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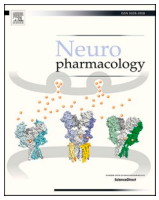

Modulation of spinal morphine pharmacokinetics and antinociception by α_2-adrenergic agonists in the male rat

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ABSTRACT

The synergistic antinociceptive effects of α_2-adrenergic agonists and intrathecal (i.t.) opioids were initially linked to pharmacodynamics. However, the α_2-agonist dexmedetomidine also enhances brain delivery of CSF-administered drugs by increasing glymphatic influx. Here, fadolmidine, a hydrophilic α_2-agonist designed for spinal analgesia, was studied for its sedative, antinociceptive, and pharmacokinetic effects with co-administered lumbar intrathecal morphine. Subcutaneous and i.t. dexmedetomidine served as comparators.

Forty-eight male Sprague-Dawley rats received i.t. lumbar catheters. Sedative effects of i.t. fadolmidine (1–10 μg) and i.t. dexmedetomidine (1–10 μg) were assessed by open field and rotarod tests. The antinociceptive effects of morphine alone (1.5 μg i.t.) and co-administered with i.t. fadolmidine (3 and 10 μg) were evaluated using the tail-flick test. Effects of i.t. fadolmidine and subcutaneous dexmedetomidine (0.2 mg/kg) on morphine concentration within CNS were assessed by liquid chromatography-tandem mass spectrometry at 60 min.

While i.t. dexmedetomidine was sedating, i.t fadolmidine was not. The antinociceptive effects of other treatment regimens weaned at latest after 90 min, whereas the combination of fadolmidine 10 μg i.t. and morphine 1.5 μg i.t. provided antinociception until the end of the measurement period (%maximum possible effect of 77.5 ± 11.5 vs saline 10.6 ± 11.1, $p = 0.0002$ at 120 min). Subcutaneous dexmedetomidine effectively targeted lumbar morphine towards the injection site resulting in a 3335-fold (95% CI: 929−11978) lower brain-to-injection site ratio, versus a 355-fold (95% CI: 196−641) difference with saline.

By improving spinal opioid targeting, α_2-adrenergic agonists dexmedetomidine and fadolmidine may reduce supraspinal side effects, enabling safe and efficacious intrathecal analgesia.

1. Introduction

Intrathecal (i.t.) administration of opioids enables an almost-complete bypass of the blood-brain-barrier (BBB), leading to a higher target concentration with considerably fewer side effects mediated by peripheral, non-spinal targets (De Andres et al., 2022). One of the factors that influence the central nervous system (CNS) disposition of intrathecally-administered opioids is their lipid solubility. For example, the hydrophilic nature of morphine promotes its retainment in the cerebrospinal fluid (CSF) but, at the same time, contributes to its rostral spread (Practice Guidelines for the Prevention, 2016). Therefore, intrathecal morphine possesses important supraspinal dose-limiting effects, the most feared one being respiratory depression (Ummenhofer et al., 2000).

Fadolmidine – earlier known as MPV-2426 or radolmidine – is an experimental α_2-adrenergic agonist investigated for spinal analgesia

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(Onttonen et al., 2000; Pertovaara, 2004). As a highly hydrophilic polar compound, the BBB penetrance of fadolmidine is poor, rendering systemic administration mostly inefficient (Eisenach et al., 1999). Compared to other intrathecally administered α_2-adrenergic agonists, such as clonidine and dexmedetomidine, fadolmidine has weaker systemic sedative and hemodynamic effects (Talke et al., 2003; Xu et al., 2000). This makes fadolmidine an appealing analgesic as the spinal antinociceptive properties of α_2-adrenergic agonists are not mediated by sedation (Pertovaara et al., 1994).

In rodents, systemic and intrathecal co-administration of an α_2-adrenergic agonist with an opioid has been shown to reduce the required opioid dose for analgesia. This synergistic analgesic effect seems to be mediated at the spinal level and has been previously suggested to occur by a pharmacodynamic interaction (Ossipov et al., 1990; Tajerian et al., 2012). However, the newly discovered glymphatic system may influence the pharmacokinetics of intrathecal drugs targeting the CNS. According to the glymphatic concept, CSF tracers reach the deep regions of the CNS along the perivascular spaces of penetrating arterioles, contributing to the delivery of molecules and solutes into the CNS (Iliff et al., 2012; Jessen et al., 2015). In preclinical models, the α_2-adrenergic agonist dexmedetomidine enhances glymphatic influx, promoting the CNS penetration of drugs and compounds administered into the cisterna magna (Lilius et al., 2019; Persson et al., 2022; Benveniste et al., 2017). Therefore, in the light of this new paradigm, the question whether the interaction between α_2-adrenergic agonists and opioids at spinal cord level could be partly mediated by a pharmacokinetic mechanism, arises. Moreover, the lumbar intrathecal administration route – more commonly employed in clinical practice – has been very little studied for these agents.

Inspired by the glymphatic concept, we hypothesized that α_2-agonists may affect the distribution and efficacy of lumbar i.t. opioids (i.e. morphine) by changes in spinal CSF dynamics. To explore this, we conducted a trial in 48 rats, comparing the effect of different fadolmidine doses on the level of alertness, nociception, and morphine concentration in different CNS compartments. Intrathecal and systemic dexmedetomidine was used as comparators, while normal saline was used as control.

2. Material and methods

2.1. Animals and ethical statement

All experiments were approved by the Southern Finland Regional State Administrative Agency (ESAVI-36258-2020). We followed the ethical guidelines of the International Association for the Study of Pain, 3R principles and the EU2010/63 directive, with adherence to the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020). A total of 48 adult, male, pathogen-free Sprague-Dawley rats (180–200 g, Envigo, Venray, Netherlands) were housed in light- and temperature-controlled rooms (temperature 23 ± 2 °C; light cycle of 12 h) in clear plastic cages that were individually ventilated. Only two animals were housed per cage. Food, water, and environmental enrichment (plastic tunnel and sizzle nest) were available ad libitum. All tests were performed during the light phase of the cycle (8 a.m.–4 p.m.).

Each animal was let to habituate in the experiment room for 2 h a day for a period of four days. To avoid damage to the i.t. catheter, rats were housed individually and the enrichments were removed after implantation. All experiments were performed in a randomized and blinded fashion. Aiming to minimize the number of used animals, when possible, a 7-day washout period was employed and then the animals were redistributed to new treatment groups and the experiment was repeated.

2.2. Drugs and solutions

Morphine hydrochloride powder, dexmedetomidine hydrochloride solution (Dexdomitor®, 0.5 mg/ml, Orion Pharma, Espoo, Finland), ketamine hydrochloride solution (Ketaminol vet®, 50 mg/ml, Intervet, Boxmeer, Netherlands), and lidocaine hydrochloride solution (Lidocain®, Orion Pharma, Espoo, Finland) were purchased from the University Pharmacy (Helsinki, Finland). Dexmedetomidine and fadolmidine powders were a kind gift from Orion Pharma (Espoo, Finland). Morphine, dexmedetomidine and fadolmidine powders were dissolved in 0.9% saline. Intrathecal administration was performed in a volume of 10 μl and the i.t. catheter flushed with 20 μl of saline.

2.3. Implantation of the intrathecal catheter

Rats were anaesthetized with s.c. injections of ketamine (30 mg/kg) and dexmedetomidine (0.2 mg/kg) before the surgical procedure that is described below. Sufficient anesthesia was confirmed with the absence of a reaction to paw pinch before the procedure. After the procedure, the rats were housed individually to avoid dislocation and/or leakage of the cannula and the catheter.

Lumbar intrathecal catheterization using the Størkson method with minor modifications was used to avoid subarachnoid space volume occupation by the indwelling i.t. catheter (Størkson et al., 1996). Fur was removed above the L6 vertebra and neck. Para-axial cutaneous incisions were made to gain access to the subcutaneous space. The spinal catheter (25 cm, PE10, 0.28 mm i.d. × 0.61 mm o.d., Clay Adams INTRAMEDIC Polyethylene, BD, Franklin Lakes, NJ) was inserted in the subarachnoid space between the L5 and L6 vertebrae with a guide cannula (20-gauge needle). After the correct localization was confirmed with a slight flick of the paw or tail, or occasionally by backflow of CSF, the catheter was carefully inserted in the subarachnoid space at the level of the caudal ribs (T13). The guide cannula was carefully removed, and the metal wire was inserted in the catheter. A retentive bead was made with a cautery pen and the catheter was attached to paravertebral muscles with 4–0 sutures to avoid dislocation of the catheter. The rest of the catheter was tunneled subcutaneously, revealed from the neck of the animal, and sutured to paravertebral muscles of the neck. The catheter was flushed and filled with saline and the tip was closed by cauterization. After confirming that there were no leaks from the catheter, cutaneous openings were closed with 4–0 sutures. On the next day, the correct placement of the catheter was confirmed with 15 μl lidocaine (20 mg/ml) flushed down by 20 μl of saline. Only rats showing reversible symmetrical paralysis of the hind limbs were accepted for the experiments. None of the operated animals had neurological symptoms after surgery and all of them exhibited symmetrical paralysis of the hind limbs after intrathecal lidocaine injection. After the procedure, seven days of recovery before experiments were assigned to each rat.

2.4. Sedation and nociceptive tests

Sedative effects were assessed before drug administration and 20, 40, 60 and 120 min after with open field and rotarod tests. Rats were habituated to experimental environment 2 h daily for 4 days and 1 h before the beginning of the experiment. The open field test was performed in a dimmed room. The animal was placed on a round box (85 cm diameter) divided into 18 areas and the number of areas animal entered in 120 s were counted. After the open field test, animal was placed on a rotating rod (speed 12 r.p.m.) of the rotarod apparatus Ugo Basile 47700 RotaRod for rats (Gemonio, Italy). The time the rat stayed on the rod was measured. The cut-off was set at 120 s.

Nociceptive behavior was assessed before drug administration and every 30 min for up to 120 min with the tail-flick test. Rats were habituated for experimental settings 2 h daily for 4 days and 1 h before beginning experimentation. Tail-flick latencies were measured with the Ugo Basile 37360 tail-flick apparatus (Gemonio, Italy). The animal was immobilized in a plastic tube covered with a dark cloth. The tail was placed on the infrared heater of the apparatus, the latency time to the flick of the tail was measured three times at each time point and the mean value was used for statistical analyses. The cut-off was set at 10 s to

avoid tissue damage. If the cut-off was reached, the response was registered as a maximum without further testing at that time point.

2.5. Sample collection and drug concentration measurements

After euthanasia by decapitation, the T13–L1 segment of the spinal cord and the brain were collected. The medulla was removed in one piece and the brain was divided in half from midline. The left hemisphere was left intact and stored, while regional CNS tissue samples were carefully dissected from the right hemisphere. Samples were immediately snap-frozen in liquid nitrogen and stored at −80 °C. The CNS tissue samples were weighed, homogenized, and suspended with water, using 1.4 mm ceramic beads and the Bead Mill 24 homogenizer (Thermo Fisher Scientific, Waltham, MA, USA). To precipitate proteins, samples were mixed with methanol containing internal standard and incubated at +6 °C for 15 min. After centrifugation, the supernatants were evaporated to dryness under air flow at 50 °C and reconstituted in 50 μl of water.

The drug concentration measurements were carried out using Sciex 6500 QTRAP with Nexera X2 liquid chromatography-tandem mass spectrometry system (AB Sciex, Toronto, ON, Canada). Morphine and the labelled internal standard morphine-d6, were purchased from Toronto Research Chemicals (North York, ON, Canada). Analytes were separated on Acquity HSS T3 (1.8 μm, 2.1 × 100 + 2.1 × 10 mm, Waters) using a flow rate of 0.4 ml/min. The gradient programme for the mobile phase B was 5–20% at 0–2.1 min, 50% at 2.2–3 min, 90% at 3.1–4 min, and balancing at 5% before next injection. The mass spectrometers were operated in electrospray positive ionization mode (ESI +) using the mass-to-charge (m/z) ion transitions of 286–152. The limit of quantification was 0.005 ng/ml for all analytes.

2.6. Experimental design

In the first experiment, 24 intrathecally catheterized rats were randomly divided into seven groups (n = 3–4). Six groups were treated with 1 μg, 3 μg or 10 μg (10 μl) i.t. dexmedetomidine or fadolmidine flushed with 20 μl of saline (5 μl/min) i.t. The control group received saline (10 μl i.t.). Sedative effects were measured before drug administration and 20, 40, 60 and 120 min after with open field and rotarod tests. After 7 days of wash out, the animals were pooled together, mixed, and the protocol repeated. Based on the first experiment, we proceeded with high fadolmidine doses as they showed no sedative effects for up to 120 min when administered intrathecally at the lumbar level.

In the second experiment, the other 24 rats were intrathecally catheterized and randomly divided in six groups (n = 4). The groups received morphine (1.5 μg i.t.) with saline, morphine (1.5 μg i.t.) with fadolmidine (3 μg i.t.), morphine (1.5 μg i.t.) with fadolmidine (10 μg i.t.), saline with saline, saline with fadolmidine (3 μg i.t.) or saline with fadolmidine (10 μg i.t.). Nociceptive behaviour was monitored with tail-flick test before, and 30, 60, 90 and 120 min after drug administration. After 7 days of wash out, the animals were pooled together, mixed, and the protocol repeated. The pharmacokinetic part was performed with 7 days of wash out period after sedation and nociceptive experiments. Thirty-eight rats were randomly allocated in four groups (n = 9–10). They received morphine (15 μg i.t.), with co-administration of saline i.t., fadolmidine (3 μg i.t.), fadolmidine (10 μg i.t.), or dexmedetomidine (0.2 mg/kg s.c.). The tissue samples were collected at 60 min, as described.

2.7. Statistical analysis, mean percentage change (%MPC), maximum possible effect (%MPE)

Group sizes were determined by the resource equation method supported by expected number of deaths or technical failures (e.g. leaking or detached catheter) of the animals (Charan and Kantharia, 2013). Tail-flick and rotarod test results are presented as maximum possible effect (%MPE) of the mean, calculated as %MPE = [(postdrug latency – baseline latency)/(cut-off time – baseline latency)] × 100%. Open field test results are presented as mean percentage change from baseline (% MPC), calculated as %MPC = [(postdrug latency – baseline latency)/baseline latency] × 100%. Data are presented as mean values with standard error of the mean (±SEM). One way or two-way analyses of variance (ANOVA) followed by Holm-Sidak post hoc tests were performed. In case of the concentration data, the geometric means with the 95% confidence intervals (95% CI) were computed, both for the absolute values and for the concentration ratios. The statistical analysis was performed on the logarithmically-transformed data (ln). The difference was considered significant at $p < 0.05$. Graphics: statistical analyses and figures were performed with GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) and biorender.com (BioRender, Toronto, ON, Canada).

3. Results

3.1. Intrathecal fadolmidine is not sedating, in contrast to intrathecal dexmedetomdine

Firstly, we assessed the sedative potential of the two α_2-adrenergic agonists, fadolmidine and dexmedetomidine, by open field and rotarod tests. Seven different drug regimens were explored: 0.9% saline i.t., fadolmidine (1, 3 or 10 μg) i.t., dexmedetomidine (1, 3 or 10 μg) i.t. (Fig. 1A).

Fadolmidine was not sedating at any of the tested doses or timepoints in the open field test (Fig. 1B). In contrast, the sedative effect of dexmedetomidine was evident at the highest two tested doses (3 μg i.t and 10 μg i.t.) already at 20 min, where the greatest differences compared to the saline were recorded (dexmedetomidine 10 μg i.t. vs saline: %MPC: −83.7 ± 6.2 vs 18.7 ± 13.6, $p < 0.0001$).

The rotarod test results were in agreement with the open field tests. Again, only dexmedetomidine at the highest tested doses (3 μg i.t. and 10 μg i.t.), caused sedation in a dose-dependent manner (Fig. 1C), with highest sedation at 20 min (%MPE: −70.7 ± 4.7 vs 0 ± 0, $p < 0.0001$ for the 3 μg i.t. and −95.3 ± 1.5 vs 0 ± 0, $p < 0.0001$ for 10 μg). These doses of dexmedetomidine had a sedative effect for the first hour (all p-values <0.0001), but only the 10 μg i.t. dose displayed a significant effect after 120 min, compared to saline (%MPE: −51.8 ± 9.6 vs 0 ± 0, $p < 0.0001$). On the other hand, none of the tested fadolmidine doses were sedating at any of the four investigated timepoints.

3.2. Intrathecal fadolmidine prolongs the antinociceptive effect of lumbar intrathecal morphine

After we established that intrathecal fadolmidine is not sedating, we explored its antinociceptive potential using the tail-flick test (Fig. 2A). Compared to saline, morphine 1.5 μg i.t. exhibited an antinociceptive effect only at the 30-min timepoint (%MPE: 63.5 ± 18.5 for morphine 1.5 μg i.t. vs 16.4 ± 17.2 for saline, $p = 0.01$), after which its effect weaned. Fadolmidine 10 μg i.t. regimen displayed an important antinociceptive effect but only for 90 min, peaking at the 60-min mark. Similar effects were seen with fadolmidine 3 μg i.t., both given alone and co-administered with morphine 1.5 μg i.t. Morphine 1.5 μg i.t. + fadolmidine 10 μg i.t. combination (Fig. 2B), produced steady, statistically significant antinociception at all tested timepoints. This effect was not statistically different from that of fadolmidine 10 μg i.t. alone, but was the only regimen superior to morphine at 120 min (%MPE: 77.5 ± 11.5 vs 20.2 ± 18.5, $p = 0.013$). Indeed, in contrast to all other regimens, morphine 1.5 μg i.t. + fadolmidine 10 μg i.t. provided effective antinociception at 120 min (%MPE: 77.5 ± 11.5 vs 10.6 ± 11.1, $p = 0.0002$). The effect of all other tested regimens waned after 90 min at latest.

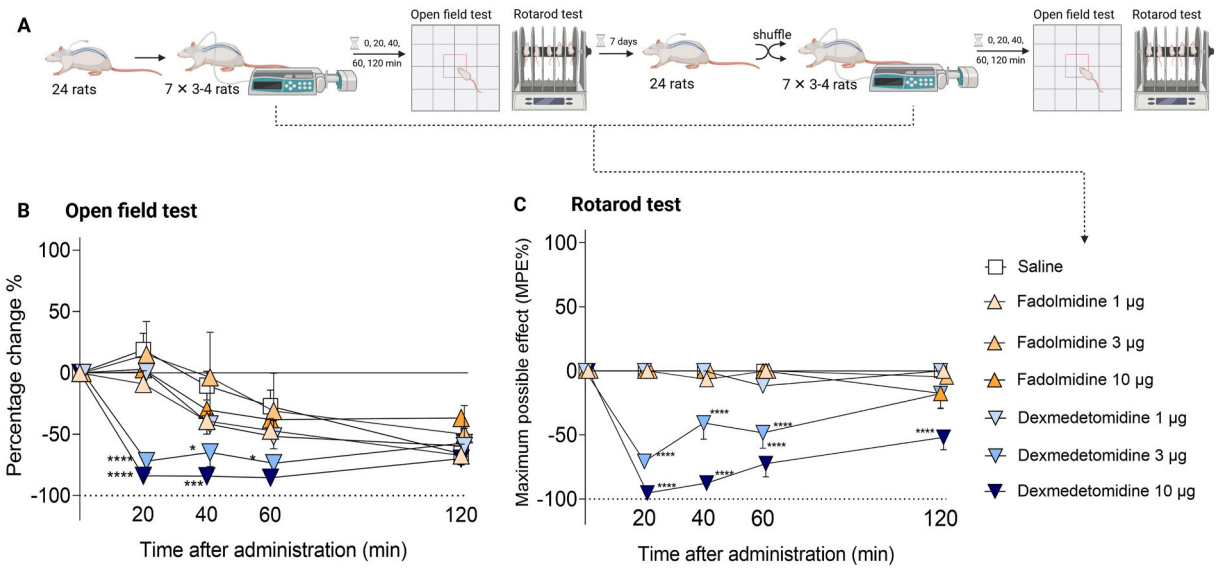

Fig. 1. Intrathecal dexmedetomidine, but not fadolmidine, exhibits sedative behavior (A) Experimental protocol. (B) Open field test results for, fadolmidine (1, 3, and 10 µg) i.t. and dexmedetomidine (1, 3, and 10 µg) i.t. at 20, 40, 60, and 120 min compared to normal saline are shown (n = 7–8). A decrease in activity in all groups, most likely due to habituation effect, was seen at 120 min. The results are plotted here as means of percentage change (%MPC). (C) Rotarod test results for fadolmidine (1, 3, and 10 µg) i.t. and dexmedetomidine (1, 3, and 10 µg) i.t. at 20, 40, 60, and 120 min compared to normal saline are shown (n = 7–8). The results are presented as means of maximum possible effect (%MPE) with a cut-off for stopping the experiments set at 120 s. Standard error of means (±SEM) are presented. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05 Two-way repeated-measures ANOVA with Holm-Sidak post-test.

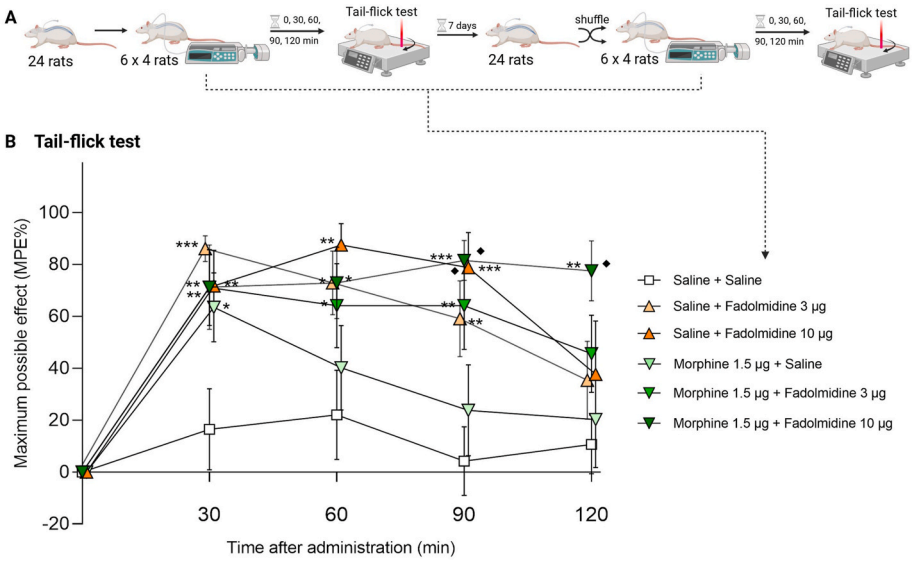

Fig. 2. Co-administration of intrathecal fadolmidine prolongs morphine antinociception (A) Experimental protocol (B) Tail-flick test results for saline + fadolmidine (3 µg or 10 µg) i.t., morphine 1.5 µg + fadolmidine (3 µg or 10 µg) i.t. compared to saline + saline or saline + morphine 1.5 µg i.t. at 0, 30, 60, 90, and 120 min (n = 8). The results are plotted as means of maximum possible effect (%MPE), the cutoff for stopping the experiment being chosen at 10 s to avoid tissue damage. Standard error of means (±SEM) are presented. When compared to the saline + saline group: ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, When compared to the morphine 1.5 µg + saline group: ◆◆◆◆ p < 0.0001, ◆◆◆ p < 0.001, ◆◆ p < 0.01, ◆ p < 0.05. Two-way repeated-measures ANOVA with Holm-Sidak post-test.

3.3. Availability of morphine is higher at the injection site with systemic dexmedetomidine compared to intrathecal fadolmidine

After observing enhancement of the antinociceptive effect of morphine by fadolmidine, we explored whether this could be caused by a change in the CNS distribution of intrathecal morphine. Therefore, we investigated the effect of intrathecal fadolmidine on the concentration of lumbar administered morphine at different CNS levels. Four different drug regimens were investigated: 0.9% saline, fadolmidine (3 µg or 10 µg i.t.) or dexmedetomidine 0.2 mg/kg s.c. in four regions of interest: T13 (i.e. the infusion site), medulla oblongata, hippocampus, and whole brain (Fig. 3A). The results are expressed in ng/g.

At the infusion level (T13), even if the geometric means of morphine tissue concentrations tended to be higher in all drug regimen groups compared to saline, the results were not statistically significant (Fig. 3B). At the supraspinal level, geometric means of morphine tissue concentrations in the three brain regions were lower in all tested regimens compared to the saline group, but only significant in the dexmedetomidine group (Fig. 3C–E). Noteworthy, the greatest effect was exhibited by dexmedetomidine in medulla oblongata, where it decreased the

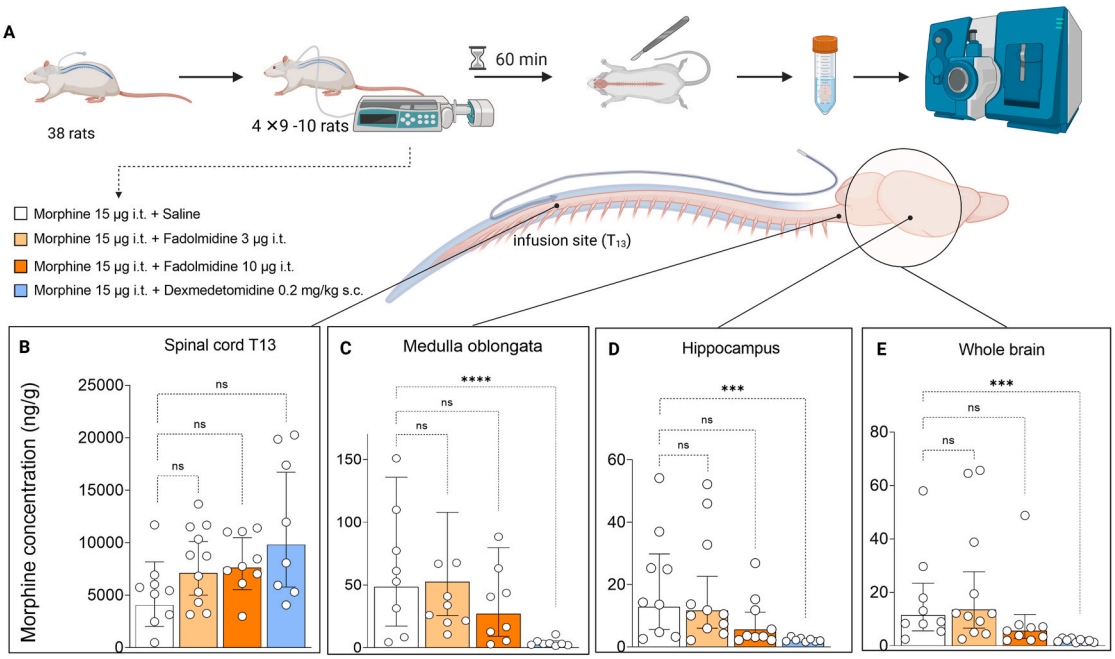

Fig. 3. α_2-adrenergic agonists decrease regional central nervous system (CNS) morphine concentrations at 60 min after intrathecal administration. (A) Experimental protocol; Morphine concentration at (B) spinal cord T13, (C) medulla oblongata, (D) hippocampus, and (E) whole brain levels, 60 min after intrathecal administration (n = 9–10). The results are plotted as geometric means with 95% CI. The statistical significance was calculated from the ln-transformed data ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, Ordinary one-way ANOVA with Holm-Sidak post-test.

morphine concentration with more than 45 ng/g compared to saline (3.4 ng/g vs 48.5 ng/g, p < 0.0001).

We next assessed the morphine concentration ratios between the injection site (T13) and the other regions of interest. Fadolmidine displayed a concentration-dependent trend to retain morphine at the infusion level (Fig. 4A–C). Fadolmidine 10 µg increased the morphine T13-to hippocampus compared with saline (concentration ratios [95% CI]: 1372 times [644–2924] lower morphine concentration vs 315-fold

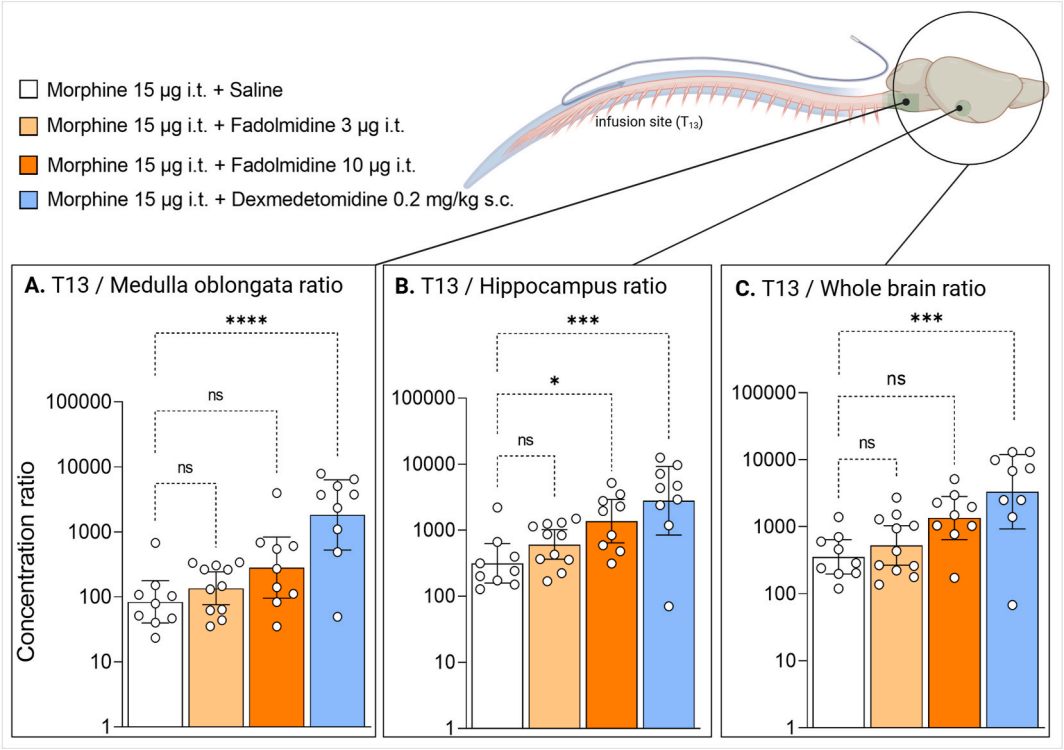

Fig. 4. Alpha2-adrenergic agonists improve the morphine concentration ratios between the injection site and different CNS compartments at 60 min after intrathecal administration. Morphine concentration ratios between the injection site (T13) and (A) Medulla oblongata, (B) hippocampus, (C) whole brain are shown. Results are plotted as geometric means with 95% CI. The statistical significance was calculated from the ln-transformed data ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, Ordinary one-way ANOVA with Holm-Sidak post-test.

[158−627] lower, p = 0.048). Dexmedetomidine also increased all the calculated ratios, compared to the saline group, but more significantly than fadolmidine. For example, a 3335-times [929−11978] lower brain concentration than at the injection site (T13) was observed in the dexmedetomidine group, and only a 355-fold [196−641] difference in the saline group ($p < 0.001$).

4. Discussion

4.1. Summary of main findings in the context of the hypothesis presented in the introduction

In this study we report a novel beneficial pharmacokinetic interaction with lumbar intrathecal morphine and α_2-adrenergic agonists. Both fadolmidine i.t. and dexmedetomidine s.c. improved targeting of morphine to the lumbar injection site, as shown by the improved concentration ratios between rostral structures and the injection point, the effect being more marked with systemic dexmedetomidine. Intrathecal fadolmidine was not sedating at any of the tested doses – as opposed to intrathecal dexmedetomidine – and was shown to prolong the antinociceptive effect of morphine.

4.2. Comparison with other work

While the majority of previous research on the topic of centrally-acting analgesics has focused on pharmacodynamic interactions at spinal level, we explored the central pharmacokinetic interactions between α_2-adrenergic agonists and lumbar intrathecal morphine (Yaksh et al., 2017). Since systemic dexmedetomidine, previously shown to improve glymphatic flow, has strong sedating properties, we wanted to test whether i.t. dexmedetomidine or fadolmidine could act as glymphatic enhancers without being sedative (Lilius et al., 2019). To address this, we compared the sedative effects of approximately equi-antinociceptive doses of i.t. fadolmidine and dexmedetomidine (1–10 μg)(Leino et al., 2009). The antinociceptive potencies of i.t. fadolmidine and dexmedetomidine have been directly correlated to their affinity to the α_2-adrenergic receptors, fadolmidine having slightly higher potency.

We found i.t. fadolmidine non-sedating, as opposed to dexmedetomidine, in agreement with Xu et al. (2000) They concluded that the observed difference is most likely explained by their different systemic and CNS distribution after i.t. administration. Leino et al. showed that lumbar i.t. fadolmidine causes sedation only at doses much higher than 10 μg (i.e. 30 μg), as opposed to dexmedetomidine and clonidine, which were strongly sedative at antinociceptive doses (Leino et al., 2009). The authors suggest this to occur because their systemic egress and subsequent brain distribution happens faster than the supraspinal redistribution of fadolmidine inside the CSF compartment. Notably, since fadolmidine is a drug designed for spinal administration with low BBB penetrance, systemic administration even at 300 μg/kg was non-sedating (Wei et al., 2002). On the other hand, systemic dexmedetomidine readily passes the BBB causing marked sedation and is widely employed clinically for this purpose (Gertler et al., 2001). After finding i.t. dexmedetomidine sedating, we proceeded to the antinociceptive tests only with the non-sedating agent – fadolmidine.

Next, we showed that fadolmidine prolongs the antinociceptive effect of i.t. morphine. In some previous studies, the synergistic effect of α_2-agonists and opioids has been explained by an action on the G protein-coupled receptors located on different spinal neuronal populations, but a possible influence of pharmacokinetics could not be excluded (Ossipov et al., 1990; Tajerian et al., 2012). Ahsan et al. reported pharmacodynamic synergism between systemic dexmedetomidine and the opioid butorphanol (Ahsan et al., 2020). Furthermore, while i.t. fadolmidine evoked dose-depended antinociception, co-administration with bupivacaine provided synergistic antinociception (Leino et al., 2021). This was, again, mainly explained by pharmacodynamics, but the vasoactive effects of α_2-agonists and possible pharmacokinetic implications were also considered. Due to their vasoconstrictive properties demonstrated in various species, spinal administration may reduce spinal cord blood flow (SCBF)(Eisenach et al., 1999; Lehtimäki et al., 2008; Leino et al., 2020). This may lead to a concomitant parenchymal volume reduction, therefore expanding the free subarachnoid and perivascular CSF compartments, turning the subarachnoid space into a reservoir. In support of this, we have reported systemic dexmedetomidine to increase both intracranial and perivascular CSF volume (Persson et al., 2024). Based on these findings, we hypothesized that the time of morphine at spinal level is prolonged and the tissue availability is increased by co-administration of α_2-agonists.

To test our hypothesis, we employed s.c. dexmedetomidine as a positive control for i.t. fadolmidine to assess their effect on the pharmacokinetics of co-administered lumbar morphine. This was based on our previous work, in which s.c. dexmedetomidine markedly improved the targeting of the intrathecal co-administered drugs to the CNS tissue at 60 min and provided stable sedation throughout the procedure (Lilius et al., 2019). The different administration routes chosen for the two α_2-agonists are also justified by their clinical use – fadolmidine designed as a spinal analgesic, while dexmedetomidine mostly employed in the intensive care units as a sedative agent. The selected dexmedetomidine dose (0.2 mg/kg s.c.) elicits systemic vasoactive effects, while i.t. fadolmidine at the studied doses (1–10 μg) has been shown to trigger only minimal changes in systemic hemodynamic parameters (Lilius et al., 2019; Leino et al., 2020). Intrathecal morphine bears no effect on the SCBF (Dohi et al., 1983). Therefore, we infer that the above-mentioned reservoir effect generated by α_2-agonists – more apparent with systemic dexmedetomidine – targets morphine towards the injection site.

To assess the influence of α_2-agonists on the pharmacokinetics of i.t. morphine, we measured tissue morphine concentrations at several CNS levels 60 min after lumbar administration. The morphine concentration ratios between lumbar injection site and the higher structures were improved by both α_2-adrenergic agents, with a markedly pronounced effect observed with systemic dexmedetomidine (Fig. 4). Morphine concentrations were measured by liquid chromatography-tandem mass spectrometry as in a previous study conducted by our group (Blomqvist et al., 2022). Throughout the investigated CNS levels, morphine concentrations in the control group were in line with our previous study, taking into account a 10-fold dose difference. Therefore, we can confirm the reliability of the employed method due to consistency in the obtained data.

Finally, α_2-adrenergic agonists are known to increase glymphatic transport of intracisternal CSF tracers and drugs (Benveniste et al., 2017; Xie et al., 2013; Hablitz et al., 2019). We previously showed that dexmedetomidine increases the spinal and supraspinal delivery of intracisternal drugs by enhancing the glymphatic CSF influx (Lilius et al., 2019). However, since the intracisternal route is not routinely used in clinical practice, we proceeded by exploring the lumbar intrathecal administration route, which is the most common route for neuraxial administration of opioids in treatment of postoperative and chronic pain (Gay, 2002; Raffaeli et al., 2006; Anderson et al., 2001). This study concludes that pharmacokinetic modulation contributes to the beneficial interaction between α_2-agonists and spinal opioids.

4.3. Methodological considerations, limitations, and suggestions for further studies

First of all, we used only male rats in this study, which could be seen as a limitation due to some differences in nociception reported in previous studies (Ro et al., 2020). Nevertheless, a study in mice showed that biological sex plays no influence on glymphatic influx, irrespective of the age (Giannetto et al., 2020).

Secondly, in a previous study we showed that, at the level of the brain, T_{max} for naloxone after intracisternal administration was at 60 min (Lilius et al., 2019). Considering the small dimensions of the spinal

cord structures, and before analyzing the data from the sedation and nociception tests, we chose the same time point for tissue sampling in this study. However, when analyzing the results of the tail-flick test, we noticed that only the combination of fadolmidine and morphine provided long-lasting antinociception until the end of the experiment (120 min timepoint). Therefore, extending the experiment time to 240 min and the tissue sampling time to 120 min could be of interest. More detailed understanding of the pharmacokinetic-pharmacodynamic interaction would need an isobolographic analysis including several tested doses of both agents (Ahsan et al., 2020).

Moreover, after administering the drugs, the rats were allowed to move freely for 60 min. The drug effects on mobility – ranging from complete lack of sedation to immobility – could explain, at least partially, the CNS morphine concentration differences seen with the two drugs. Exploring the effect of immobility on the distribution of spinal drugs can be the topic of further research in humans.

Finally, as described in the previous section, we suspect that α_2-agonists, through vasoconstriction and concomitant parenchymal volume reduction, lead to an expanded CSF compartment which plays the role of a reservoir for the co-administered morphine at the injection site. This may decrease the egress of morphine to systemic circulation and its supraspinal distribution after intrathecal administration. To confirm this hypothesis, CSF and plasma morphine levels after intrathecal administration could be co-analyzed in a future study.

4.4. Clinical implications and future perspectives

Current findings contribute to a better understanding of the pharmacokinetic interactions between α_2-adrenergic agonists and opioids and could lead to promising clinical implications. Parturient and obese female patients are at an increased risk of cephalad distribution of i.t. drugs. This may be due to decreased subarachnoid space volume following increased intra-abdominal pressure and a shorter spinal cord length compared to male patients (Ni et al., 2018). Even if the benefits of i.t. opioids are undeniable, side effects caused by supraspinal spread of the drug, such as respiratory depression and cardiovascular effects, can be life-threatening (D'Angelo et al., 1994; DeBalli and Breen, 2003). Depending on the desired anatomical level of analgesia, but also to avoid the serious side effects generated by its supraspinal distribution, the spread of the opioid from the injection site can be modulated by adding an α_2-adrenergic agonist. For example, co-administration of i.t. fadolmidine and an opioid could prove beneficial during labor and delivery.

On another plane, intrathecal drugs such as nusinersen – an antisense nucleotide medication used to treat spinal muscular atrophy –, and baclofen – an antispastic agent administered mostly by intrathecal pump infusion – are designed to act spinally (Bennett, 2019; Finkel et al., 2016; Rivera Díaz et al., 2013). Co-administration of nusinersen with an α_2-adrenergic agonist could improve its targeting at the spinal level, reducing its systemic egress. Similarly, coupling baclofen with an α_2-agonist could reduce its supraspinal distribution and concomitant adverse effects (Aronson, 2016).

To conclude, s.c. dexmedetomidine, although strongly sedating by itself, prevents supraspinal distribution of intrathecal morphine from its administration site, while i.t. fadolmidine prolongs its antinociceptive properties without causing sedation. Thus, co-administration of fadolmidine with a lumbar opioid such as morphine, could prove beneficial when prolonged analgesia is required without sedation. On the other hand, by improving the targeting of intrathecal opioids at spinal level, dexmedetomidine could reduce the adverse events caused by their supraspinal distribution, leading to a safer and more efficacious intrathecal analgesia in the future. Therefore, α_2-adrenergic agonists should be considered as valuable tools in the kit of spinal anesthesia.

CRediT authorship contribution statement

Radu G. Copie: Writing – review & editing, Writing – original draft, Formal analysis. **Kim Blomqvist:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Melina Farzaneh Kari:** Investigation. **Mika Kurkela:** Investigation. **Mikko Niemi:** Conceptualization. **Pekka V. Rauhala:** Conceptualization. **Terhi J. Lohela:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis. **Marko Rosenholm:** Writing – review & editing, Investigation. **Tuomas O. Lilius:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

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Data availability

Data will be made available on request.

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