* = 16)	6.4 ± 1.8	37.5 ± 8.0	5.9 ± 1.4***	30.6 ± 6.8***
Bupivacaine 300 (*n* = 8)	—	—	2.5 ± 0.7	52.5 ± 8.0

Values are presented as mean ± SD. of the maximum motor score (12 = normal muscle tone, 0 = muscle tone absent) and the duration of action (min). Effects on the motor score were noted every 10–15 min after drug dosing. The duration of action on the motor score is the first measurement time point after drug dosing when the motor score is the normal baseline score being 12. -, no data. *N* = 8–16 rats/dose. Statistical analysis across the bupivacaine doses of 30, 50 and 100 µg in the study 1 and the bupivacaine doses of 100 and 300 µg in the study 2 were performed by using Kruskal–Wallis test for ranks and Dwass, Steel, Critchlow-Figner procedure for pairwise comparisons against highest dose of bupivacaine.

p-values for pairwise comparisons: *$p < .05$, **$p < .01$, ***$p < .001$.

to saline (Table 4). No significant differences (covariance model for repeated-measures one-way ANOVA, $p = .1075$) were detected in the body temperature of the four groups (Table 4). No major effects on sedation were noted after fadolmidine alone or the combination bupivacaine and fadolmidine. However, in one dog, after the combination, sedation was scored as 1 at the 2 h time point. In the other treatment groups and time points, the sedation score values were 0 (data not shown). Vomiting (once) was observed at 13 min after fadolmidine administration in one dog.

3.6 | Concentrations of fadolmidine in plasma and spinal cord in rats

Total and dose-corrected (after intrathecal administration in plasma) radioactivity in plasma and the corresponding concentration in mass equivalent of ^{3}H-fadolmidine (free base) in spinal cord after intrathecal and intravenous administration at the dose of about 3 µg/kg to rats are presented in Figure 7. Mass equivalents of the intrathecal dosing were corrected (dose-corrected) according to the radioactive dose ratio in order to allow for comparison

TABLE 3 The time course of the bupivacaine at the dose of 300 μg, fadolmidine at the doses of 0.3, 1, 3 and 10 μg and the combination bupivacaine 300 μg and fadolmidine 0.3, 1, 3 and 10 μg on motor score (12 = normal muscle tone, 0 = muscle tone absent), rotarod performance (s, maximum measurement time was 120 s) and body temperature (°C) were measured after intrathecal injection in rats

Dose (μg)	0.5 h	1 h	2 h	4 h	7 h
Motor score (0–12)					
Fado[a] 0.3	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0
Fado[a] 1	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0
Fado[a] 3	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0
Fado[a] 10	12 ± 0	12 ± 0	12 ± 0	12 ± 0	12 ± 0
Bupivac[b] 300	3 ± 0	10 ± 2	12 ± 0	12 ± 0	12 ± 0
Bupivac[b] 300 + Fado[a] 0.3	3 ± 0	8 ± 2	12 ± 0	12 ± 0	12 ± 0
Bupivac[b] 300 + Fado[a] 1	3 ± 0	4 ± 2	9 ± 1	12 ± 0	12 ± 0
Bupivac[b] 300 + Fado[a] 3	3 ± 0	3 ± 0	9 ± 2	11 ± 1	12 ± 0
Bupivac[b] 300 + Fado[a] 10	3 ± 0	3 ± 0	8 ± 2	11 ± 2	12 ± 0
Rotarod performance (s)					
Fado[a] 0.3	120 ± 0	120 ± 0	120 ± 0	120 ± 0	120 ± 0
Fado[a] 1	120 ± 0	120 ± 0	120 ± 0	120 ± 0	120 ± 0
Fado[a] 3	118 ± 6	120 ± 0	120 ± 0	120 ± 0	120 ± 0
Fado[a] 10	91 ± 44	94 ± 39	106 ± 23	112 ± 22	120 ± 0
Bupivac[b] 300	0 ± 0	41 ± 51	116 ± 12	120 ± 0	120 ± 0
Bupivac[b] 300 + Fado[a] 0.3	0 ± 0	1 ± 2	81 ± 54	120 ± 0	120 ± 0
Bupivac[b] 300 + Fado[a] 1	0 ± 0	1 ± 3	27 ± 39	66 ± 48	116 ± 13
Bupivac[b] 300 + Fado[a] 3	0 ± 0	0 ± 0	24 ± 40	73 ± 45	106 ± 29
Bupivac[b] 300 + Fado[a] 10	0 ± 0	0 ± 0	14 ± 31	63 ± 45	91 ± 42
Body temperature (°C)					
Saline (10 μl)	37.8 ± 0.6	37.9 ± 0.3	38.0 ± 0.3	37.9 ± 0.5	37.6 ±0.3
Fado[a] 0.3	38.0 ± 0.3	38.4 ± 0.4	38.1 ± 0.4	38.1 ± 0.3	37.7 ± 0.5
Fado[a] 1	37.5 ± 0.5	38.0 ± 0.4	37.9 ± 0.4	37.8 ± 0.6	37.7 ± 0.3
Fado[a] 3	37.2 ± 0.4	37.0 ± 0.3	37.7 ± 0.4	37.7 ± 0.6	37.6 ± 0.5
Fado[a] 10	36.8 ± 0.5*	36.7 ± 0.5**	37.3 ± 0.6*	37.7 ± 0.4	37.4 ± 0.3
Bupivac[b] 300	36.6 ± 0.8**	37.4 ± 0.5	37.9 ± 0.3	37.9 ± 0.4	37.6 ± 0.5
Bupivac[b] 300 + Fado[a] 0.3	37.0 ± 0.6	37.2 ± 0.6	37.9 ± 0.5	37.6 ± 0.4	37.7 ± 0.3
Bupivac[b] 300 + Fado[a] 1	36.3 ± 0.8***	37.2 ± 0.8	37.5 ± 0.4	37.7 ± 0.2	37.6 ± 0.4
Bupivac[b] 300 + Fado[a] 3	36.0 ± 0.8***	36.5 ± 1.3***	37.3 ± 0.7*	37.6 ± 0.5	37.6 ± 0.3
Bupivac[b] 300 + Fado[a] 10	35.5 ± 0.4***	35.7 ± 0.6***	36.7 ± 0.7***	37.4 ± 0.7	37.3 ± 0.6

Values are presented as mean ± SD, n = 8/dose group.

*p < .05, **p < .01, ***p < .001, significantly different from corresponding saline response.

[a]Fado: fadolmidine.

[b]Bupivac: bupivacaine.

with the intravenous dosing. Conversion to mass equivalents is based on the specific activity of the formulations. The radioactive doses were 4.198 MBq/kg (1.194 MBq/rat) after intrathecal and 2.918 MBq/kg (1.004 MBq/rat) after intravenous administration. The corresponding doses were 3.564 μg/kg (1.013 μg/rat) after intrathecal and 2.538 μg/kg (0.873 μg/rat) after intravenous administration. The tested dose of fadolmidine 1 μg/rat, i.t. was approximately antinociceptive ED_{50} dose (1.2 μg/rat, i.t). The results were shown that the circulating level of ^{3}H-fadolmidine -related radioactivity in rats reached its maximum rapidly. Total ^{3}H-fadolmidine related radioactivities (intravenous and intrathecal) rapidly declined from their initial (2–3 ng-equivalents/g) level with graphically estimated half-lives of 0.33 h.

After intrathecal administration the concentration of ^{3}H-fadolmidine in plasma was very low. Systemic elimination of fadolmidine seems to be faster than elimination from the intrathecal space.

4 | DISCUSSION

In this study, the effects of intrathecal fadolmidine, an α_2-adrenergic agonist with a local anesthetic bupivacaine, on the sensory-motor

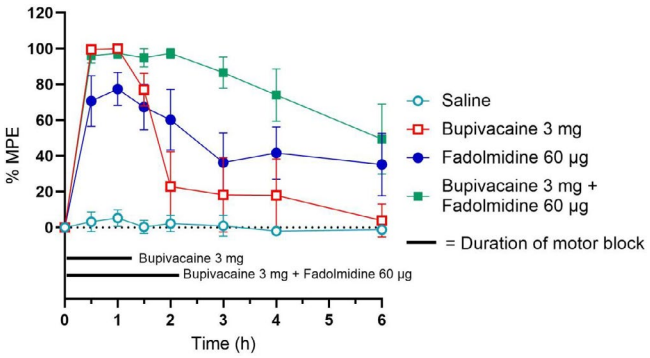

FIGURE 6 Time course of the antinociceptive effect (%MPE; percent maximum possible effect) and the duration of motor block (line, the values are mean) of intrathecal fadolmidine 60 µg and saline (0.5 ml) alone and combined with bupivacaine 3 mg in the skin twitch test in dogs (n = 5). The antinociceptive effects were statistically significantly increased between fadolmidine 60 µg (p < .001), bupivacaine 3 mg (p < .05), and the combination bupivacaine 3 mg and fadolmidine 60 µg (p < .0001) groups compared to saline during the measuring time (6 h). Each % MPE point represent mean ± SEM

block were evaluated in rats and dogs. In addition, the effects of the compounds on safety parameters as MAP, HR, respiratory rate and body temperature were evaluated in dogs.

Co-administration of intrathecal fadolmidine with bupivacaine produced an increase in the magnitude and duration of the antinociceptive response (an increase in thermal response latency) when compared to that evoked by both compounds on their own. Additionally, the co-administration prolonged the duration of bupivacaine-induced motor block but did not affect its onset time when compared to the value for bupivacaine alone. Furthermore, the duration of sensory block was much longer than the duration of motor block. The isobolographic analysis of the rat data revealed that the interaction of nociceptive response of fadolmidine and bupivacaine was synergistic in its nature.

Previously, intrathecally administered fadolmidine has been reported to induce antinociception in a rat tail-flick test[33,49,61] and in a dog skin twitch test.[34] Furthermore, the combination of intrathecal α_2-adrenoceptor agonist (e.g. clonidine, tizanidine) and a local anesthetic is known to produce a synergistic antinociceptive action in rats[26,42] and dogs as well as prolonging the duration of the motor block,[10,36] but other systemic effects have not been so well characterised.

The analgesic effect of spinally administered α_2-adrenoceptor agonist is mediated through α_2-adrenergic receptors located on the small primary afferents C and A-delta fibres present on nociceptive specific cells and on wide dynamic range neurons[16,23,63] and in the descending inhibitory medullospinal pathways in the dorsal horn of the spinal cord.[7,37] Furthermore, an α_2-adrenoceptor-induced antinociception has been reported to be sex-specific being attenuated by oestrogen in the female and requires testosterone in male rats.[5,39] It is suggested that α-adrenoceptor agonist responsiveness could be lower in females than males.[12,50] Thus, the present findings in the antinociception in male subjects might not be directly applicable to female subjects.

The local anesthetic bupivacaine intrathecally has been demonstrated to evoke sensory-motor block in rats and dogs[28,42] by blocking voltage sensitive sodium channels in efferent autonomic and motor nerves. The mechanism behind the prolongation of a nerve block duration of the combination of a local anesthetic with an α_2-adrenoceptor agonist, clonidine[27] and dexmedetomidine[11] has been postulated to be attributable to inhibition of the hyperpolarization-activated cation current and not via an α_2-adrenoreceptor mediated mechanism. Fadolmidine combined with intrathecal bupivacaine increased clearly both the magnitude and duration of antinociceptive response in the rat tail-flick test and in the dog skin twitch test. The intrathecal combination of bupivacaine and clonidine has been reported to produce also synergistic analgesia in the rat tail-flick test and in the formalin test but however not to prolong the duration of antinociceptive effects, suggesting that the duration of the effect of bupivacaine was long enough to allow it to be modified by clonidine.[42] Nonetheless, in humans, when clonidine has been administered as an adjuvant with local anesthetics for peripheral nerve and plexus blocks, it has been reported to prolong the duration of analgesia and sensory block by about 2 h.[51]

Spinally administered local anesthetics induce hypotension by blocking the voltage sensitive sodium channels in efferent autonomic nerves. In the present study, however, bupivacaine had no effects on MAP in dogs. The combination bupivacaine and fadolmidine increased MAP and bupivacaine attenuated the HR decrease seen with fadolmidine on its own. Intrathecal fadolmidine increases initial MAP[34] probably by redistributing from the injection site to the periphery and further acting on α_2-adrenergic receptors in the peripheral vasculature.[24,30] Furthermore, α_2-adrenergic agonists are known to reduce blood pressure by acting both on sympathetic preganglionic neurons in the spinal cord and by decreasing norepinephrine release in specific brainstem nuclei such as the locus coeruleus[13,24,55] and decreasing HR by inhibiting sympathetic and stimulating parasympathetic pathways.[24,30,35] It has been claimed that the intrathecal combination of lidocaine and clonidine[26] and bupivacaine and clonidine at antinociceptive doses might decrease systemic blood pressure in rats.[42] In this study, the combination of bupivacaine and fadolmidine decreased HR but not MAP in dogs. Clonidine allows usage of lower doses of spinal bupivacaine thus diminishing the risk for hypotension evoked at higher bupivacaine concentrations in humans.[8,54] However, in the clinic, the combination of a local anesthetic and clonidine has evoked side effects such as hypotension, bradycardia and sedation.[41,56,57]

α_2-Adrenergic agonists induce centrally mediated effects such as sedation, a hypothermic response and vomiting in both experimental animals and humans.[24,38,53] In dogs, at antinociceptive doses no sedation and a hypothermic response were observed either after fadolmidine or the combination bupivacaine and fadolmidine dosing. Furthermore, fadolmidine induced emesis only once in a single dog, although dogs are known to be a very sensitive species to α_2- adrenergic agonist-induced emesis.[53] The locus coeruleus has been reported to

TABLE 4 Effects of intrathecal saline (0.5 ml), bupivacaine 3 mg, fadolmidine 60 μg and the combination bupivacaine 3 mg + fadolmidine 60 μg on mean arterial blood pressure, heart rate, respiratory rate and body temperature in dogs

Parameters	Saline 0.5 ml	Bupivacaine 3 mg	Fadolmidine 60 μg	Bupivacaine 3 mg + Fadolmidine 60 μg
Mean arterial blood pressure (mmHg)				
0 h	98 ± 14	97 ± 32	98 ± 20	98 ± 10
0.17 h	94 ± 7	91 ± 31	104 ± 18	107 ± 6
0.33 h	93 ± 10	88 ± 30	103 ± 18	111 ± 13
0.5 h	95 ± 12	92 ± 23	100 ± 19	112 ± 16
0.67 h	102 ± 8	94 ± 27	92 ± 18	113 ± 20
0.83 h	99 ± 11	91 ± 27	96 ± 16	112 ± 19
1 h	99 ± 14	98 ± 24	92 ± 17	115 ± 10
1.5 h	97 ± 9	90 ± 36	95 ± 22	101 ± 15
2 h	97 ± 12	94 ± 28	93 ± 17	105 ± 18
3 h	104 ± 17	99 ± 27	100 ± 24	103 ± 17
4 h	98 ± 11	99 ± 26	97 ± 16	100 ± 19
6 h	94 ± 9	103 ± 23	78 ± 19	101 ± 13
ANOVA; *p* value for group effect treatment, *p* = .0292				*
Heart rate (beats/min)				
0 h	84 ± 24	103 ± 26	89 ± 21	91 ± 12
0.17 h	88 ± 41	109 ± 23	70 ± 23	82 ± 34
0.33 h	84 ± 27	96 ± 31	65 ± 30	93 ± 35
0.5 h	88 ± 26	96 ± 16	66 ± 29	91 ± 33
0.67 h	102 ± 41	97 ± 22	51 ± 25	79 ± 25
0.83 h	79 ± 33	85 ± 8	56 ± 20	86 ± 22
1 h	88 ± 50	93 ± 23	55 ± 21	88 ± 20
1.5 h	82 ± 39	78 ± 11	57 ± 18	58 ± 11
2 h	78 ± 21	78 ± 12	62 ± 21	64 ± 18
3 h	76 ± 30	79 ± 18	66 ± 23	66 ± 21
4 h	75 ± 27	100 ± 21	59 ± 15	78 ± 21
6 h	78 ± 28	92 ± 11	73 ± 14	69 ± 12
ANOVA; *p* value for group effect treatment, *p* = .0529				
Respiratory rate (breaths/min)				
0 h	23 ± 2	25 ± 4	26 ± 5	27 ± 5
0.5 h	24 ± 5	21 ± 4	23 ± 4	20 ± 4
1 h	26 ± 7	18 ± 5	22 ± 2	21 ± 3
1.5 h	24 ± 4	17 ± 1	21 ± 3	20 ± 6
2 h	25 ± 3	17 ± 3	20 ± 6	18 ± 4
3 h	24 ± 6	18 ± 5	20 ± 5	16 ± 3
4 h	23 ± 4	19 ± 3	19 ± 3	17 ± 3
6 h	24 ± 8	20 ± 6	21 ± 5	21 ± 2
ANOVA; *p* value for group effect treatment, *p* = .0308		**		*
Body temperature (°C)				
0 h	38.6 ± 0.1	38.7 ± 0.4	38.6 ± 0.4	38.8 ± 0.3
1 h	38.6 ± 0.6	37.8 ± 1.2	37.9 ± 0.3	37.8 ± 1.2
2 h	38.3 ± 0.4	37.4 ± 1.8	37.4 ± 0.8	36.6 ± 1.6

(Continues)

TABLE 4 (Continued)

Parameters	Saline 0.5 ml	Bupivacaine 3 mg	Fadolmidine 60 µg	Bupivacaine 3 mg + Fadolmidine 60 µg
3 h	38.1 ± 0.3	37.4 ± 1.9	37.0 ± 0.8	36.4 ± 1.9
4 h	38.3 ± 0.4	37.4 ± 1.6	37.1 ± 0.9	36.7 ± 2.2
6 h	38.2 ± 0.5	38.2 ± 0.2	37.8 ± 1.0	37.4 ± 1.5
ANOVA; p value for group effect treatment, p = .1075				

Values are presented as mean ± SD, n = 5. Comparisons are $^{*}p < .05$, $^{**}p < .01$ significant difference from saline response.

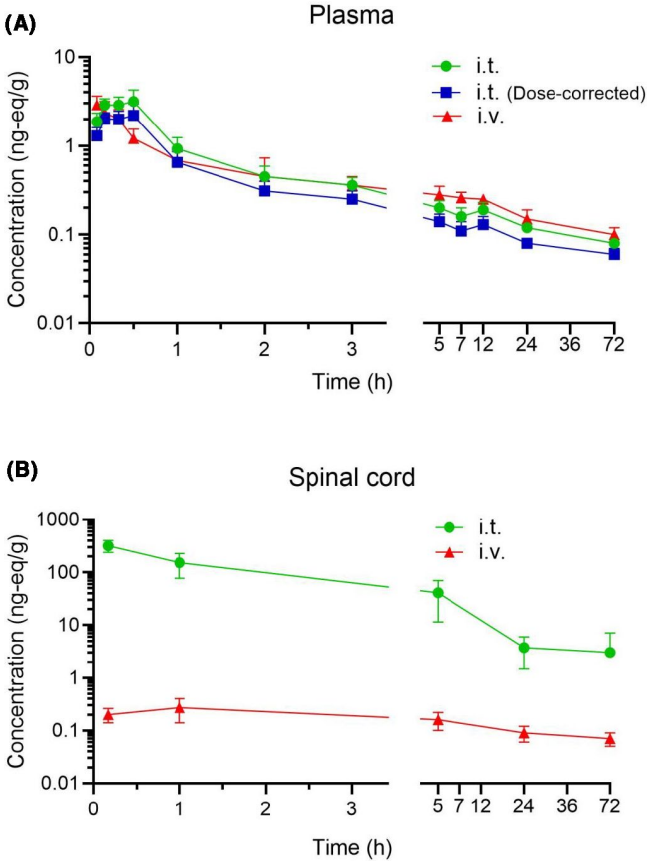

FIGURE 7 Drug-related total and dose-corrected (after intrathecal [i.t.] administration in plasma) radioactivity in rat plasma (A) and spinal cord (B) concerted to mass equivalents of ^{3}H-Fadolmidine base after an intrathecal or intravenous (i.v.) dose of about 3 µg/kg of the compound (hydrochloride). For the dose-correction, the radioactive doses were 4.198 and 2.918 MBq/kg after intrathecal and intravenous administration, respectively. Values are presented as Mean ± SD. (n = 6 per time point)

be the site in the brain mediating the sedative effects of fadolmidine.[62] The results are further support for our belief that fadolmidine has a weaker ability to redistribute to the supraspinal space after its spinal administration. Furthermore, in rats, fadolmidine (0.3–3 µg) alone exerted no effects on motor function, sedation and body temperature but did accentuate the motor function impairment and the hypothermic response induced by a high dose of bupivacaine (300 µg). The high dose of 300 µg was selected for the combination because this dose evoked more prominent and longer duration of effects that at the lower doses on motor function, sedation and body temperature at the maximum antinociceptive time of fadolmidine. In addition, bupivacaine and the combination of bupivacaine and fadolmidine slightly decreased the respiratory rate in dogs. The mechanism behind the decline in the respiratory rate seen with α_2-adrenergic agonists is not clear, but it likely reflects calmness and mild sedation.[53]

In experiments conducted in rats, it has been suggested that clonidine may decrease spinal cord blood flow via vasoconstriction and thereby affect the pharmacokinetics of bupivacaine and alter its hemodynamic effects in the periphery[14] and this may well occur also in humans.[56,57] Fadolmidine induces vasoconstriction in rats[32,33] dogs[34] and sheep,[20] and further reduces spinal cord blood flow in sheep[20] and could affect the distribution of co-administered bupivacaine or vice versa. The results show that a good sensory-motor block of the combination (bupivacaine and fadolmidine) was achieved without effects on MAP, sedation and body temperature. Furthermore, most of the drugs both intrathecal bupivacaine and fadolmidine remaining at the site of application and, rapid clearance of the fadolmidine after reaching systemic circulation was noted. However, to avoid any additional disturbances to the animals, no samples of plasma and spinal fluid were taken for determining the concentrations of fadolmidine and bupivacaine and thus the ability of the compounds to redistribute from the spinal space to periphery and supraspinal spaces after dosing of the combination. Fadolmidine[61] is a polar and a less lipophilic compound than either clonidine or dexmedetomidine.[1] Yaksh et al.[65] have suggested that a more polar α_2-adrenoceptor agonist might have virtues for spinal drug delivery. These current results together with the earlier reports indicate that after systemic[32] and intrathecal[33,34,47] administrations, fadolmidine does not pass readily across the blood-brain barrier nor does it further distribute in the central nervous system.[62] It seems that fadolmidine has a local mode of action with minor central nervous system mediated adverse effects.

In summary, intrathecal fadolmidine as an adjuvant to bupivacaine produces antinociceptive effect and prolongs the duration of motor block of bupivacaine without causing sedation in either rats or dogs or hypotension in dogs. These results indicate that the polar α_2-adrenoceptor agonist fadolmidine, when added to intrathecal bupivacaine, enhances the antinociceptive effect without causing hypotension and could thus represent a suitable combination for spinal anesthesia in humans.

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DISCLOSURE

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AUTHOR CONTRIBUTIONS

Leino, Viitamaa, Salonen, and Haapalinna *participated in research design*.

Leino, Viitamaa, and Salonen *conducted experiments and performed data analysis*.

All authors *wrote or contributed to the writing of the manuscript*.

None *contributed new reagents or analytic tools*.

DATA AVAILABILITY STATEMENT

The generated and analyzed data that support the findings of this study are available from the corresponding author upon reasonable request.

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