Orexin receptor type-1 antagonist SB-334867 decreases morphine-induced antinociceptive effect in formalin test

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A B S T R A C T

Orexin-A and orexin-B are two neuropeptides selectively synthesized in the lateral hypothalamus (LH), a region involved in morphine induced analgesia and pain modulation. Furthermore, orexin-A has been reported to produce an analgesic effect in pain models, which was blocked by orexin-1 receptor antagonist SB-334867, but not naloxone. We studied the effects of intracerebroventricular (ICV) injection of SB-334867, a selective orexin receptor type-1 antagonist, on morphine-induced antinociceptive effect in formalin test in rats. Morphine injection at a dose of 1.5 mg/kg caused a significant decrease in the formalin-induced nociceptive behaviors in phase 1, interphase, and phase 2A, whereas at doses of 3, 6, and 10 mg/kg, a significant reduction in the formalin-induced nociceptive behaviors was observed in all phases. The ICV injection of SB-334867 alone had no effect on the formalin-induced nociceptive behaviors. Pre-treatment with SB-334867 at a dose of 0.5 nmol significantly attenuated the analgesia induced by morphine (at dose 1.5 mg/kg of morphine; interphase and phase 2B and at dose 3 mg/kg of morphine just phase 2B of formalin test). Also, pre-treatment with SB-334867 at a dose of 5 nmol considerably attenuated the morphine-induced analgesia (at dose 1.5 mg/kg of morphine; phase 1, interphase, and phase 2, at dose 3 and 6 mg/kg of morphine just phase 2 of formalin test). Pre-treatment with SB-334867 at a dose of 50 nmol remarkably attenuated the morphine-induced analgesia (at dose 1.5 and 3 mg/kg of morphine; in phase 1, interphase, and phase 2 and also at dose 6 mg/kg of morphine; phase 1 and phase 2B of formalin test). These data suggest that the antinociceptive effects of morphine in formalin test might be associated with orexin receptor type-1. Our findings reveal a new role for the lateral hypothalamus orexin neurons in the morphine-induced analgesia.

1. Introduction

Morphine, the most abundant alkaloid found in opium, is a potent analgesic and frequently used drug for the treatment of both acute and chronic pain. There are spinal and supra-spinal mechanisms governing the analgesic effect of systemically injected morphine (Barton et al., 1980). Morphine activates the top-down descending modulating inhibitory systems in the supraspinal regions such as the rostral ventromedial medulla (RVM), which finally inhibited the first synapse in the dorsal horn neurons (Bashaum and Fields, 1984). Also, through another mechanism, morphine directly affects the first synapse of dorsal horn neurons, inhibiting the transmission of nociceptive information to supra-spinal centers (Yaksh and Noueihed, 1985).

The lateral hypothalamus (LH) that exclusively expresses orexin has a very important role in the modulation of nociceptive behavior (Dafny et al., 1996; Franco and Prado, 1996; Holden and Pizzi, 2008). The stimulation of LH produces an antinociceptive effect in both acute and tonic pain (Dafny et al., 1996; Franco and Prado, 1996; Holden and Naleway, 2001). Additionally, the stimulation of LH elicits antinociception via relays to the brain stem sites such as; periaqueductal gray matter (PAG) and the RVM, which ultimately effect on descending inhibitory pathways (Behbehani et al., 1988). Morphine microinjection into the LH significantly decreases nociceptive behaviors on the tail flick and formalin-induced nociceptive behaviors (Fuchs and Melzack, 1995; Dafny et al., 1996; Franco and Prado, 1996), suggesting that the LH can be considered as one of the centers in the supraspinal region that modulates nociceptive behaviors (Lopez et al., 1991; Lopez and Cox, 1992; Fuchs and Melzack, 1995; Dafny et al., 1996; Franco and Prado, 1996). In a study by Jain et al., it was shown that the LH lesions produced a hyperalgesic effect as demonstrated by a decrease in the tail flick latency (Jain et al., 2001).

Recently, two new neuropeptides; the orexin-A and orexin-B (also called hypocretin-1 and hypocretin-2) have been described in the neurons of the LH and perifornical area. These neuropeptides are reported to have broad projections, leading to the recognition of their roles in...
the regulation of a variety of functions including feeding, sleep–wake cycle, cardiovascular function, hormone secretion (Ferguson and Samson, 2003; Siegel, 2003; Sakurai, 2005; Samson et al., 2005), and more recently the modulation of nociceptive processing (Bingham et al., 2001; Yamamoto et al., 2003a; Suyama et al., 2004; Mobarakeh et al., 2005b; Azhdari-Zarnehri et al., 2011; Sadeghi et al., 2013). The antinociceptive effect of orexin-A which is blocked by orexin-1 receptor antagonist SB-334867 but not nalorex, ruled out the involvement of endogenous opiate system in these effects (Bingham et al., 2001; Yamamoto et al., 2002; Cheng et al., 2003). Furthermore, the stress-induced antinociceptive effect was prevented by SB-334867 (Heidari-Oranjaghi et al., 2012) and the antagonism of orexin receptor 1 decreased the development of morphine tolerance and physical dependence (Erami et al., 2012b).

In another study, we showed that the orexinergic projections from the LH to the ventral tegmental area and the nucleus accumens are direct/indirectly involved in the antinociception induced by LH chemical stimulation, and orexin-1 and -2 receptors in the ventral tegmental area have a more substantial role in this phenomenon (Sadeghi et al., 2013; Azhdari-Zarnehri et al., 2013). Orexin-A and their receptors are localized in the areas of the brain and spinal cord associated with nociceptive processing such as the periaqueductal gray (PAG) and locus coeruleus (LC) (Peyron et al., 1998). These studies that emphasize the analgesic effect of orexin-A (Bingham et al., 2001; Chiou et al., 2010; Azhdari-Zarnehri et al., 2011; Ho et al., 2011; Erami et al., 2012a) and the pattern of localization of both peptide and receptor in the areas involved in pain modulation provide a strong evidence for orexin-A in having a role in the modulation of morphine-induced analgesia. Recently, using in-situ hybridization, Georgescu et al. (2003) have demonstrated that all orexin-A cells that respond to chronic morphine administration and opiate antagonist-precipitated morphine withdrawal also express the μ-opioid receptor, suggesting a direct mode of action (Georgescu et al., 2003).

The stimulation of LH or the microinjection of morphine into the LH produces an analgesic effect (Holden et al., 2002; Holden et al., 2005). Moreover, orexin-A is exclusively produced in the LH and since it has an essential role in pain modulation, and that the orexin induced antinociceptive effect mediated through orexin receptor 1, we hypothesized that the blockade of orexin receptor type-1 by SB-334867 prevents the morphine-induced antinociceptive effect in the formalin test. We used the formalin test as a tonic pain test to quantify the effect of the antagonism of orexin receptor type-1 on the morphine induced analgesia. The formalin-induced nociceptive behavior was used because it is a model of inflammation that lasts long enough, during which the experimental animals show a spontaneous response. Moreover, the pain stimulus is a continuous rather than a transient one and may thus have resemblance to some kinds of clinical pain and that the observations are made on animals that are restrained lightly or not at all.

2. Materials and methods

2.1. Subjects

All experiments involving the animals were conducted according to the policy of Iranian Convention for the Protection of Vertebrate Animals Used for the Experimental Purposes, and the protocol was approved by the Ethics Committee of the School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran. Adult Wistar rats (220–300 g) were purchased from Razi Institute (Karaj, Iran). Animals were housed in groups of 8 rats per big cage (cage size was 58 × 40 × 22 cm), at a temperature-controlled room, under a 12 h light–dark cycle with lights on at 7:00 to 19:00. Food and water were provided ad libitum. In all experiments, attention was paid to the regulations of the local authorities for handling of laboratory animals.

2.2. Drugs

Two percent formalin was diluted (Temad, Iran) with sterile physiological saline solution (Soha, Iran). Morphine sulfate (Temad, Iran) was dissolved in saline. OXR1 antagonist; SB-334867 (N-(2-methyl-6-benzoxazolyl)-N-1.5-naphthyridin-4-yl urea); (molecular weight = 356, Tocris), was dissolved in dimethyl sulfoxide (DMSO, stock solution) and diluted in saline on the day of experiment (DMSO was initially prepared as 10/100 in 0.9% w/v saline solution and further diluted 1/100 and 1/1000). All drugs were diluted in saline immediately before use on the morning of experiment.

2.3. Surgical preparation for ICV microinjections

To perform direct ICV administrations of drugs or respective vehicle, the guide cannula was implanted 7 days before the experiments. Rats were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg) and a 23-gauge stainless steel guide cannula, 2 mm long was stereotaxically (Stoelting) lowered until its tip was 2 mm above the right cerebroventricular by applying coordinates from the atlas of Paxinos and Watson (2005) (10.9 mm caudal to bregma, 1.8 mm lateral to the midline and 3.6 mm depth). Using dental cement, the cannula was anchored on two stainless steel screws in the skull. Immediately after waking from surgery, rats were returned to their home cages to await the formalin test procedure. On the day of experiment, rats were transferred to individual cages and allowed to acclimatize for 60 min before they were put in an acrylic observation chamber. Direct ICV administration of drugs or respective vehicle, was conducted with a stainless steel cannula (30G, 0.3 mm outer diameter) connected by a polyethylene tube to a 26-gauge Hamilton syringe, inserted through the guide cannula and extended 2 mm beyond the tip of the guide cannula to reach the right cerebroventricular area. SB-334867 (0.5, 5 and 50 nmol; ICV) was microinjected 5 min (Heidari-Oranjaghi et al., 2012; Erami et al., 2012b) before morphine injection (1.5, 3, 6, and 10 mg/kg, S.C.) followed by the performance of formalin test 30 min later. Drug solutions or vehicles (5 μl) were injected ICV over a period of 60 s and the injection cannula was gently removed 1 min later. After 30 min, formalin was injected into the plantar surface of the right hind paw using a disposable insulin syringe with a fixed 30G needle.

2.4. Formalin test

Rats were moved to the test room at least 1 h before the commencement of experiment. Formalin tests were performed in clear plastic boxes (30 × 30 × 30 cm) with a mirror placed underneath at 45° angle to allow an unimpeded view of the animals’ paws. Initially, rats were acclimatized for 30 min in an acrylic observation chamber followed by injecting 50 μl of 2% formalin subcutaneously into the plantar surface of the right hind paw using a 30 gauge needle. To ensure stable scores from the formalin-induced nociceptive behaviors, it was necessary to make sure that the needle was inserted through the skin and run for 5 mm under the skin. Each rat was immediately returned to the observation box and the behavioral recording began. Behavioral scoring was performed by a subtle modification of the method originally described by Dubuisson and Dennis (1977) in which they employed four behavioral categories. Nociceptive behaviors were scored as follows: 0, the injected paw was not favored; 1, the injected paw had little or no weight placed on it; 2, the injected paw was elevated and not in contact with any surface; and 3, the injected paw was licked or bit. Recording of nociceptive behaviors started immediately after formalin injection (time 0) and continued for 90 min. The scores of nociceptive behaviors for each 3-minute interval were calculated as the weighted average of the number of seconds, spent in each nociceptive behavior, from the beginning of experiment. The scores were recorded in vehicle rats as well as those receiving the drug. In each group, the average behavioral responses of each rat during the first (1–7 min), inter-phase (8–14),...
2.5. Experimental protocols

The formalin test was the experimental procedure used in our study to examine the effect of antagonizing OX1R on the morphine antinoceptive effect. Three sets of experiments were considered in the formalin test: (Experiment 1) Rats were given only morphine injection (1.5, 3, 6 and 10 mg/kg) followed by the performance of formalin test. (Experiment 2) Rats had ICV microinjection of SB-334867 (0.5, 5 and 50 nmol) or 1% DMSO followed by morphine injection (1.5 mg/kg) and formalin test was pursued 30 min later. (Experiment 3) Rats were given SB-334867 (0.5, 5 and 50 nmol) by ICV microinjection or 1% DMSO followed by morphine injection (6 mg/kg) and after 30 min, the formalin test was carried out. In all experiments, morphine was microinjected subcutaneously; SB-334867 microinjected into the right cerebroventricular area, and formalin injected into the plantar surface of the right hind paw.

2.6. Data analysis

Data were presented as mean ± SEM and analyzed by one-way analysis of variance between the groups. The total time points of 90 minutes duration were divided into four phases as phase 1 (minutes 0 to 7), interphase (minutes 8 to 14), phase 2A (minutes 15 to 60), and phase 2B (minutes 61 to 90). The Tukey’s test was employed to determine where a significant difference occurred. The defined level for statistical significance was P < 0.05.

3. Results

3.1. Effects of different doses of morphine through subcutaneous injection on formalin-induced nociceptive behaviors

Fig. 1 indicates the results of experiment 1. Formalin produced typical biphasic pain responses. The first and second phases were separated by a brief inter-phase where little or no nociceptive behavior was observed in the control group (Fig. 1). Morphine injection at 1.5 mg/kg 30 min before the formalin test significantly decreased formalin-induced nociceptive behaviors in phase 1 (p < 0.05), interphase (p < 0.001), and phase 2A (p < 0.05) compared with the control group.

Fig. 1: Formalin-induced nociceptive behaviors (mean ± SEM of 6 rats per group) following subcutaneous injection of morphine (1.5, 3, 6 and 10 mg/kg) measured every 3 min for 90 min (A) and the bar chart (B). The columns represent the average nociceptive scores in each phase: phase 1 (minutes 1–7), inter-phase (minutes 8–14), phase 2A (minutes 15–60), and phase 2B (minutes 61–90). Rats received morphine 30 min before formalin injection. Recording of nociceptive behaviors began immediately after formalin injection (time 0) and continued for 90 min. **p < 0.05; ***p < 0.01; ****p < 0.001 compared with control group.

3.2. Effects of different doses of SB-334867 (ICV) on morphine-induced analgesia in formalin test

Fig. 2 demonstrates the results of experiment 2. Morphine injection at 1.5 mg/kg before the formalin test, significantly decreased formalin-induced nociceptive behaviors in phase 1 (p < 0.05), interphase (p < 0.001), and phase 2A (p < 0.05) compared with the control group (Fig. 2). Pre-treatment with orexin receptor type-1 antagonist, SB-334867 at a dose of 0.5 nmol significantly attenuated morphine-induced analgesia in interphase (p < 0.05, Fig. 2), and phase 2B compared with the control group (p < 0.05, Fig. 3). Pre-treatment with SB-334867 at a dose of 5 nmol, significantly attenuated morphine-induced analgesia in interphase (p < 0.05) and when used at a higher dose (50 nmol), morphine-induced analgesia was considerably attenuated in phase 1 (p < 0.05), interphase (p < 0.01), and the first and second parts of phase 2 compared with the control group (p < 0.01, Fig. 2).

Fig. 3 shows the results of experiment 3. The injection of 3 mg/kg of morphine alone produced antinociceptive behaviors in the formalin test as demonstrated by a substantial decrease in the nociceptive behavior in phase 1 (p < 0.05), interphase (p < 0.01), and the first and second parts of phase 2 compared with the control group (p < 0.001, Fig. 3). Also, pre-treatment with SB-334867 at a dose of 0.5 nmol remarkably attenuated morphine-induced analgesia in phase 2B when compared with the control group (p < 0.05). In addition, the administration of SB-334867 at a dose of 5 nmol significantly attenuated morphine-induced analgesia in phase 1 (p < 0.05), interphase (p < 0.05), and the first (p < 0.05) and the second parts of phase 2 (p < 0.01). Furthermore, pre-treatment with SB-334867 at a dose of 50 nmol notably attenuated morphine-induced analgesia in phase 1 (p < 0.001), interphase (p < 0.05), and the first and second parts of phase 2 when compared to the control group (p < 0.01, Fig. 3).

Finally, Fig. 4 illustrates the results of experiment 4 in which the injection of 6 mg/kg of morphine alone produced antinociceptive behaviors in the formalin test as revealed by a considerable decrease in nociceptive behavior in phase 1 (p < 0.001), interphase (p < 0.001), and the first and second parts of phase 2 compared with the control group.
Moreover, pre-treatment with SB-334867 at a dose of 0.5 nmol produced no significant attenuation of morphine-induced antinociceptive effect in the formalin test (p > 0.05, Fig. 4). Also, pre-treatment with SB-334867 at a dose of 5 nmol considerably attenuated morphine-induced analgesia at the first (p < 0.05) and second parts of phase 2 (p < 0.01). Furthermore, SB-334867 at a dose of 50 nmol significantly attenuated morphine-induced analgesia in phase 1 (p < 0.001) and phase 2B when compared to the control group (p < 0.001, Fig. 4).

To investigate the effect of different doses of orexin receptor type-1 on the antinociceptive effect of different doses of morphine, we analyzed our data for phase 1 (min 0–7) and phase 2 (min 15–90) and the results were included in Table 1 in which the reversal percentages of morphine-induced antinociception by different doses of SB-334867 are shown. As demonstrated in Table 1, the ICV administration of SB-334867 at 0.5 nmol failed to produce any effect on antinociceptive responses of morphine (1.5, 3 and 6 mg/kg) in phase 1 [F (2, 16) = 0.552, P = 0.590], and phase 2 [F (2, 16) = 3.852, P = 0.079], revealed by one-way ANOVA and the Newman–Keuls multiple comparison tests. The ICV administration of SB-334867 at 5 nmol failed to produce any effect on the antinociceptive responses of morphine (1.5, 3 and 6 mg/kg) in phase 1 [F (2, 17) = 1.466, P = 0.281] but showed a significant effect on phase 2 [F (2, 17) = 6.873, P = 0.052], demonstrated by one-way ANOVA and the Newman–Keuls multiple comparison tests. One-way ANOVA followed by the Newman–Keuls multiple comparison tests showed that the antinociceptive response of morphine (1.5, 3 and 6 mg/kg) was suppressed by the injection of SB-334867 at 50 nmol concentration in phase 1 [F (2, 15) = 15.489, P = 0.000] and phase 2 [F (2, 15) = 12.313, P < 0.001] (Table 1).

The injection of 10 mg/kg morphine completely suppressed formalin-induced nociceptive behaviors in both phases 1 and 2 (p < 0.001, Fig. 1). Pre-treatment with SB-334867 at doses of 0.5, 5, and 50 nmol produced no significant attenuation of morphine-induced antinociceptive effect at 10 mg/kg in the formalin test (p > 0.05, data not shown).

4. Discussion

It is well-known that morphine induced analgesia is mediated through (direct and indirect) spinal and supraspinal sites (Yaksh and Noueihed, 1985; Dafny et al., 1996; Franco and Prado, 1996). Investigations reported from several laboratories support the involvement of orexin-A in the modulation of nociceptive behavior (Yamamoto et al., 2003b; Holland et al., 2005; Mobarakeh et al., 2005a,b; Chiou et al.,
Table 1

<table>
<thead>
<tr>
<th>SB-334867</th>
<th>SB-334867 0.5 nmol</th>
<th>SB-334867 5 nmol</th>
<th>SB-334867 50 nmol</th>
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<tr>
<td><strong>Morphine</strong></td>
<td><strong>Phase 1</strong></td>
<td><strong>Phase 2</strong></td>
<td><strong>Phase 1</strong></td>
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<tr>
<td>Mor 1.5</td>
<td>0.3 ± 1.11</td>
<td>37.1 ± 16.1</td>
<td>12.6 ± 10.3</td>
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<tr>
<td>Mor 3</td>
<td>−0.6 ± 22.5</td>
<td>943.7 ± 296.2</td>
<td>24.3 ± 8.1</td>
</tr>
<tr>
<td>Mor 6</td>
<td>−7.5 ± 16.1</td>
<td>−10.8 ± 43.4</td>
<td>7.1 ± 8.2</td>
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Fig. 4. Formalin-induced nociceptive behaviors (mean ± SEM of 6–8 rats per group) following ICV administration of SB-334867 (0.5, 5 and 50 nmol) + morphine 6 mg/kg, measured every 3 min for 90 min (A), the bar chart for each phase (B). In B section, the columns represent the average nociceptive scores in each phase: phase 1 (minutes 1–7), inter-phase (minutes 8–14), phase 2A (minutes 15–60) and phase 2 (minutes 61–90). Rats received morphine 30 min before formalin injection. Recording of nociceptive behaviors began immediately after formalin injection (time 0) and continued for 90 min. *P < 0.05; **P < 0.01 and ***P < 0.001 compared with control group. †P < 0.05; ††P < 0.01 and †††P < 0.001 compared with morphine induced analgesia group.
through GABAergic disinhibition (Akashi et al., 2000). Furthermore, synap tic disinhibition is one possible mechanism by which morphine increases neurotransmitter release in the brain nuclei (Zhu et al., 2008).

Orexin-induced analgesia involves both spinal and supraspinal mechanisms. The dens projections of orexin cells to the marginal zone, lamina 1, and the entire segments of the spinal cord proposed that orexin may be implicated in pain processing and thermal sensation (van den Pol, 1999). In some sections of Van Den Pol's study, some cells of the marginal zone were restricted by the orexin-immunoreactive boutons, suggesting that orexin fibers selectively innervate subpopulations of cells in the marginal zone which might either be associated with a spatial sensation, pain information, or modulating specific ascending fibers (Cedarbaum and Aghajanian, 1978; Narotzky and Kerr, 1978). Supraspinal analgesia involves effects of orexin-A at the level of the PAG. The intra-PAG administration of orexin A produced an antinociceptive effect which was blocked by SB 334867 (Azhdari-Zarmehri et al., 2011; Ho et al., 2011) or AM 251 (Ho et al., 2011). In the slices of the ventrolateral PAG, orexin-A, through the receptor 1, suppressed the GABAergic induced inhibitory postsynaptic current. The postsynaptic OX1 receptor activation in the ventrolateral PAG can engage a Gq-protein coupled PLC-DAGLx enzymatic pathway to produce 2-AG, leading to a disinhibition phenomenon followed by activating the top-down descending pain inhibitory pathway, and these in turn explain the analgesic effect of orexin-A in the ventrolateral PAG (Ho et al., 2011).

Our result also showed that the ICV injection of SB-334867 alone had no effect on the formalin test at the doses usually used to antagonize the analgesic effect of orexin-A in the formalin test. In this respect, our results are consistent with a previous study that showed that the intrathecal injection of SB-334867 alone had no effect in both formalin and hot plate tests (Yamamoto et al., 2002). However, our findings are incompatible with those of Bingham et al. (2001) in which it was demonstrated that the intraperitoneal injection of SB-334867 produced a pro-hyperalgesic effect in the mouse carrageenan-induced thermal hyperalgesia test (Bingham et al., 2001). Based on a report by Yamamoto et al. in 2002, these data might suggest that a tonic orexin-A inhibitory system does not exist in the central nervous system during the formalin test (Yamamoto et al., 2002) however, there may be a tonically activated orexin-A inhibitory system present during a carrageenan-induced thermal hyperalgesia test in mice (Bingham et al., 2001). To justify this difference, there are several possibilities: Although both subcutaneous formalin injection and carrageenan injection induce a localized inflammation, nevertheless the formalin injection of the paw induces a nociceptive behavior for about 1 h (Wheeler-Aceto et al., 1990; Yamamoto and Yakhsh, 1992) whereas the edema induced by the paw carrageenan injection lasts longer than the formalin test and in addition, the thermal hyperalgesia in the carrageenan test occurs about 2 h after the injection (Wheeler-Aceto et al., 1990; Yamamoto and Yakhsh, 1992; Yamamoto et al., 2002). This may reflect the strain differences, different routes of administration, differences between the models used (carrageenan hyperalgesia and formalin tests), and methodological differences (e.g. lighting, noise, odors, handling stress or anesthesia prior to formalin injection and might differences in data collection times between laboratories; all known to influence the test).

In conclusion, based on the findings of both the previous and the present studies, we would like to suggest that the orexergic system might provide a possible pathway for morphine induced analgesia, promising a new potential therapeutic target in the treatment of pain. However, further in vitro and in vivo studies are necessary to clarify how the orexergic system is involved in morphine pain modulation.

Conflict of Interest

There is no financial or other conflict of interest associated with the present study.

Acknowledgments

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References


Yamamoto T, Yamakawa A. Comparison of the antinociceptive effects of pre- and posttreatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat. Anesthesiology 1992;77:575–63.


