Differential Effects of \( \omega \)-Conotoxin GVIA, Nimodipine, Calmidazolium and KN-62 Injected Intrathecally on the Antinociception Induced by \( \beta \)-Endorphin, Morphine and \([\text{D-Ala}^2,\text{N-MePhe}^4,\text{Gly-ol}^5]\)-enkephalin Administered Intracerebroventricularly in the Mouse

HONG W. SUH, DONG K. SONG, SUNG R. CHOI, SUNG O. HUH and YUNG H. KIM

Department of Pharmacology, Institute of Natural Medicine, College of Medicine, Hallym University, Chunchon, Kangwon-Do, 200–702, South Korea

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ABSTRACT

We previously reported that \( \beta \)-endorphin and morphine administered supraspinally produce antinociception by activating different descending pain-inhibitory systems. To determine the role of spinal calcium channels, calmodulin and calcium/calmodulin-dependent protein kinase II in the production of antinociception induced by morphine, \([\text{D-Ala}^2,\text{N-MePhe}^4,\text{Gly-ol}^5]\)-enkephalin (DAMGO) or \( \beta \)-endorphin administered supraspinally, the effects of nimodipine (an L-type calcium channel blocker), \( \omega \)-conotoxin GVIA (an N-type voltage-dependent calcium channel blocker), calmidazolium (a calmodulin antagonist) or KN-62 (a calcium/calmodulin-dependent protein kinase II inhibitor) injected intrathecally (i.t.) on the antinociception induced by morphine, DAMGO or \( \beta \)-endorphin administered supraspinally were examined in the present study. Antinociception was assessed by the mouse tail-flick test. The i.t. injection of nimodipine (from 0.024 to 2.4 pmol), \( \omega \)-conotoxin GVIA (from 0.0033 to 0.33 pmol), calmidazolium (from 0.0015 to 0.15 pmol) or KN-62 (from 0.0014 to 0.14 pmol) alone did not affect the basal tail-flick latencies. The i.t. pretreatment of mice with nimodipine, \( \omega \)-conotoxin GVIA, calmidazolium or KN-62 dose dependently attenuated the inhibition of the tail-flick response induced by \( \beta \)-endorphin administered i.c.v. However, the inhibition of the tail-flick response induced by morphine or DAMGO administered i.c.v. was not changed by i.t. pretreatment with nimodipine, \( \omega \)-conotoxin GVIA, calmidazolium or KN-62. The results suggest that spinally located L- and N-type calcium channels, calmodulin and calcium/calmodulin-dependent protein kinase II may be involved in the modulation of antinociception induced by \( \beta \)-endorphin, but not morphine and DAMGO, administered supraspinally.

Antinociception can be produced by injection of opioid agonists, such as morphine and \( \beta \)-endorphin, into the supraspinal ventricular space or spinal subarachnoid space (Suh and Tseng, 1988; Tseng, 1981; Tseng \textit{et al.}, 1979; Yaksh, 1981; Yaksh and Rudy, 1978). The periventricular, periaqueductal gray and rostral ventromedial medulla of the brain and the dorsal horn of the spinal cord are rich in endorphin and opioid receptors, which are compartments involved in the antinociception (Hokfelt \textit{et al.}, 1977, 1979; Mayer and Price, 1976). Although the exact neuronal circuits involved in antinociception are not completely understood, it has been demonstrated that opioid agonists applied to supraspinal brain sites produce their antinociceptive effects through activation of descending pain-inhibitory systems (Dubner and Bennett, 1983; Fields and Basbaum, 1978). In addition to these indirect opioid actions at supraspinal sites, there is evidence of a direct spinal action of opioids (Suh \textit{et al.}, 1988; Yaksh, 1981; Yaksh and Rudy, 1977).

We previously demonstrated that i.c.v. morphine and \( \beta \)-endorphin produce their antinociceptive effects by the stimulation of different types of opioid receptors followed by the activation of different descending pain control systems that use different neurotransmitters and receptors in the spinal cord. The antinociception induced by morphine is mediated by the stimulation of \( \mu \) opioid receptors and release of norepinephrine and serotonin acting on \( \alpha_2 \)-adrenoceptors and serotonin receptors in the spinal cord (Jung \textit{et al.}, 1988).
Previous studies have demonstrated that calcium may play an important role in modulating nociception. For example, i.t. injection of ω-conotoxin GVIA (an N-type calcium channel blocker) potentiates i.t. injected morphine- and clonidine-induced inhibition of the tail-flick response (Roerig and Wei, 1995). In addition, the systemic injection of L-type calcium channel blockers such as nimodipine, nifedipine, verapamil and diltiazem produces antinociception in the formalin, writhing and hot-plate tests (Miranda et al., 1992). Malmberg and Yaksh (1994, 1995) reported that both acute i.t. injection and continuous i.t. infusion of ω-conopeptides produce antinociception in the forminal and hot-plate tests. On the other hand, i.t. injection of calcium paradoxically potentiates morphine-induced antinociception in the tail-flick test (Lux et al., 1988). Furthermore, calcium injected i.t. also produces antinociception (Hornfeldt et al., 1992; Lux et al., 1988). However, the roles of spinal calcium channels and calcium-associated proteins such as calmodulin and calcium/calmodulin-dependent protein kinase II in the regulation of antinociception induced by opioids administered supraspinally have not been characterized. The present study was designed to examine the effects of ω-conotoxin GVIA, nimodipine (an L-type calcium channel blocker), calmidazolium (a calmodulin antagonist) and KN-62 [(a calcium/calmodulin-dependent protein kinase II inhibitor); (S)-5-isoquinolinesulfonic acid,4-[2-[(5-isoquinolinyl- sulfonyl)methylamino]3-oxo-3-(4-phenyl-1-piperazinyl)-propyl]phenyl ester] injected i.t. on the inhibition of the tail-flick response induced by morphine, DAMGO or β-endorphin administered i.c.v. in mice.

Methods

Experimental animals. Male ICR mice weighing 23 to 25 g were used for all experiments. The animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-hr light/dark cycle. Food and water were available ad libitum. Animals were used only once.

Assessment of antinociception. Antinociception was determined by the tail-flick test (D’Amour and Smith, 1941). For measurement of the latency of the tail-flick response, mice were gently held with one hand with the tail positioned in the apparatus (model TP6; EMDIE Instrument Co., Maidens, VA) for radiant heat stimulation. The tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The intensity of heat stimulus in the tail-flick test was adjusted so that the animal flicked its tail within 3 to 5 sec. The tail-flick latency was measured before (T0) and after (T1) the injection of opioid agonists. The inhibition of the tail-flick response was expressed as percent maximal possible effect (% MPE), which was calculated as [(T1 - T0)/T2 - T0] × 100, where the cutoff time (T2) was set at 10 sec.

The i.c.v. and i.t. injections. The i.c.v. administration was performed according to Haley and McCormick (1957). The i.t. administration was performed according to Hylden and Wilcox (1980) using a Hamilton syringe with a 30-gauge needle. The i.c.v. and i.t. injection volumes were 5 μl. The i.c.v. injection sites were verified by injecting the same volume of 1% methylene blue and observing the distribution of injected drugs or dye in the ventricular space and spinal cord. The dye injected i.c.v. was found to be distributed in ventricular spaces and ventral surface of the brain, and the dye was found in upper cervical portion of the spinal cord. The dye injected i.t. was distributed both rostrally and caudally but at a short distance (~1 cm), and no dye was found in the brain. When the success rate for injection was consistently ~95%, the experiment was performed.

Experimental protocol. In the first group, mice were pretreated i.t. with nimodipine (0.024–2.4 pmol), ω-conotoxin GVIA (0.0033–0.33 pmol), calmidazolium (0.0015–0.15 pmol) or KN-62 (0.0014–0.14 pmol) for 10 min. Then, morphine (3 nmol), DAMGO (10 pmol) or β-endorphin (0.3 nmol) was administered i.c.v. The second group of mice was injected i.t., with a fixed dose of nimodipine (2.4 pmol), ω-conotoxin GVIA (0.33 pmol), calmidazolium (0.15 pmol) or KN-62 (0.14 pmol) for 10-min. Then, various doses of morphine, DAMGO or β-endorphin were administered i.c.v. The tail-flick response was tested 30, 20 and 30 min after the i.c.v. injection of morphine, DAMGO and β-endorphin, respectively. The times used were chosen based on preliminary time course studies; at these times, mice produced a maximal inhibition of the tail-flick responses induced by each opioid agonist.

Statistical analysis. Values are mean ± S.E.M. One-way analysis of variance, followed by Dunnett’s multiple-comparison test when more than one dose was administered, was used for statistical evaluation. The median antinociceptive doses (ED50) and their 95% confidence intervals were calculated according to Litchfield and Wilcox (1949), with the aid of a computer program described by Tallarida and Murray (1981). Values of P < .05 were considered to indicate statistical significance.

Drugs. Morphine hydrochloride was purchased from Sam-Sung Pharm. Co. (Seoul, Korea). β-Endorphin and DAMGO were purchased from Peninsula Laboratory Inc. (Belmont, Calif.). Nimodipine, ω-conotoxin GVIA, calmidazolium chloride and KN-62 were purchased from Research Biochemicals Inc. (Natick, MA). Morphine, β-endorphin, ω-conotoxin GVIA and DAMGO were dissolved in sterile saline (0.9% NaCl solution). Nimodipine chloride, calmidazolium and KN-62 were dissolved in 20% dimethylsulfoxide.

Results

Involvement of spinal calcium channels in the production of antinociception induced by opioids administered supraspinally. To determine whether spinal L- and N-type calcium channels are involved in the antinociception induced by opioids administered supraspinally, the effect of nimodipine or ω-conotoxin GVIA i.t. pretreatment on the inhibition of the tail-flick response induced by β-endorphin, DAMGO or morphine administered i.c.v. was examined. The tail-flick latencies in mice pretreated i.t. with nimodipine or ω-conotoxin GVIA alone were not significantly different from those in mice injected i.t. with vehicle (figs. 1 and 2). The tail-flick response was measured at 30, 20 and 20 min after i.c.v. administration of β-endorphin, morphine and DAMGO, respectively. β-Endorphin (0.6 nmol), morphine (3 nmol) and DAMGO (10 pmol) increased the inhibition of the tail-flick response (fig. 1 and data not shown). Pretreatment of mice i.t. with nimodipine or ω-conotoxin GVIA dose-dependently attenuated the inhibition of the tail-flick response induced by β-endorphin administered i.c.v. (figs. 1 and 2). However, the inhibition of the tail-flick response induced by morphine or DAMGO administered i.c.v. was not changed by the i.t. pretreatment with nimodipine or ω-conotoxin GVIA (data not shown). In the dose-dependent experiments, pretreatment of mice i.t. with nimodipine or ω-conotoxin GVIA antagonized the tail-flick inhibition induced by β-endorphin administered
The ED50 values for morphine or DAMGO administered i.c.v. (figs. 3 and 4). However, the inhibition of the tail-flick response induced by morphine or DAMGO administered i.c.v. was not changed by the i.t. pretreatment with calmidazolium or KN-62 (figs. 3 and 4). In the dose-dependent experiments, the ED50 value of β-endorphin for tail-flick inhibition was increased ~4-fold above the controls in mice pretreated i.t. with calmidazolium or KN-62 (table 1). However, the ED50 values of morphine and DAMGO administered i.c.v. for tail-flick inhibition in mice pretreated i.t. with calmidazolium or KN-62 were not significantly different from those in mice treated i.t. with vehicle (table 1).

**Discussion**

We previously reported that the antinociceptive effects of morphine and β-endorphin administered supraspinally are mediated by the stimulation of different descending pain inhibitory systems. In addition, several lines of evidence have demonstrated that calcium channels located in the spinal cord may play important roles in the regulation of antinociception. In the present study, we found that spinally injected nimodipine, α-conotoxin GVIA, calmidazolium or KN-62 effectively attenuated the inhibition of the tail-flick response induced by β-endorphin administered supraspinally. However, spinal injection of nimodipine, α-conotoxin GVIA, calmidazolium or KN-62 did not affect the inhibition of the tail-flick response induced by morphine or DAMGO administered supraspinally. The results of the present study indicate that spinal L- and N-type calcium channels, calmodulin and calcium/calmodulin-dependent protein kinase II may be involved in the production of antinociception induced by β-endorphin, but not morphine and DAMGO, administered supraspinally, further supporting the hypothesis that morphine and β-endorphin administered supraspinally produce their antinociception by activating different pain-inhibitory systems.

We and others have previously hypothesized that the antinociception induced by morphine given supraspinally is mediated by the stimulation of mu opioid receptors and the activation of descending serotonergic and noradrenergic pathways and subsequent stimulation of serotonergic and alpha-2 adrenergic receptors in the spinal cord for the production of antinociception (Suh et al., 1988, 1989, 1992b; Suh and Tseng, 1990a, 1990b). On the other hand, the antinociception induced by β-endorphin given supraspinally is mediated by the stimulation of epsilon opioid receptors and by releasing [Met5]enkephalin from the spinal cord with subsequent stimulation of opioid receptors in the spinal cord for the production of antinociception (Suh et al., 1988, 1989; Suh and Tseng, 1990a, 1990b, 1990c). The results of the present study raise the possibility that supraspinally administered nimodipine, α-conotoxin GVIA, calmidazolium or KN-62 may modulate β-endorphin-induced antinociception by several actions. First, nimodipine, α-conotoxin GVIA, calmidazolium or KN-62 may modulate, presynaptically, the release of [Met5]enkephalin from descending neurons activated by β-endorphin administered supraspinally. Llinas et al. (1991) previously demonstrated that calcium/calmodulin-dependent protein kinase II in the spinal cord may play important roles in the regulation of antinociception.
The effects of nimodipine, \( \omega \)-conotoxin GVIA, calmidazolium and KN-62 injected i.t. on \( \text{ED}_{50} \) values for \( \beta \)-endorphin, morphine and DAMGO administered i.c.v. for the tail-flick inhibition.

<table>
<thead>
<tr>
<th></th>
<th>( \beta )-Endorphin</th>
<th>Morphine</th>
<th>DAMGO</th>
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<tbody>
<tr>
<td></td>
<td>i.c.v.</td>
<td>i.c.v.</td>
<td>i.c.v.</td>
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<tr>
<td>Control</td>
<td>0.19</td>
<td>1.10</td>
<td>0.0059</td>
</tr>
<tr>
<td>(0.11–0.38)(^a)</td>
<td>(0.42–1.98)</td>
<td>(0.0028–0.0127)</td>
<td></td>
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<tr>
<td>Nimodipine (2.4 pmol)</td>
<td>0.75</td>
<td>1.14</td>
<td>0.0065</td>
</tr>
<tr>
<td>(0.40–1.50)(^c)</td>
<td>(0.54–2.09)</td>
<td>(0.0030–0.0130)</td>
<td></td>
</tr>
<tr>
<td>( \omega )-Conotoxin GVIA (0.33 pmol)</td>
<td>0.77</td>
<td>1.21</td>
<td>0.0070</td>
</tr>
<tr>
<td>(0.39–1.48)(^c)</td>
<td>(0.57–2.54)</td>
<td>(0.0031–0.0143)</td>
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</tr>
<tr>
<td>Calmidazolium (0.15 pmol)</td>
<td>0.82</td>
<td>1.18</td>
<td>0.0062</td>
</tr>
<tr>
<td>(0.42–1.63)(^c)</td>
<td>(0.47–2.45)</td>
<td>(0.0027–0.0132)</td>
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<tr>
<td>KN-62 (0.14 pmol)</td>
<td>0.85</td>
<td>1.20</td>
<td>0.0058</td>
</tr>
<tr>
<td>(0.43–1.72)(^c)</td>
<td>(0.50–2.51)</td>
<td>(0.0029–0.0131)</td>
<td></td>
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</table>

\(^a\) \( \text{ED}_{50} \) values were calculated according to Litchfield and Wilcoxon (1949).

\(^b\) Numbers in parentheses indicate 95% confidence interval.

\(^c\) Significantly different from control, \( P < .05 \).

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Fig. 3. Effect of calmidazolium injected i.t. on the inhibition of the tail-flick response induced by \( \beta \)-endorphin administered i.c.v. After pretreating the mice with either vehicle (5 \( \mu l \)) or calmidazolium (0.0015–0.15 pmol), we administered \( \beta \)-endorphin (0.6 nmol) i.c.v. Vertical bars, S.E.M. The number of animals used for each group was 10. \( \ast P < .05 \) compared with group of mice injected with vehicle i.t. and \( \beta \)-endorphin i.c.v.

Fig. 4. Effect of KN-62 injected i.t. on the inhibition of the tail-flick response induced by \( \beta \)-endorphin administered i.c.v. After pretreating the mice with either vehicle (5 \( \mu l \)) or KN-62 (0.0014–0.14 pmol), we administered \( \beta \)-endorphin (0.6 nmol) i.c.v. Vertical bars, S.E.M. The number of animals used for each group was 10. \( \ast P < .05 \) compared with group of mice injected with vehicle i.t. and \( \beta \)-endorphin i.c.v.
Tseng, 1988, 1990a). Furthermore, a single injection of morphine or \( \beta \)-endorphin induces acute antinociceptive tolerance to its own distinctive opioid receptor and does not induce cross-tolerance to other opioid agonists with different opioid receptor specificities (Suh and Tseng, 1990d).

The results of the present study showed that i.t. injection of nimodipine, \( \omega \)-conotoxin GVIA, calmidazolium or KN-62 alone did not affect base-line pain sensitivity in the tail-flick test. This finding suggests that L- and N-type calcium channels, calmodulin and KN-62 located at the spinal level may not be tonically involved in the antinociceptive process. The activation of spinal calcium channels, calmodulin and calcium/calcium-dependent protein kinase II may occur when the descending pain-inhibitory systems are activated by an opioid receptor agonist such as \( \beta \)-endorphin, leading to the production of antinociception.

In addition to the antagonism of nimodipine, \( \omega \)-conotoxin GVIA, calmidazolium and KN-62 against the i.c.v. administered \( \beta \)-endorphin-induced antinociception in the present study, we found recently that i.t. injection of \( \omega \)-conotoxin GVIA, calmidazolium or KN-62 pretreated i.t. effectively attenuated the inhibition of the tail-flick response induced by cold-water swimming stress.\(^{2}\) Mizoguchi et al. (1995) have shown that the spinal [Met\(^5\)]enkephalin and delta-2 opioid receptors are involved in cold-water swimming stress-induced antinociception. This contention is supported by the findings that the blockade of spinal delta-2 opioid receptors by naltindole effectively antagonizes cold-water swimming stress-induced antinociception (Mizoguchi et al., 1995). In addition, the same group found that either i.t. pretreatment with the antibody against [Met\(^5\)] enkephalin attenuates the antinociception induced by cold-water swimming stress, suggesting that cold-water swimming stress causes the release of [Met\(^5\)]enkephalin from the spinal cord, leading to the production of antinociception via activation of spinal delta opioid receptors. Vanderah et al. (1993) previously demonstrated that supraspinal delta-2 opioid receptors also mediate cold-water swimming stress antinociception. This finding and the previous findings that antinociception induced by \( \beta \)-endorphin administered i.c.v. is mediated by spinal delta opioid receptors (Suh et al., 1994; Suh and Tseng, 1990b, 1990c) suggest that supraspinally located epsilon or delta-2 opioid receptors may be involved in cold-water swimming stress-induced antinociception. Thus, it can be speculated that spinally injected \( \omega \)-conotoxin GVIA, calmidazolium and KN-62 may exert their antagonistic effects secondarily against the supraspinally administered \( \beta \)-endorphin-induced antinociception by modulating spinal delta opioid systems. However, the possibility that supraspinally administered \( \beta \)-endorphin may produce antinociception by stimulating supraspinal delta-2 opioid receptors should be further assessed.

References


Send reprint requests to: Hong-Won Suh, Ph.D., Department of Pharmacology, College of Medicine, Hallym University, 1 Okchun-Dong, Chuncheon, Kangwon-Do, 200–702, South Korea. E-mail: hwsuh@sun.hallym.ac.kr