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RESEARCH PAPER

Systemic morphine produce antinociception mediated by spinal 5-HT$_7$, but not 5-HT$_{1A}$ and 5-HT$_2$ receptors in the spinal cord

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Background and purpose: The serotonergic system within the spinal cord have been proposed to play an important role in the analgesic effects of systemic morphine. Currently, seven groups of 5-HT receptors (5-HT$_{1-7}$) have been characterized. One of the most recently identified subtypes of 5 HT receptor is the 5-HT$_7$ receptor. We aimed to examine the role of spinal 5-HT$_7$ receptors in the antinociceptive effects of systemic morphine.
Experimental approach: The involvement of spinal 5-HT$_7$ receptor in systemic morphine antinociception was compared to that of the 5-HT$_{1A}$ and 5-HT$_2$ receptors by using the selective 5-HT$_7$ receptor antagonist, SB-269970, the selective 5-HT$_{1A}$ receptor antagonist, WAY 100635, the selective 5-HT$_2$ antagonist ketanserin as well as the non-selective 5-HT$_{1,2,7}$ receptor antagonist, metergoline. Nociception was evaluated by the radiant heat tail-flick test.
Key results: I.t. administration of SB-269970 (10 μg) and metergoline (20 μg) completely blocked the s.c. administered morphine-induced (1, 3, 5 and 10 mg kg^{-1}) antinociception in a time-dependent manner. Additionally, i.t. administration of SB-269970 (1, 3, 10 and 20 μg) and metergoline (1, 5, 10 and 20 μg) dose dependently inhibited the antinociceptive effects of a maximal dose of morphine (10 mg kg^{-1}, s.c.). I.t. administration of WAY 100635 (20 μg) or ketanserine (20 μg) did not alter morphine-induced (1, 3, 5 and 10 mg kg^{-1}, s.c.) antinociception.
Conclusion and implications: These findings indicate that the involvement of spinal 5-HT$_7$, but not of 5-HT$_{1A}$ or of 5-HT$_2$ receptors in the antinociceptive effects of systemic morphine.
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Keywords: serotonin; 5-HT$_7$ receptor; opioid; descending pathways; analgesia; spinal cord

Introduction

Morphine has been considered to be the gold standard of analgesic drug therapy and is often administered systemically for the treatment of moderate and severe pain. Systemically administered morphine produces a strong analgesic effect through activation of opioid receptors in the brain, spinal cord and at peripheral sites (Matos *et al*., 1995; Advokat *et al*., 1997; Taylor and Basbaum, 2003). The antinociceptive effects of systemic administered opioids are attenuated followed by spinal transection, suggesting a crucial role of supraspinal sites for the antinociceptive efficacy of systemically administered opioids (Advokat *et al*., 1997). It has been reported that fibers descending from supraspinal sites via the dorsolateral funiculus to the dorsal horn of the spinal cord are important mediators of the analgesic effect of morphine (Chiang and Zhuo, 1989; Gilbert and Franklin, 2002). The dorsal horn of the spinal cord is a critical relay with regard to nociceptive transmission as well as its modulation (Chen *et al*., 2005). Nociceptive inputs entering the spinal dorsal horn are subjected to descending modulation from supraspinal centers (Millan, 1997; Millan, 2002; Chen *et al*., 2005). Noradrenergic and serotonergic systems comprise major components of these descending pain modulatory systems (Yaksh *et al*., 1981; Millan, 1997; Ochi and Goto, 2000). Considerable evidence demonstrate that 5-HT is an important modulator of nociceptive transmission in the spinal cord (Yaksh and Wilson, 1979; Furst, 1999; Kristensen *et al*., 2000; Jeong *et al*., 2004). The marked attenuation of the antinociceptive effects of systemic morphine that was observed following the depletion of 5-HT in the spinal cord indicated that the descending serotonergic pain modulatory pathway played a key role in systemic opioid-induced antinociception (Kuraishi *et al*., 1983; Giordano and Barr, 1988). Additionally, it has been reported that systemic and supraspinal opioid administration evokes the release of 5-HT in the spinal cord (Yaksh and Tyce, 1979; Matos *et al*., 1992; Sorkin *et al*., 1993; Kawamata *et al*., 2002). Thus, it has been proposed that 5-HT

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receptors in the spinal cord can be responsible for the antinociceptive effects of systemic morphine (Liu *et al*., 2002). However, the role of the serotonergic receptor subtypes in systemic morphine-induced antinociception is controversial. Some studies have shown that the blockade of the spinal 5-HT$_{1A}$ (Liu *et al*., 2002) or 5-HT$_3$ (Kawamata *et al*., 2002) receptors reduce the antinociceptive effect of systemic morphine. In contrast, others have found that the blockade of spinal 5-HT$_{1A}$ (Bardin and Colpaert, 2004) or 5-HT$_3$ (Xu *et al*., 1994: Liu *et al*., 2002) receptors did not alter systemic morphine antinociception.

Currently, there are seven families of 5-HT receptors (5-HT$_{1-7}$), which may serve either excitatory or inhibitory functions in neurons (Millan, 2002). One of the most recently identified subtypes is the 5-HT$_7$ receptor (Meuser *et al*., 2002; Thomas and Hagan, 2004). Surprisingly, there have been no attempts to directly examine the role of spinal populations of 5-HT$_7$ receptor in the antinociceptive effects of systemic morphine. The 5-HT$_7$ receptors show some similarities in their pharmacological profile to that for the 5-HT$_{1A}$ receptor subtype (Meuser *et al*., 2002). However, attributing pharmacologic properties to a particular subtype with reference to another must be performed cautiously (Bonaventure *et al*., 2002). For example, 8-OH-DPAT has been shown to exhibit a relatively high affinity for the 5-HT$_7$ receptor, and several functions formerly attributed to a 5-HT$_{1A}$ receptor may be likely attributable to the 5-HT$_7$ subtype (Bonaventure *et al*., 2002). The recent development of a novel 5-HT$_7$ receptor antagonist that displays both high affinity and selectivity for the 5-HT$_7$ receptor (Lovell *et al*., 2000) now provides an opportunity to establish more definitive evidence for the physiological roles of 5-HT$_7$ receptors in the spinal processing of nociceptive inputs. The potent and selective 5-HT$_7$ receptor antagonist, SB-269970 displayed at least 100-fold selectivity for the 5-HT$_7$ receptor subtype over the 5-HT$_{1A}$ subtype (Hagan *et al*., 2000; Lovell *et al*., 2000). Metergoline has a high affinity for the 5-HT$_7$ receptor, but lacks selectivity, and is considered to be a nonselectice, 5-HT$_{1,2,7}$ antagonist (Hagan *et al*., 2000).

The present study was undertaken to examine the contribution of spinal 5-HT$_7$ receptor with comparison of 5-HT$_{1A}$ receptor subtype in systemic morphine antinociception in mice by using the selective 5-HT$_7$ receptor antagonist, SB-269970, the 5-HT$_{1,2,7}$ antagonist metergoline and the 5-HT$_{1A}$ selective receptor antagonist, WAY 100635 (Forster *et al*., 1995). In addition to 5-HT$_{1A}$ receptor antagonist, and in order to differentiate any possible involvement of the 5-HT$_2$ receptors with regard to the effect of metergoline, the effects of intrathecally (i.t.) administered ketanserine, a selective 5-HT$_2$ receptor antagonist, on the antinociceptive effects of systemically administered morphine was also examined.

Methods

Adult male Balb-C mice (25–30 g) were used. They were placed in a quiet, humidity controlled room (22±3°C and 60±5%, respectively) in which a 12/12 h light–dark cycle was maintained (08:00–20:00 hours light).

The radiant heat tail-flick test (Columbus, OH, USA; Type 812) was used to assess antinociception. The intensity of the beam was adjusted to produce mean control reaction times of 2–3 s. A cutoff time of 6 s was used to prevent tissue damage. All drugs were freshly prepared by dissolving in saline. Morphine hydrochloride was given in a volume 5 mg kg^{-1} of body weight for subcutaneous (s.c.) injection. I.t. injections were performed according to a previously described method (Hylden and Wilcox, 1980) were a needle is inserted into the lumbar space between the L5 and L6 vertebrae of unanesthetized mice and a volume of 10 μl is injected. Control animals received 0.9 % sterile saline. Morphine was administered s.c. into different groups of mice. Fixed doses of SB-269970, metergoline or WAY 100635 were given i.t. 30 min after the s.c. administration of different doses of morphine. In order to compare antagonist potencies, a maximal dose of morphine (10 mg kg^{-1}, s.c.) was injected into the different groups of animals, and different doses of antagonists were given i.t. 30 min after morphine.

Baseline tail-flick latencies (BL) for each mouse was determined before treatment. After the drug injections, the test latencies (TL) were measured. Dose–response curves were constructed using the latencies determined 30 min after i.t. administration of SB-269970, metergoline, WAY 100635 and ketanserine (i.e.; 60 min after s.c. morphine administration). Dose–response curves were constructed for the antagonists against the maximal dose of morphine (10 mg kg^{-1}, s.c.) from the data collected 30 min after the i.t. administration of the 5-HT receptor antagonists. In order to generate a dose–response curve, data were converted to % Antinociception by the formula % Antinociception = ((TL–BL)/(cutoff latency–BL)) × 100.

All animals were treated in accordance with the guidelines set forth by International association for the study of Pain (Zimmermann, 1983).

Drugs

Morphine HCl was obtained from Sandoz (Switzerland), metergoline from Tocris (USA) and SB-269970, ketanserine and WAY-100635 were obtained from Sigma Chemical Co. (USA). Morphine HCL, SB-269970 and ketanserine were dissolved in % 0.9 saline and metergoline was dissolved in 1% ascorbic acid.

Statistical analysis

The data were expressed as the mean±s.e.m. in all cases. Groups of 8–12 mice were tested at each dose. A nonparametric method of statistical analysis was used. Statistical significance of more than two groups were evaluated by Kruskall–Wallis test ($P<0.05$), followed by Dunnett's multiple test for individual comparisons. Three to four doses of each drug were used to determine the ED$_{50}$ or ID$_{50}$ value. The ED$_{50}$ or ID$_{50}$ values and their 95% confidence limits (95% CL) were calculated from the dose–percent inhibition relations by computer log-linear regression analysis (Tallarida and Murray, 1987).

Results

The mean pooled baseline tail-flick latency of all the treatment groups was 2.47 ± 0.12 s. Systemic administration of morphine (1, 3, 5 and 10 mg kg^{-1}) produced a significant dose-dependent increase in the tail-flick latencies (Figure 1a). Morphine-induced antinociception was indicated by prolongation of tail-flick latencies and reached peak effect 30 min (approaching the cutoff latency of 6 s in the 10 mg kg^{-1} group) following the morphine injection. The morphine-induced increase in the tail-flick latencies lasted beyond 180 min when given in doses of 5 and 10 mg kg^{-1}. I.t. injection of saline or ascorbic acid (10 μl) did not alter morphine-induced antinociception While i.t. administered SB-269970 (10 μg), metergoline (20 μg) alone did not produce any significant effect in tail-flick latencies, the i.t. injection of SB-269970 and of metergoline 30 min after the morphine injection produced a significant, time-dependent reduction in the effect of morphine on tail-flick latencies (Figure 1a and b). The effects of SB-269970 began within 10 min and peaked at 30 min post administration. Inhibition of the antinociceptive effect of morphine lasted 90 min and the tail-flick latencies returned to the corresponding response latencies for 3, 5 and 10 mg kg^{-1} of morphine 120 min after administration of SB-269970 (Figure 1a). In contrast to SB-269970, the blocking effects of i.t. administered metergoline (10 μg) began within 10 min and peaked at 30 min and remained throughout the entire observation period (Figure 1b).

I.t. injection of WAY-100635 (20 μg), a 5-HT$_{1A}$ antagonist, and of ketanserine (20 μg) alone did not produce any significant effect in the baseline tail-flick latencies (Figure 2a and b). In contrast to SB-269970 and metergoline, the i.t. administration of WAY-100635, and ketanserine did not significantly alter the antinociceptive effect of systemic morphine during the entire 180 min observation period (Figure 2a and b).

Figures 1c and 2c illustrate the dose–response curves generated from the data at 30 min after the i.t.administration of SB-269970, metergoline and WAY-100635. The ED$_{50}$ values and 95% CL for systemic morphine, with SB-269970 (10 μg), metergoline (20 μg) and WAY-100635 (10 μg) are shown in Table 1. The ED$_{50}$ values for morphine and WAY-100635 or ketanserine-morphine were not significantly different from those for morphine and saline. However, the

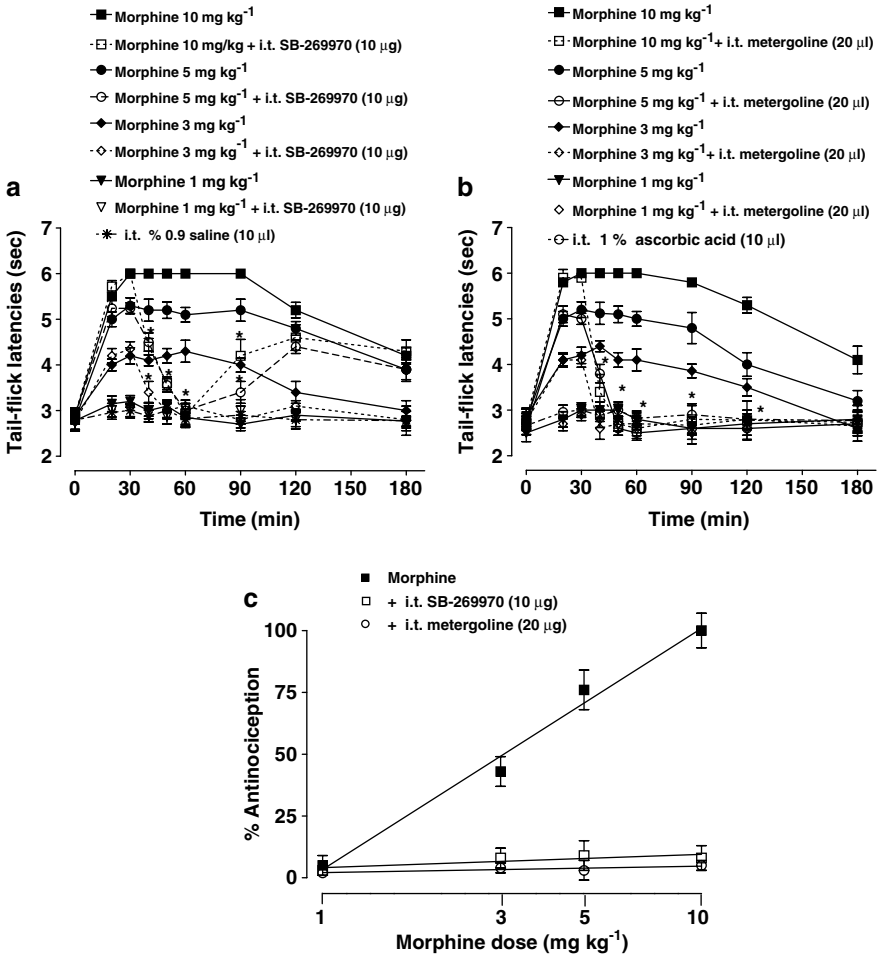

Figure 1 The effects of i.t. injection of SB-269970 (10 μg) (**a**) and metergoline (**b**) on systemic morphine-induced prolongation of tail-flick latencies. SB-269970 was given i.t. 30 min following s.c. morphine administration. Results are presented as mean $\pm$ s.e.m. Tail-flick latencies at the time of peak blocking effects (30 min) were converted to % Antinociception in order to generate the dose–response curve (**c**). $N = 8$–12 per group. *Differences corresponding dose of morphine alone $P < 0.05$.

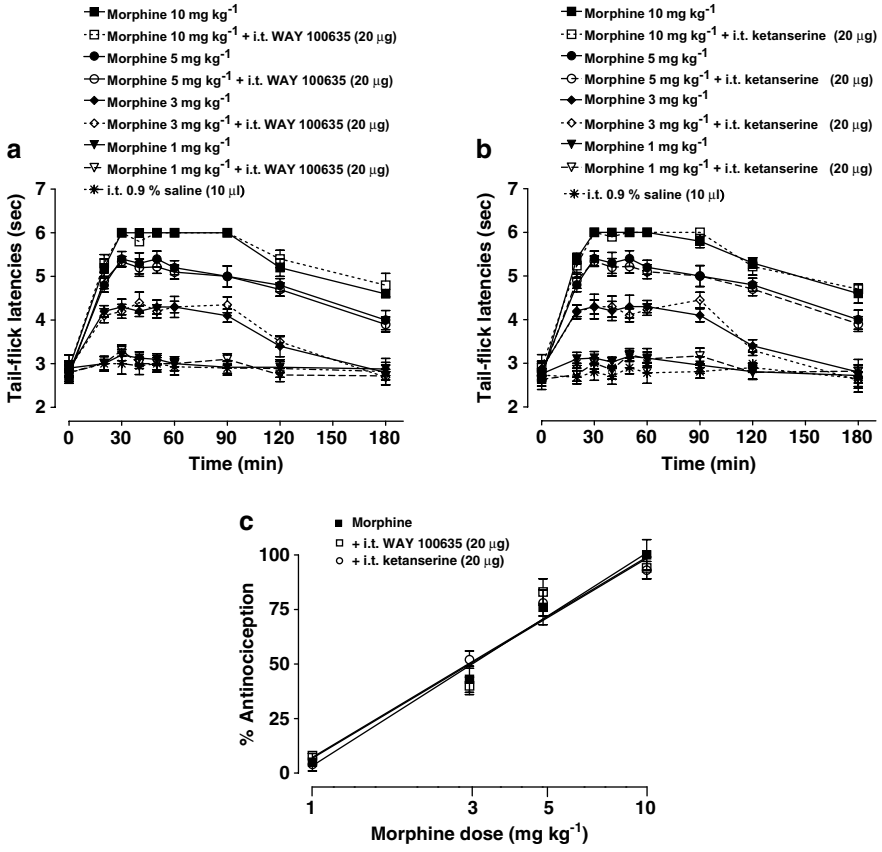

Figure 2 The effects of i.t. injection of WAY 100635 (20 μg) (**a**) and ketanserin (20 μg) (**b**) on systemic morphine-induced prolongation of tail-flick latencies. WAY 100635 (10 μg) and ketanserin (20 μg) were given i.t. 30 min following s.c. morphine administration. Results are presented as mean $\pm$ s.e.m. (**a**). Tail-flick latencies recorded at the time of peak blocking effects (30 min) were converted to % Antinociception in order to generate the dose–response curve for ketanserin (**c**). $N = 8$–12 per group. *Differences corresponding dose of morphine alone $P < 0.05$.

Table 1 The effects of spinally administered SB-269970, a 5-HT$_7$ receptor antagonist and WAY-100635, a 5-HT$_{1A}$ antagonist on s.c. morphine-induced antinociception followed by 30 min after i.t. SB-269970 or WAY-100635 administration

Drug	*Antagonist*	*ED50 and 95% CL (μg mouse−1, i.t.)*
Morphine	Saline i.t.	2.62 (2.16–3.19)
Morphine	SB-269970 10 μg mouse^{-1}, i.t.	>80
Morphine	WAY-100635 20 μg mouse^{-1}, i.t.	2.93 (2.57–3.33)
Morphine	Metergoline 10 μg mouse^{-1}, i.t.	>80
Morphine	Ketanserine 20 μg mouse^{-1}, i.t.	2.87 (2.35–3.34)

Abbreviation: i.t., intrathecal.

ED_{50} values for morphine and i.t. SB-269970 or i.t. metergoline were significantly greater than those for morphine and saline (Table 1).

Next, to examine the potency of i.t. administered SB-269970 and metergoline on systemic morphine-induced antinociception, the different dose of SB-269970 (1, 3 and 10 μg, i.t.) and metergoline (5, 10 and 20 μg, i.t.) were tested against the maximal antinociceptive dose of morphine (10 mg kg^{-1}, s.c) (Figure 3a and b). While the i.t. injection of SB-269970 and of metergoline alone did not produce any significant change in tail-flick latencies (data not shown), the i.t. administration of SB-269970 and of metergoline both produced dose-dependent inhibition of the morphine-induced elevations in tail-flick latencies (Figure 3a and b). The inhibitory effects of i.t. SB-269970 and of metergoline on systemic morphine antinociception began within 10 min and peaked at 30 min after administration. The inhibitory effect of spinal injection of 1 μg of SB-269970 was minimal and lasted 30 min. However, the blocking effect of the spinal injections of 3 and 10 μg peaked at 30 min, were reduced at 60 min and were no longer present at 90 min, as indicated by a return of tail-flick latencies to those observed with the morphine control group (Figure 3a). The 5 μg dose of i.t. metergoline did not alter the antinociceptive effect of systemic morphine. However, the inhibitory effects of i.t. injections of 10 and 20 μg of metergoline peaked at 30 min and lasted 150 min without a reduction of its blocking effect (Figure 3b). The dose–response curves for SB-269970 and for metergoline against the maximal fixed dose of morphine were generated 30 min after the injection of either antagonist (Figure 3c). The ID_{50} value of SB 269970 against morphine was 3.13 (2.67–3.67) μg and was significantly different from, and threefold more potent than that of metergoline, which was 10.07 (9.70–10.44) μg.

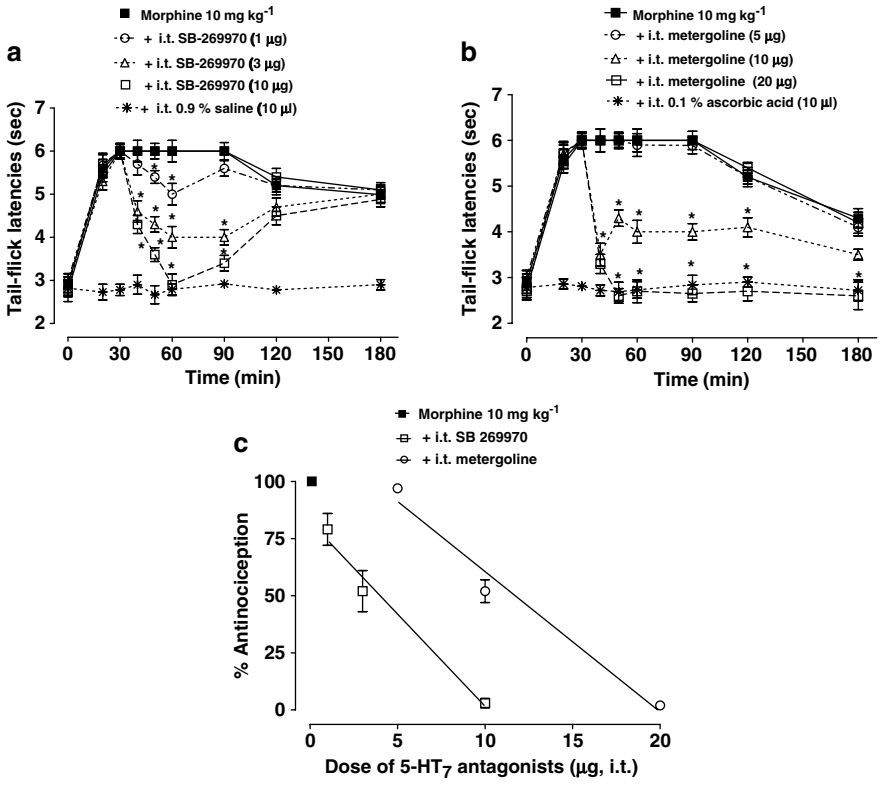

Figure 3 The effects of i.t. injection of different doses of SB-269970 (1, 3 and 10 μg) (**a**) and metergoline (5, 10 and 20 μg) (**b**) on the fixed maximal dose of systemic morphine (10 mg kg^{-1}, s.c.)-induced prolongation of tail-flick latencies. SB-269970 (1, 3 and 10 μg) and metergoline (5, 10 and 20 μg) were given i.t. 30 min following s.c. administration of morphine (10 mg/kg). Results are presented as mean $\pm$ s.e.m. *Differences morphine alone $P < 0.05$. Tail-flick latencies at 30 min following i.t. administration of different dose of SB-269970 and metergoline against a fixed dose of morphine (10 mg kg^{-1}, s.c.) were converted to % Antinociception in order to generate dose–response curve (**c**). $N = 8$–12 per group.

Discussion

The link between opioid analgesia and 5-HT has been suggested for many years and it has been reported that descending serotonergic pathways play an important role in the opioid analgesia. In the present study, we observed that the spinal administration of SB-269970, a selective 5-HT$_7$ receptor antagonist (Hagan *et al*., 2000), completely blocked the antinociceptive effects of systemic morphine. Metergoline, which has a high 5-HT$_7$ receptor affinity, but lacks selectivity (Hagan *et al*., 2000; To *et al*., 2000), also attenuated the antinociceptive effects of systemic morphine. In contrast, the spinal administration of WAY-100635, which selectively block 5-HT$_{1A}$ receptors, and ketanserine, the selective 5-HT$_2$ receptor antagonist, did not alter the antinociceptive effects of systemic morphine. The doses of SB-269970, metergoline, WAY-100635 and ketanserine used here exerted no effect on thermal thresholds when given alone. Thus, the current study demonstrated that spinal 5-HT$_7$ receptors, in contrast to the spinal 5-HT$_{1A}$ or 5-HT$_2$ receptors, appears to play an important role in the antinociceptive effects of systemic morphine.

It is well known that following systemic administration, morphine is evenly distributed throughout the spinal cord and brain, including the potential anatomical sites of action of morphine (Matos *et al*., 1995; Taylor and Basbaum, 2003). Nevertheless, the analgesic actions of systemically administered morphine were in large part mediated by descending pathways from the brain stem to the spinal cord (Advokat *et al*., 1997; Heinricher *et al*., 2001). Serotonergic systems comprise one of the major components of descending pain inhibitory pathways (Yaksh *et al*., 1981; Millan, 1997; Ochi and Goto, 2000). Considerable evidence suggests that the antinociceptive activity of various analgesics depend on integrity of descending serononergic system (Millan, 2002). It has been known that systemic and supraspinal opioid administration evokes the release of 5-HT in the spinal cord and that most, if not all, of the serotonergic innervation of spinal cord is derived from supraspinal sources (Proudfit and Anderson, 1975; Oliveras *et al*., 1977; Yaksh *et al*., 1977; Sorkin *et al*., 1993; Millan, 2002; Gilbert *et al*., 2003). In this regard, it is possible that there is an existence of specific neuronal circuits whereby systemic morphine can modulate nociception through activation of a descending serotonergic system to the spinal cord and acting on serotonergic receptors in the dorsal horn. Seven families of 5-HT receptors (5-HT$_{1-7}$) have been identified, and all of them appear to present in the spinal cord (Millan, 2002). In previous studies, it has been reported that a variety of nonselective 5-HT receptor antagonist have been shown to reduce the antinociceptive effects of systemic or supraspinal applied opioids (Liu *et al*., 2002; Nemmani and Mogil, 2003; Lo *et al*., 2004).

In the present study, the finding that spinal selective blockade of 5-HT$_7$ receptors by SB-269970 completely inhibits the antinociceptive effect of systemic morphine suggests that an important contribution of the spinal 5-HT$_7$ receptors. In support of this hypothesis, in the present study, metergoline, which has high affinity but lacks selectivity for 5-HT$_7$ receptor also significantly blocked the antinociceptive effect of systemic morphine. The lack of effect of spinal administration of the selective 5-HT$_{1A}$ and 5-HT$_2$ receptor antagonists on the antinociceptive effect of systemic morphine supports the suggestion that spinally administered metergoline attenuated systemic morphine-induced antinociception through blockade of 5-HT$_7$ receptors. In our study, the failure of spinal 5-HT$_{1A}$ and 5-HT2 receptor antagonism to inhibit morphine antinocicepion is in agreement with previous studies (Millan and Colpaert, 1991; Liu *et al*., 2002). However, in another study, Bardin and Colpaert (2004) reported that spinal blockade of 5-HT$_{1A}$ receptor increased systemic morphine antinociception. On the other hand, in a recent study, Rocha-Gonzales *et al*. (2005) showed that spinal 5-HT$_7$ receptors play a pronociceptive, but not antinociceptive role. These observations are in contrast with our finding. The reason for these discrepancies regarding the effects of spinally administered WAY-100635 on systemic morphine antinociception and pronociceptive role of spinal 5-HT$_7$ receptor are not clear. Nevertheless, Bardin and Colpaert (2004) and Rocha-Gonzales *et al*. (2005) used rats as experimental animals. Additionally, unlike our study, they used either paw pressure or formalin as the nociceptive stimuli rather than radiant heat. It has been well known that spinal depletion of 5-HT differently affect systemic morphine antinociception in different tests of nociception (Kuraishi *et al*., 1983). The 5-HT$_{1A}$ receptor was identified as participating in nociceptive mechanisms through electrical stimulation, but not through thermal stimulation (Nadeson and Goodchild, 2002). Thus, species differences or differences in nociceptive testing may account for the discrepancy.

In our study, the inhibitory potency of spinal SB-269970 against systemic morphine-induced antinociception was 3-fold higher than that of the spinal metergoline. The lower inhibitor effect of metergoline on systemic morphine antinociception when compared with SB-269970 may be due to lower antagonist potency of metergoline against 5-HT$_7$ receptors. Consistent with this hypothesis, it has been reported that SB-269970 has higher 5-HT$_7$ receptor-binding affinity in the guinea-pig cortex, indicated by the pK_i of 9.2 compared with the pK_i of metergoline, which was 8.0 (Hemedah *et al*., 1999; Thomas *et al*., 2002). An interesting finding in the present study was that SB-269970, which is structurally different from metergoline showed a different time course of effect on systemic morphine antinociception. The blocking effect of spinal SB-269970 (10 μg) against systemic morphine was weakened at 60 min and totally lasted 90 min after its administration. In contrast, the inhibitory effects of spinal metergoline (20 μg) against systemic morphine persisted longer, and was present at 150 min. Our results correlate with studies of Forbes *et al*. (2002) who showed that SB-269970 had extremely high clearance rate and possessed short half-life of less than 0.5 h when compared to that of metergonine, which was 60 min (Martini *et al*., 1983) when given systemically. Additionally, relative slight effects of lower dose of SB-269970 (3 μg) and the inefficacy of 1 μg of SB-269970 against systemic morphine are the most likely explained by the limited duration of action of SB-269970.

The biologic role of spinal 5-HT receptors is based on results from studies on the anatomical distribution of 5-HT receptors. Although anatomical distribution of 5-HT$_7$ receptors in the brain is well reported (Stowe and Barnes, 1998; Vanhoenacker *et al*., 2000), only a few studies about the distribution of 5-HT$_7$ receptors in spinal cord have been performed (Meuser *et al*., 2002; Jordan and Schmidt, 2002; Doly *et al*., 2005). A immunocytochemistrical study found that 5-HT$_7$ receptors are localized in the superficial layers of spinal cord dorsal horn (Meuser *et al*., 2002), and another study showed that 5-HT$_7$ receptors are highly concentrated in the intermediate regions of the spinal cord (Jordan and Schmidt, 2002). In a recent study, Doly *et al*. (2005) showed the cellular and subcellular distribution of the 5-HT$_7$ receptor in the spinal cord. It was found that 5-HT$_7$ receptors were mainly localized on primary afferent fibers, peptidergic interneurons in laminae I and II, and on glial cells. Functionally, the 5-HT$_7$ receptors have been shown to stimulate cAMP formation and cause neuronal excitation (Cardenas *et al*., 1999). Thus, it is unlikely that the systemic morphine-induced increase in serotonin level in spinal cord causes activation of 5-HT$_7$ receptors localized on primary afferent in order to directly inhibit pain-induced neuronal responses in the spinal cord. However, serotonergic system may interact with other neurotransmitters in the modulation of nociception (Jeong *et al*., 2004). It is possible that systemic morphine-induced increase in serotonin level in spinal cord activate 5-HT$_7$ receptors localized on inhibitory enkephalinergic or GABAergic interneurons that then evoke the release of enkephaline or GABA, which may in turn inhibit nociceptive transmission at sites either presynaptic or postsynaptic to the terminals of primary afferent fibers. Consistent with this hypothesis, it has been demonstrated that a GABAergic system contributed to spinal serotonin-mediated antinociception and intense labeling for GABA receptors are found in the superficial layers of spinal cord dorsal horn (Persohn *et al*., 1991; Charles *et al*., 2001; Kawamata *et al*., 2002; Sands *et al*., 2003). Further studies needed to show the colocalization of 5-HT$_7$ receptors in peptidergic interneurons in laminae I and II to better understand the role of spinal 5-HT$_7$ receptors in the systemic morphine antinociception.

In conclusion, ours result support the notion that systemically administered morphine activate the descending serotonergic pathways and 5-HT$_7$ receptors in the spinal cord plays an important role in the systemic morphine antinociception.

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Conflict of interest

The authors state no conflict of interest.

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