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Research Paper

Systemic daily morphine enhances the analgesic effect of intrathecal dexmedetomidine via up-regulation of alpha 2 adrenergic receptor subtypes A, B and C in dorsal root ganglion and dorsal horn

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Abstract

Objectives It has been reported that the effect of intrathecally administered $\alpha 2$ adrenergic receptor ($\alpha 2$ AR) agonists is enhanced in mice that are chronically tolerant to systemic morphine. However, contributory factors have not been identified. Here we examined whether repeated systemic morphine affected the analgesic potency of intrathecal dexmedetomidine and the expression of subtype A, B and C $\alpha 2$ AR ($\alpha 2$A, $\alpha 2$B and $\alpha 2$C AR) in the dorsal root ganglion and dorsal horn in mice.

Methods After subcutaneous injection of morphine or saline for two weeks, dexmedetomidine was administered intrathecally to evaluate its antinociceptive effect. Also, the $\alpha 2$ AR subtypes and μ-opioid receptor mRNA expression in lumbar dorsal root ganglion was quantified using PCR, and $\alpha 2$A and $\alpha 2$C AR in lumbar dorsal root ganglion and dorsal horn were examined by immunohistochemistry.

Key findings Daily morphine enhanced the antinociceptive effect of intrathecal dexmedetomidine, increased all the $\alpha 2$ AR subtypes but decreased the μ-opioid receptor mRNA expression in dorsal root ganglion and increased immunoreactivity of $\alpha 2$A and $\alpha 2$C AR in dorsal root ganglion and dorsal horn.

Conclusions These results suggest that systemic daily morphine enhances the analgesic effect of intrathecal dexmedetomidine via up-regulation of the $\alpha 2$A, $\alpha 2$B and $\alpha 2$C AR in lumbar dorsal root ganglion and dorsal horn.

Keywords alpha2 adrenergic receptor; dexmedetomidine; dorsal horn; dorsal root ganglion; morphine

Introduction

It has been reported that the effect of $\alpha 2$ adrenergic receptor ($\alpha 2$ AR) agonist clonidine was enhanced in mice with tolerance to morphine.[1] However its mechanisms have not been elucidated.

Morphine and synthetic opioid derivatives are commonly used for the treatment of severe pain, but troubling side effects, development of tolerance and dependence, and the risk of addiction limit the usefulness of this class of drugs, especially for prolonged use.

While the analgesic efficacy and most of the typical undesired opioidergic effects of morphine are mainly mediated by the μ-opioid receptor, the noradrenergic system also plays an important role in opioid actions.[2] The $\alpha 2$ AR antagonist yohimbine attenuates the analgesic effect of opioids in rats.[3] Coadministration of $\alpha 2$ adrenergic with opioid receptor agonists results in a multiplicative or greater-than-additive analgesic effect, which has been characterized as adrenergic–opioidergic synergy.[4–6] The μ-opioid receptor is activated to a greater extent when it coexists with $\alpha 2$A ARs than when it exists alone.[7] The synergistic interaction of $\alpha 2$ AR agonists with opioids is important in clinical pain management.[8–12] To minimize side effects, co-administration of $\alpha 2$ AR agonists can help to reduce effective opioid dosage.[13] Co-administration may also be useful in the treatment of opioid-insensitive chronic pain states.[14]

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Correspondence to the pharmacological subtypes, three subtypes of α2 AR – α2A, α2B and α2C – have been cloned in humans, rats and mice.[15–18] And morphine has been shown to interact with the three subtypes.[19]

To elucidate the mechanisms of the enhanced effect of spinal α2 AR agonist under tolerance to morphine, we examined the effect of daily morphine on the antinociception induced by intrathecal α2 AR agonist dexmedetomidine and the spinal expression of μ-opioid receptors and the three subtypes of α2 ARs by quantitative PCR and immunohistochemistry.

Materials and Methods

Animals

After receiving approval of the experimental protocol from the Animal Care and Use Committee at Osaka University, 48 adult male C57BL/6 mice (8–10 weeks old, body weight 23–25 g) were used in the current study. All efforts were taken to minimize the number of mice used and adequate measures were taken to minimize their pain and discomfort. The mice for the control and treatment groups originated from the same litter and were stratified to match weight distribution as closely as possible. All mice were housed under standard laboratory conditions with free access to food pellets and water. Lighting followed a regular light–dark cycle, with lights on from 8:00 to 20:00 h.

Drugs

Morphine hydrochloride (Takeda Pharmaceutical Company Limited, Osaka, Japan) and dexmedetomidine (Maruishi Pharmaceutical Co., Osaka, Japan) were used. Both drugs were dissolved in physiological saline and normal saline was used as a control. Morphine was administered subcutaneously in a dose of 10 mg/kg or 0.1 mg/kg once daily for 14 days, and dexmedetomidine was administered intrathecally in a dilution of 30 ng/3 μl. For intrathecal injection, a previously described method, with the head of the mouse placed in a plastic cap and the body held with one hand, was used.[20] A 27-gauge needle attached to a Hamilton microsyringe was inserted into the subarachnoid space between the L5 and L6 vertebrae of the conscious mouse and 3 μl of drug solution was slowly injected, as described by Hylden and Wilcox.[21] Accurate placement of the needle was confirmed by a quick 'flick' of the mouse's tail.[22]

Antinociceptive test

Thermal nociceptive thresholds were assessed by a radiant heat test[23] in which the centre of the plantar surface of the hind paw was exposed to a projector-light beam. The time for purposeful withdrawal of the paw from the light beam was registered. A 15-s cut-off time was established to prevent tissue damage. In this antinociceptive test, the mean of three consecutive measurements separated by a period of 1 min was used.

Real-time PCR

After each mouse was euthanized under sevoflurane anaesthesia, the fifth lumbar dorsal root ganglion was immediately removed. Tissue samples were disrupted and homogenized. In a final volumes of 12 μl, total RNA was isolated from the samples using RNeasy Kit (RNeasy Mini Kit, Quiagen, Valencia, USA) and the cDNA synthesis reaction was performed using SensiscriptRT Kits (Quiagen) according to the manufacturer's protocols. In total reaction volumes of 35 μl, amplification reactions were carried out in a 7300 Real-Time PCR System (Applied Biosystems, CA, USA) using components of the TaqMan PCR Core Reagent Kit (Applied Biosystems), according to the kit manufacturer's protocol. Commercially available primers (TaqManGene expression assays, adrenergic-receptor-alpha2a, adrenergic-receptor-alpha2b, adrenergic-receptor-alpha2c and opioid-receptor-mu1; Applied Biosystems) were used for cDNA amplification. The amplification conditions were 2 min at 50°C and 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. For relative quantification, we used β-actin (TaqManGene expression assays, actin-beta-cytoplasmic; Applied Biosystems) as a housekeeping gene. The instrument's software normalizes the signal to an internal reference ([DELTA]Rn) and calculates the threshold cycle (CT) at which an increase in reporter fluorescence above a baseline signal can first be detected. Calculation of the relative expression of target mRNA is normalized to the values obtained for β-actin mRNA.

Immunohistochemistry

Mice were deeply anaesthetized with 5% sevoflurane. A needle was inserted into the left ventricle, and quickly the right auricle was cut as an exit for blood, and 100 ml fixative containing 4% paraformaldehyde in phosphate-buffered saline (PBS) was perfused. The fifth lumbar dorsal root ganglion and lumbar enlargement of spinal cord were removed immediately after perfusion, post-fixed in the same fixative for 12 h, and then cryoprotected in 30% sucrose in PBS for 72 h. Using a cryostat (Leica 3050S, Leica Microsystems, Tokyo, Japan), the spinal cord and the dorsal root ganglion were transversely sectioned into 10-μm frozen slices. Immunohistochemical staining was performed with staining kits. (VECTASTAIN ABC Goat IgG Kit; Vector Laboratories, Peterborough, UK) Free-floating sections were washed in PBS. Following elimination of endogenous peroxidase activity with 0.3% hydrogen peroxide in 30% methanol and preblocking with normal rabbit serum for 30 min, sections were incubated with the primary antibody (1 : 100; polyclonal goat anti-alpha2a-adrenergic-receptor, anti-alpha2c-adrenergic-receptor; Santa Cruz Biotechnology, Santa Cruz, USA) for 72 h at 4°C; then, after incubation for 30 min with diluted biotinylated secondary antibody solution, VECTASTAIN ABC Reagent was added and left for 30 min at room temperature. Finally, a nickel-intensified diaminobenzidine reaction was developed until the desired stain intensity was obtained. Samples from saline-treated mice and morphine-treated mice were stained in parallel for the same duration. Samples were then mounted on the slide glass. The immunoreactivity was observed using light microscope. After immunohistochemistry, the immunoreactivity was photographed with an Olympus digital photomicroscope. Computerised image analysis was performed to quantify the immunoreactive area in the samples and analysed using WinROOF (version 5.5) (Mitani corpora-

tion, Fukui, Japan). Five random fields per tissue section in dorsal root ganglion and spinal dorsal horn were scanned. Positive immunostaining was highlighted by setting the gray-level detection limits to threshold and the highlighted immunoreactivity obtained as percentage area of the field scanned.

Experiment 1

To investigate the effect of intrathecal dexmedetomidine on mice receiving long-term daily doses of morphine, the mice were assigned to three groups ($n = 6$ per group). Morphine (10 mg/kg or 0.1 mg/kg) or saline was injected subcutaneously once a day for 14 consecutive days. Twenty-four hours after the final injection, radiant heat tests were performed. After determination of withdrawal threshold base value, dexmedetomidine (30 ng/3 μl) was injected intrathecally and the withdrawal threshold was measured at 15, 30, 45, 60 and 90 min after dexmedetomidine injection.

Experiment 2

To investigate the effect of long-term daily administration of morphine on the expression of μ-opioid receptors and α2A, α2B, and α2C ARs in dorsal root ganglion, the mice were assigned to three groups ($n = 6$ per group). Morphine (10 mg/kg or 0.1 mg/kg) or saline was injected subcutaneously once a day for 14 consecutive days. To quantify the mRNA expression of μ-opioid receptors and α2 ARs, on the day following the last injection, the dorsal root ganglion was removed and examined.

Experiment 3

As an increase in α2A, α2B, and α2C AR mRNA expression in dorsal root ganglion for the 10 mg/kg morphine group was observed in experiment 2 compared with that for saline group, the next study was conducted to examine the expression for protein level and also to evaluate the effect on spinal dorsal horn as well as dorsal root ganglion because the intrathecal dexmedetomidine in experiment 1 might activate both spinal cord and dorsal root ganglion neurons. The immunoreactivity of α2A and α2C ARs in lumbar fifth dorsal root ganglion and lumbar dorsal horn was examined after daily injection of 10 mg/kg morphine.

Statistics

The values for thermal nociceptive thresholds in antinociceptive tests were expressed as means ± SEM. The data were statistically compared using two-way repeated-measure analysis of variance (factors: morphine treatment and time after intrathecal dexmedetomidine injection) and Fisher's Protected Least Significant Difference test.

Calculated relative mRNA levels in different treatment groups were expressed as mean ± SD and the data were analysed using Kruskal–Wallis test followed by Scheffe's post-hoc test. A value of 100% represents the relative expression of target mRNA normalized to β-actin mRNA in the dorsal root ganglion of saline-treated mice.

The percentage area of highlighted immunoreactivity in the saline and 10 mg/kg morphine group was expressed as mean ± SD and the data were analysed using Mann–Whitney *U*-test. The criterion for statistical significance was $P < 0.05$.

Results

Experiment 1

Figure 1 shows the thermal paw withdrawal test after intrathecal dexmedetomidine. Significant difference was found for two factors: morphine doses ($F = 15.7$, $P = 0.0001$) and time after intrathecal dexmedetomidine injection ($F = 5.45$, $P = 0.0056$). Although the intrathecal injection of dexmedetomidine demonstrated an analgesic effect in all three groups and withdrawal latency was significantly extended at 15, 30 and 45 min after injection, the analgesic effect in the 10 mg/kg morphine group was greater than that in saline group and 0.1 mg/kg morphine group. There was no apparent difference between the morphine groups and saline group for basal withdrawal latency (before the intrathecal injection of dexmedetomidine; time 0 in Figure 1), which showed no analgesic effect was observed 24 h after daily morphine for 14 days.

Experiment 2

Table 1 shows the comparison of mRNA expression for α2A, α2B and α2C ARs and μ-opioid receptors in lumbar dorsal root ganglion after daily saline or morphine treatment for 14 days. The mRNA expression in 10 mg/kg morphine group was increased to 263% for α2A, to 856% for α2B, and to 229% for α2C AR compared with that in the saline group (α2A $^*P = 0.0023$, α2B $^*P = 0.0054$, α2C $^*P = 0.0062$). Daily 0.1 mg/kg morphine did not affect the mRNA expression of any subtype of α2 AR (α2A $P = 0.7179$, α2B $P = 0.9672$, α2C $P = 0.9991$).

On the other hand, μ-opioid receptor mRNA expression was reduced to 57% in the 10 mg/kg morphine group

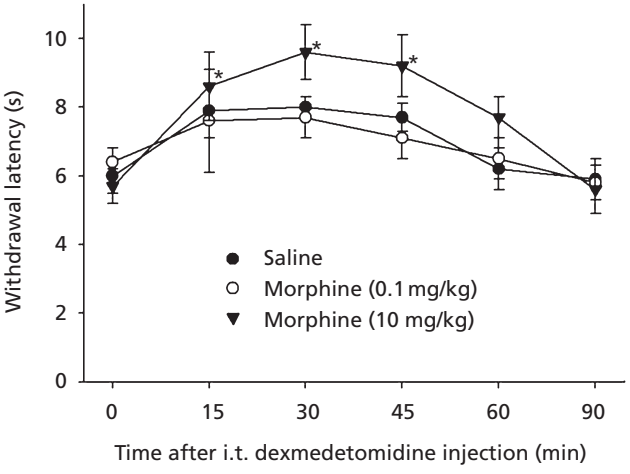

Figure 1 Analgesic effect of intrathecal dexmedetomidine in mice after daily morphine for 14 days. Withdrawal latency is shown. Withdrawal latency was extended at 15, 30 and 45 min after intrathecal injection of dexmedetomidine ($^*P < 0.05$ compared with before injection). The 10 mg/kg morphine-treated group had a greater extension of withdrawal latency than the saline group. Each value is expressed as mean ± SEM and the data were compared using two-way repeated analysis of variance, followed by Fisher's Protected Least Significant Difference. The criterion for statistical significance was $P < 0.05$. Each group consisted of six mice.

Table 1 Altered mRNA expression of α2A, α2B and α2C adrenergic receptors and μ-opioid receptors in the fifth lumbar dorsal root ganglion after daily morphine for 14 days to mice

	Saline	Morphine 0.1 mg/kg	Morphine 10 mg/kg
α2A Adrenergic receptor	1 ± 0.374	0.870 ± 0.374	3.64 ± 2.61*
α2B Adrenergic receptor	1 ± 0.493	1.110 ± 0.552	9.56 ± 8.98*
α2C Adrenergic receptor	1 ± 0.328	1.005 ± 0.335	3.30 ± 1.29*
μ-Opioid receptor	1 ± 0.258	0.717 ± 0.263	0.578 ± 0.207*

The ratio that represents the relative expression of target mRNA normalized to β-actin mRNA is compared between groups. Each value is expressed as mean ± SD and the data are analysed by Kruskal–Wallis non-parametric test followed by Scheffe's post-hoc test. The criterion for statistical significance is $*P < 0.05$. Each group consists of six mice.

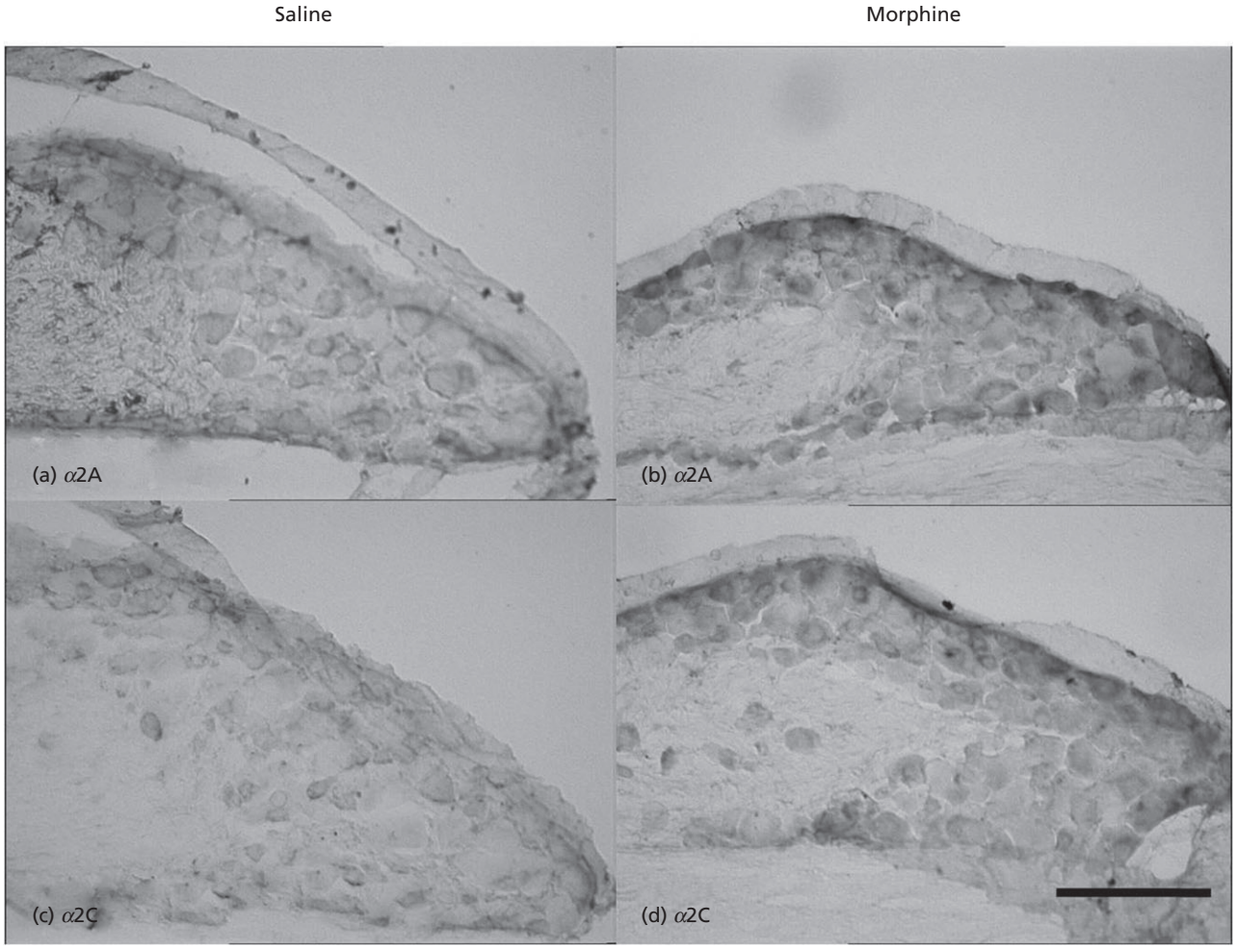

Figure 2 Increased expression of α2A and α2C adrenergic receptors in the fifth lumbar dorsal root ganglion after daily morphine for 14 days. (a, c) The immunoreactivity of α2A and α2C adrenergic receptor of daily saline group. (b, d) The immunoreactivity of α2A and α2C adrenergic receptor of daily morphine group. The expression of both α2A and α2C adrenergic receptor in the daily morphine group was greater than that in the saline group. The scale bar represents 100 μm for all images.

compared with that in saline group ($*P = 0.0186$). Daily 0.1 mg/kg morphine was also associated with less expression of μ-opioid receptor mRNA, but a statistically significant difference was not found between the saline and 0.1 mg/kg morphine groups ($P = 0.1870$).

Experiment 3

Figures 2 and 3 shows the α2A and α2C AR protein expression in the lumbar dorsal root ganglion (Figure 2) and dorsal horn (Figure 3) upon immunohistochemical examination and

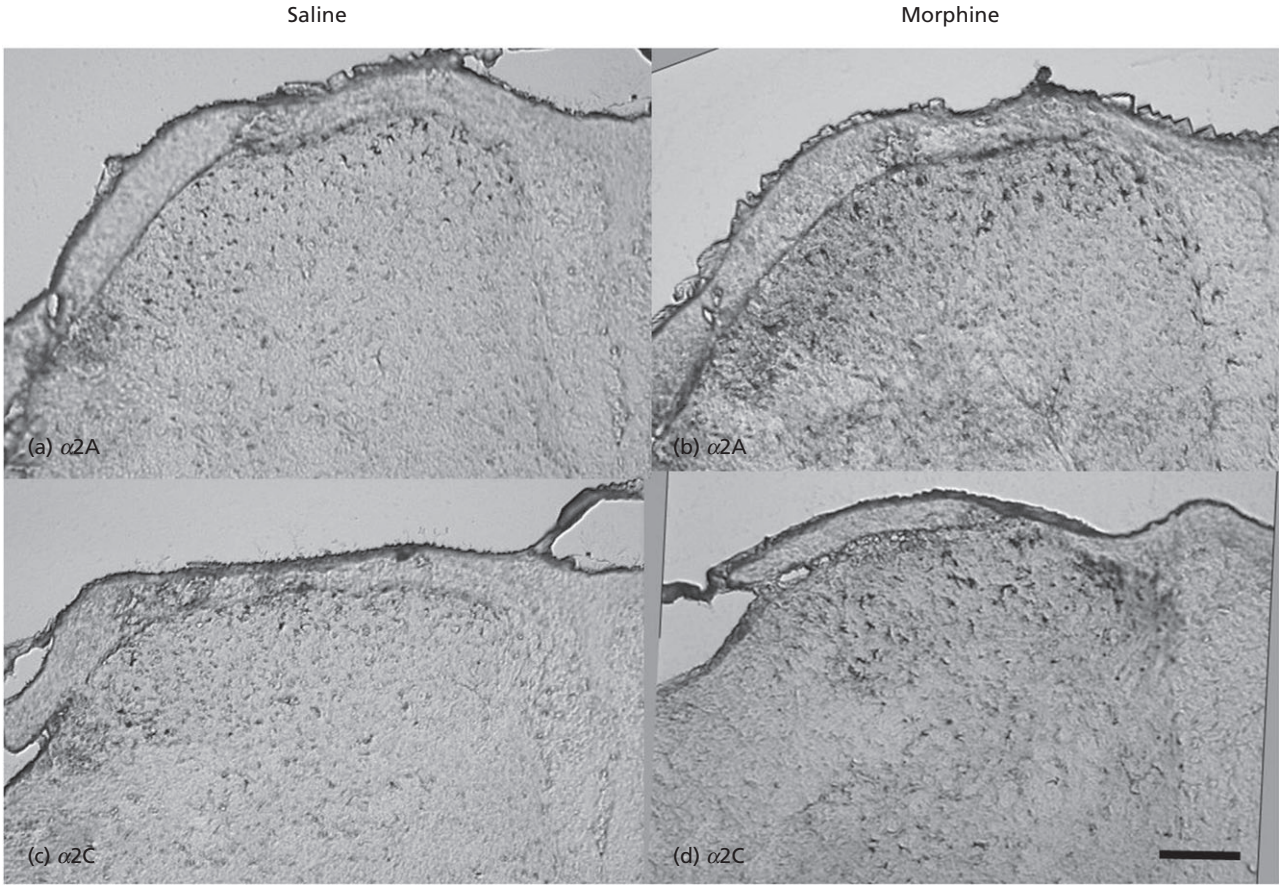

Figure 3 Increased expression of α2A and α2C adrenergic receptor in the fifth lumbar spinal dorsal horn after daily morphine for 14 days. (a, c) The immunoreactivity of α2A and α2C adrenergic receptor of daily saline group. (b, d) The immunoreactivity of α2A and α2C adrenergic receptor of daily morphine group. The expression of both α2A and α2C adrenergic receptor in the daily morphine group was greater than that in the saline group. The scale bar represents 100 μm for all images.

Table 2 Image analysis of α2A and α2C adrenergic receptor in the fifth lumbar dorsal root ganglion and dorsal horn after daily morphine for 14 days to mice

	Dorsal root ganglion		Dorsal horn	
	Saline	Morphine	Saline	Morphine
α2A Adrenergic receptor	4.93 ± 2.13	22.2 ± 11.5*	1.11 ± 0.58	5.32 ± 2.85*
α2C Adrenergic receptor	8.93 ± 4.66	29.6 ± 9.5*	1.32 ± 0.61	5.77 ± 1.64*

The immunoreactivity obtained as % area is compared between the saline and 10 mg/kg morphine groups. Each value is expressed as mean ± SD and the data is analysed by Mann–Whitney *U*-test. The criterion for statistical significance is $*P < 0.05$. Each group consists of six mice.

the results are presented in Table 2. In the dorsal root ganglion neuron, both α2A and α2C ARs were stained and the immunoreactivity was more intense in the 10 mg/kg morphine group than in the saline group. The percentage area of highlighted immunoreactivity of α2A and α2C ARs in the 10 mg/kg morphine group was greater than that in the saline group (α2A $*P = 0.0104$ and α2C $*P = 0.0065$). In the spinal cord section, the expression of both α2A and α2C ARs was observed in the dorsal horn. Greater expression of both α2A and α2C ARs was detected in the 10 mg/kg morphine group than in the saline group. The percentage area of highlighted immunoreactivity of α2A and α2C ARs in the 10 mg/kg morphine group was greater than that in the saline group (α2A $*P = 0.0082$ and α2C $*P = 0.0039$).

Discussion

Daily 10 mg/kg morphine for 14 days increased the withdrawal latency of mouse hind paw induced by intrathecal dexmedetomidine, which suggests that systemically repeated morphine enhanced the spinal analgesia induced by dexmedetomidine. This result is consistent with the findings of a previous report in which the effect of the α2 AR agonist clonidine was enhanced in mice with tolerance to morphine.[1]

In the clinical setting, 10 mg/kg/day morphine is around the maximum dose and 0.1 mg/kg/day is around the initial dose. Daily 0.1 mg/kg morphine for 14 days did not affect the spinal antinociception induced by intrathecal dexmedetomidine.

In the dorsal and ventral horns of the lumbar spinal cord, although α2B AR mRNA signalling is weaker than the other two types of α2 AR signal, all subtypes of α2 AR have been found in in-situ hybridization (ISH) studies.[24–27] In dorsal root ganglion neurons, however, only the α2A and α2C subtypes have been localized in ISH studies.[25,28] In this study, using real-time PCR, in dorsal root ganglion samples we detected mRNA expression of not only α2A and α2C, but also of α2B ARs. This discrepancy might be due to a lower level of expression of the α2B subtype in dorsal root ganglion. After 10 mg/kg morphine for 14 days, however, α2B mRNA expression increased tenfold (Table 1) and this amplification also clarified its existence in dorsal root ganglion. Regarding protein level, the expression of α2A and α2C ARs was found in the superficial layer of spinal dorsal horn and dorsal root ganglion. Daily morphine for 14 days enhanced the immunoreactivity of α2A and α2C ARs in the dorsal root ganglion and spinal dorsal horn. Previous studies have shown the immunoreactivity of α2A and α2C ARs in the superficial layer of the spinal dorsal horn.[29] No report, however, has shown the protein expression of α2B subtype using anti-α2B antibody in spinal cord and dorsal root ganglion. We could not demonstrate α2B AR expression in dorsal root ganglion or spinal cord by protein level. This might be due to the low expression level of α2B ARs.

Daily 10 mg/kg morphine for 14 days increased α2A, α2B and α2C AR mRNA in the dorsal root ganglion and the immunoreactivity of α2A and α2C ARs in the spinal dorsal horn. Daily 0.1 mg/kg morphine for 14 days, however, did not change the expression in the dorsal root ganglion. These results were consistent with antinociception induced by intrathecal dexmedetomidine in the group receiving daily 10 mg/kg morphine for 14 days, but its absence in the group receiving daily 0.1 mg/kg morphine. Interactions of morphine with α2 ARs has been suggested. A recent study, using α2A, α2B and α2C AR knockout mice showed that morphine acted as an agonist at all three subtypes in a competitive fashion, and it was concluded that morphine had a higher affinity for α2B and α2C ARs than α2A ARs.[19] On the other hand, the μ-opioid receptor exists not only singly but as a heterodimer with α2A AR and morphine triggers conformational change of α2A ARs inducing cell signalling.[30] Secondary cell signalling induced by μ-opioid receptor activation by morphine in co-existence with α2A AR was greater than that in the absence of α2A AR. Co-localization of μ-opioid receptors and α2A ARs were also observed in hippocampus neuron.[7] From these previous studies, we might be vulnerable to speculation that regulation of α2 ARs is associated with regulation of μ-opioid receptors and that when the μ-opioid receptor is down-regulated by repeated morphine, the α2 AR is also going to be down-regulated. Our result suggested, however, that the α2 AR was up-regulated when the μ-opioid receptor was down-regulated by repeated morphine and agreed with the behavioral data that the enhanced and prolonged effect of dexmedetomidine was observed in mice with daily 10 mg/kg morphine for 14 days.

Daily 10 mg/kg morphine for 14 days reduced μ-opioid receptor expression in the dorsal root ganglion to 57% compared with expression in the daily saline group. This result is similar to the findings of a study in which chronic morphine treatment reduced the expression of μ-opioid receptor mRNA in rat dorsal root ganglion.[31] On the contrary, other studies have reported that, in mouse spinal cord, although high-efficacy opioid agonists such as etorphine induce μ-opioid receptor down-regulation,[32–36] low-efficacy agonists (e.g. morphine) induce tolerance but do not produce μ-opioid receptor down-regulation.[32,34] In rat brain, chronic morphine did not change μ-opioid receptor mRNA expression.[37] In our study, the reduction in μ-opioid receptor expression was found to be 43% in the 10 mg/kg morphine group using the quantitative PCR method, which was sensitive enough for detection of a mild change in mRNA expression, and this mild reduction might account for the different results in various reports.

Daily 10 mg/kg morphine for 14 days induced less μ-opioid receptor mRNA expression than saline ($P = 0.0186$), but 0.1 mg/kg morphine for 14 days did not ($P = 0.1870$). The difference in μ-opioid receptor mRNA expression between the 10 mg/kg and 0.1 mg/kg morphine groups was not statistically significant ($P = 0.610$) (Table 1). Without statistical significance, 0.1 mg/kg morphine for 14 days showed a tendency to reduce μ-opioid receptor mRNA expression. On the other hand, the difference in α2A, B and C AR mRNA between the 0.1 mg/kg morphine group and 10 mg/kg group was apparent ($P < 0.01$). This suggests that less morphine is needed to alter the mRNA expression for the μ-opioid receptor than for the α2 AR and that μ-opioid receptors are more sensitive to morphine than α2 ARs.

Daily 10 mg/kg morphine for 14 days enhanced the antinociception induced by intrathecal dexmedetomidine, and increased expression of α2 ARs in dorsal root ganglion and dorsal horn. This increased expression of α2 ARs may suggest that either the dorsal root ganglion or spinal cord, or both, is involved in intrathecal antinociception, although which subtype of α2 AR contributes has not been determined.

Spinal adrenergic–opioidergic synergy has been examined mainly by intrathecal administration, where intrathecal coadministration of an opioid and an α2 AR agonist may activate both spinal cord and dorsal root ganglion neurons.[5,29] Coadministration of α2 adrenergic and opioid receptor agonists results in synergy.[5,6] Investigations into which subtype of α2 AR is involved in adrenergic–opioidergic synergy have been made. One study suggested that α2C AR mediated adrenergic–opioidergic synergy;[38] another suggested that α2A ARs were essential to opioid synergy.[29] By contrast, another report found that α2B or α2C ARs were important.[19,39] A recent report presented evidence that α2A and non-α2A ARs were involved in synergy.[40] These various results may be due to the lack of α2 AR-agonist-subtype specificity. Dexmedetomidine, clonidine, ST-91, UK14304, moxonidine and other α2 AR agonists are available, and the subtype specificity of each has not yet been clarified. We used dexmedetomidine, a highly selective α2 AR agonist that binds to all subtypes of α2 AR[41]. Although most studies have suggested that the α2A AR is the primary mediator of the sedative, analgesic and anaesthetic-sparing responses to

dexmedetomidine,[42–52] others have suggested that α2B and α2C ARs also contribute.[53] In light of these previous reports, we suggest that the enhanced antinociceptive effect induced by daily morphine is primarily due to the increased α2A ARs in the spinal cord. Various subtype-selective agonists are, however, reported to have synergy with opioids. Development of subtype-selective agents would enable better elucidation of specific contributions to spinal adrenergic–opioidergic synergy, and mutant studies may reveal which subtypes are involved in spinal analgesia.

Conclusion

Daily morphine for 14 days increased the expression of all subtypes of α2 AR in mouse dorsal root ganglion. The enhanced antinociceptive effect of intrathecal dexmedetomidine may be via up-regulation of α2 ARs in the spinal cord. The α2 AR agonists, clonidine, dexmedetomidine, etc., may have the capacity to relieve pain in the morphine tolerant state, although this needs further studies in models of neuropathic pain and cancer pain.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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