Differential Antinociceptive Effects of Endomorphin-1 and Endomorphin-2 in the Mouse

LEON F. TSENG, MINORU NARITA, CHIAKI SUGANUMA, HIROKAZU MIZOGUCHI, MASAIRO OHSAWA, HIROSHI NAGASE, and JOHN P. KAMPINE

Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin (L.F.T., M.N., C.S., H.M., J.P.K.); and Basic Research Laboratories, Toray Industries Inc., Kamakura, Japan (H.N.)

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ABSTRACT

Two highly selective \( \mu \)-opioid receptor agonists, endomorphin-1 and endomorphin-2, have been identified and postulated to be endogenous \( \mu \)-opioid receptor ligands. We determined the antinociceptive effects of these two ligands at the supraspinal level in mice with the tail-flick and hot-plate responses. The i.c.v. injection of endomorphin-1 and -2 inhibited the tail-flick and hot-plate responses in a dose-dependent manner. The endomorphin-1 was found to be 3.3- and 2.4-fold more potent than endomorphin-2 in inhibiting the tail-flick and hot-plate responses, respectively. The antinociception induced by endomorphin-1 was blocked by i.c.v. pretreatment with the \( \mu \)-opioid receptor antagonist \( \beta \)-funaltrexamine but not by the \( \kappa \)-opioid receptor antagonist nor-binaltorphimine, the \( \delta \)-opioid antagonist 7-benzylidene naltrexamine, or the \( \delta_2 \)-opioid receptor antagonist naltriben. In contrast, the antinociception induced by endomorphin-2 was blocked by i.c.v. pretreatment with \( \beta \)-funaltrexamine or nor-binaltorphimine but not by 7-benzylidene naltrexamine or naltriben. The inhibition of the tail-flick response induced by endomorphin-2 was blocked by pretreatment with an antisera against dynorphin A(1-17) but not by antisera against Met-enkephalin, Leu-enkephalin, or \( \beta \)-endorphin. None of these antisera reduced the endomorphin-1-induced tail-flick inhibition. We propose that endomorphin-1 produces antinociception by stimulating one type of \( \mu \)-opioid receptor, whereas endomorphin-2 initially stimulates different \( \mu \)-opioid receptors, which subsequently induce the release of dynorphins that act on \( \kappa \)-opioid receptors to produce antinociception.

Since the initial demonstration of \( \mu \)-opioid receptors more than 25 years ago, investigators have searched for their endogenous ligands. The search led to the discovery of enkephalins, endorphins, and dynorphins in the 1970s (Hughes et al., 1975; Bradbury et al., 1976; Li and Chung, 1976; Goldstein et al., 1979, 1981), yet they have either low selectivity or efficacy at the \( \mu \)-opioid receptors (Corbett et al., 1993; Zadina et al., 1997). The enkephalins are the endogenous ligands for \( \delta \)-opioid receptors, and dynorphin A(1-17) is the endogenous ligand for \( \kappa \)-opioid receptors (Knapp et al., 1995; Nock, 1995). \( \beta \)-Endorphin has been proposed to be an endogenous ligand for the \( \epsilon \)-opioid receptor (Nock, 1995; Tseng, 1995, Narita and Tseng, 1998). However, it binds equally well to \( \mu \)- and \( \delta \)-opioid receptors with high affinity (Reisine, 1995). Thus, many investigators believe these peptides were not the endogenous ligands for \( \mu \)-opioid receptors due to their selectivity profiles.

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ABBREVIATIONS: \( \beta \)-FNA, \( \beta \)-funaltrexamine; NTB, naltriben; BNTX, 7-benzylidene naltrexamine; nor-BNI, nor-binaltorphimine; DPDPE, \([\alpha\text{-Pen}^\beta,\alpha\text{-Pen}^\delta]\)-enkephalin; DAMGO, \([\alpha\text{-Ala}^\beta,\text{N-MePhe}^\alpha,\text{Gly}-\text{ol}^\delta]\)-enkephalin.
tinociception induced by endomorphin-2 was potentiated by the coadministration of dipeptidyl IV inhibitor Ala-pyrrolidonyl-2-nitrile and the enzyme-resistant peptide n-Pro\(^\circ\)-endomorphin-2 was more potent than endomorphin-2 in producing antinociception, indicating that dipeptidyl peptidase IV plays a role in inactivation of the endomorphin-2 in vivo (Shane et al., 1999). To further characterize these peptides, we extended these initial studies to determine whether there are any differential actions of endomorphin-1 and endomorphin-2 administered supraspinally on the production of antinociception in the mouse. We found that although the antinociception induced by both endorphins is mediated by the selective stimulation of \(\mu\)-opioid receptors, the antinociception induced by endomorphin-2 contains an additional component, which is mediated by the release of dynorphin A(1-17) and the subsequent stimulation of \(\kappa\)-opioid receptors.

Materials and Methods

**Animals.** Male ICR mice weighing 25 to 30 g (Sasco, Inc., Omaha, NE) were used for the study. Animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light/dark cycle. Food and water were available ad libitum. Animals were used only once in all experiments.

**Drugs and Antisera.** Endomorphin-1 (Tyr-Pro-Trp-Phe-NH\(_2\)), endomorphin-2 (Tyr-Pro-Phe-Phe-NH\(_2\); Zadina et al., 1997), naltrexone (NTB; Portoghese et al., 1992), 7-benzylidene naltrexamine (BNTX; Portoghese, 1991), and \(\beta\)-FNA (Takemori et al., 1981) were synthesized in the laboratory of H.N. (Basic Research Laboratories, Bell, CA). [\(\beta\)-Ala\(^2\)]Deltorphin-II was obtained from Molecular Research Laboratories (Durham, NC). [\(\beta\)-Pen\(^2\),\(\beta\)-Pen\(^3\)]enkephalin (DPPDE), [\(\beta\)-Ala\(^2\),\(\beta\)-MePhe\(^2\),\(\beta\)-Gly-\(\beta\)-]enkephalin (DAMGO), and morphine sulfate were obtained from Mallinkrodt Chemical Works (St. Louis, MO). Nor-binaltorphimine dihydrochloride (nor-BNI) was obtained from Research Biochemicals Inc. (Natick, MA). All drugs for i.c.v. administration were dissolved in 0.9% NaCl containing 0.01% Triton X-100.

The antisera against dynorphin A(1-17), Met-enkephalin, Leu-enkephalin, and \(\beta\)-endorphin were produced according to the method of Höltt et al. (1978). The specificity of these antisera has been determined with the radioimmunoassay or ELISA tests. Briefly, the antisera against dynorphin A(1-17) did not show cross-reactivity with dynorphin-2; dynorphin A(1-8), (2-5), (2-8), (6-17), or (1-6); Met-enkephalin; Leu-enkephalin; Met-enkephalin-Lys; \(\beta\)-endorphin; or \(\beta\)-neoendorphin. It showed a small degree of cross-reactivity with dynorphin A(1-9) (6.1%) and A(1-10) (5.5%). However, it showed 100% cross-reactivity with dynorphin A(1-13). The antisera against Met-enkephalin did not cross-react with dynorphin A(1-13), dynorphin A(1-17), or \(\beta\)-endorphin. However, it showed 29.4% cross-reactivity with Leu-enkephalin. The antisera against Leu-enkephalin did not cross-react with \(\beta\)-endorphin, dynorphin A(1-13), or dynorphin A(1-17); it showed 14% cross-reactivity with Met-enkephalin. The antisera against \(\beta\)-endorphin did not cross-react with Leu-enkephalin, Met-enkephalin, or the dynorphins.

**Assessment of Antinociceptive Response.** Antinociceptive responses were determined with the tail-flick test (D’Amour and Smith, 1941) and hot-plate test (Eddy and Leimbach, 1953). 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 TF6; 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 the heat stimulus in the tail-flick test was adjusted so the animal flicked its tail within 3 to 5 s. For the hot-plate test, mice were individually placed on the hot-plate (55°C), and the reaction time starting from the placement of the mouse on the hot-plate to the time of licking either the forepaw or the hindpaw was measured. The dimensions of the hot-plate apparatus were 30 × 30 × 30 cm (model 39; IITC Inc., Woodland Hills, CA). Control latencies for the paw-licking were approximately 4 to 9 s. The latency of the tail-flick and hot-plate responses was measured before \(T_0\) and at various times after \(T_1\) i.c.v. injection of endorphins. The inhibition of the tail-flick and hot-plate responses to endorphins was expressed as a percentage of the maximum possible effect (% MPE), which was calculated as \(\frac{[T_1 - T_0]}{T_2 - T_0} \times 100\), where the cut-off time, \(T_2\), was set at 10 s for the tail-flick response and 30 s for the hot-plate response. To establish the dose-response curves, at least four drug doses were used with at least 10 mice at each dose.

**Experimental Protocols.** Various doses of endorphin-1 and endorphin-2 were injected i.c.v. according to the procedure of Haley and McCormick (1957), and the tail-flick and hot-plate responses were measured at different times after the injection. Complete dose-response curves were then established for the analysis of 50% antinociceptive dose (AD\(_{50}\)) values and slope function. In other experiments, mice were pretreated i.c.v. with the selective opioid antagonists \(\beta\)-FNA or nor-BNI (Spanagel et al., 1994) 24 h or with BNTX or NTB (Mizoguchi et al., 1995) 10 min before i.c.v. challenge with opioid agonists. The effectiveness of the doses of the antagonists used in blocking the respective opioid receptors was verified in other experiments in which the same doses of the antagonists significantly blocked the antinociception induced by its own opioid receptor agonist (see Table 2). The tail-flick response was measured 5 min after the injection of endorphins or 10 min after the injection of morphine, DPDPE, [\(\beta\)-Ala\(^2\)]deltorphin-II, or U50,488H. Antisera to dynorphin A(1-17), Met-enkephalin, Leu-enkephalin, or \(\beta\)-endorphin were administered 60 min before the i.c.v. administration of the agonists. These measurement times were selected based on time course studies, which determined the time of maximum effect after the injection of opioid agonists (Tseng and Collins, 1993; Xu and Tseng, 1997).

**Statistical Analysis.** The Student’s \(t\) test (for comparisons between two groups), ANOVA followed by Dunnett’s test (for comparison of multiple groups with one saline control group), and ANOVA followed by the Newman-Keuls test (for comparisons among multiple groups) were used to indicate the significance among groups. To construct the dose-response curves, at least four doses were used with 10 mice at each dose. The AD\(_{50}\) values and their 95% confidence limits were determined by using the graded dose-response procedure described by Tallarida and Murray (1987).

**Results**

**Time Courses of Tail-Flick Response to i.c.v. Administration of Endorphin-1 and Endorphin-2.**

Groups of mice were injected i.c.v. with saline or different doses of endorphin-1 or endorphin-2, and the tail-flick and hot-plate responses were measured 5, 10, 15, and 20 min after injection. The i.c.v. injection of endorphin-1 or endorphin-2 caused a dose-dependent increase of the inhibition of the tail-flick and hot-plate responses. The inhibition reached its peaks 5 min after injection, rapidly declined, and returned to the preinjection level 20 min after injection (Figs. 1 and 2). The duration of the tail-flick inhibition induced by endorphin-1 appeared to be longer than that of endorphin-2 (Fig. 1, A and B). The 5-min measurement interval after i.c.v. injection of endorphins was therefore determined for the experiments described in the next sections.

Endorphin-1 and endorphin-2 inhibited the tail-flick
and hot-plate responses in a dose-dependent manner at 5 min after i.c.v. injection (Fig. 3). The AD50 values and their slope functions for endomorphin-1 and endomorphin-2 for inhibition of the tail-flick and hot-plate responses are shown in Table 1. Endomorphin-1 was found to be about 3.3- and 2.4-fold more potent than endomorphin-2 in inhibiting the tail-flick and hot-plate responses, respectively. The slope of the dose-response curve of endomorphin-2 for inhibition of the tail-flick response was significantly steeper than that of endomorphin-1, whereas the slope functions of both endomorphins for inhibition of the hot-plate response were parallel. The different slope functions of the dose-response lines for endomorphin-1 and endomorphin-2 for inhibition of the tail-flick response suggest that these two peptides produce antinociception through different modes of action.

**Effects of i.c.v. Pretreatment with β-FNA, nor-BNI, BNTX, or NTB on Inhibition of Tail-Flick Response Induced by i.c.v. Administration of Endomorphin-1, Endomorphin-2, Morphine, DPDPE, [D-Ala2]deltorphin-II, and U50,488H.** The i.c.v. pretreatment with β-FNA at doses from 0.5 to 2 nmol for 24 h dose dependently blocked the tail-flick inhibition induced by the i.c.v. administration of endomorphin-1 (24.7 nmol) or endomorphin-2 (35 nmol; Figs. 4, 5A, and 6A). Similarly, the hot-plate inhibition induced by endomorphin-1 or endomorphin-2 was also blocked by the pretreatment with β-FNA (2 nmol; Figs. 5B and 6B). The same pretreatment with β-FNA was found to block the inhibition of the tail-flick and hot-plate responses induced by morphine (Table 2). Pretreatment with nor-BNI at doses from 1.3 to 6.6 nmol dose dependently attenuated the tail-flick inhibition induced by
endomorphin-2 (35 nmol; Fig. 7). Pretreatment with nor-BNI at a dose 6.6 nmol significantly attenuated both the tail-flick and hot-plate inhibition induced by endomorphin-2 (35 nmol for the tail-flick response and 17.5 nmol for the hot-plate response) by 41.5 and 26.0%, respectively (Fig. 6, A and B). Pretreatment with nor-BNI (1.3–6.6 nmol) did not affect the inhibition of either the tail-flick or hot-plate response induced by endomorphin-1 (24.6 nmol; Fig. 8). However, the same pretreatment with nor-BNI (6.6 nmol) was found to effectively block the inhibition of the tail-flick and hot-plate responses induced by U50,488H (Table 2). Pretreatment with BNTX (2.0 and 9.8 nmol in the tail-flick and hot-plate tests, respectively) or NTB (9.4 nmol in both tests) did not block the inhibition of the tail-flick and hot-plate responses induced by endomorphin-1 (24.6 and 6.6 nmol in the tail-flick and hot-plate tests, respectively) and endomorphin-2 (35.0 and 17.5 nmol in the tail-flick and hot-plate tests, respectively; Figs. 5 and 6). However, the same pretreatment with BNTX and NTB markedly blocked the respective inhibition of the tail-flick and hot-plate responses induced by DPDPE (15.5 nmol in both tests) and &Ala2&deltorphin-II (25.6 and 12.8 nmol in the tail-flick and hot-plate tests, respectively) (Table 2).

Effects of i.c.v. Pretreatment with Antisera to Dynorphin A(1-17), Met-Enkephalin, Leu-Enkephalin, or &beta;-Endorphin on Inhibition of Tail-Flick Response Induced by Endomorphin-1 and Endomorphin-2. Because dynorphin A(1-17) has been proposed to be the endogenous opioid ligand for &kappa;-opioid receptors, the finding that antinociception induced by endomorphin-2 was blocked by the &kappa;-opioid receptor antagonist nor-BNI suggests that endomorphin-2 causes the release of dynorphins, which subsequently act on &kappa;-opioid receptors to produce antinociception.

Pretreatment of mice with an antiserum against dynorphin A(1-17) at doses of 10 to 100 mg for 1 h dose dependently attenuated the tail-flick inhibition induced by endomorphin-2 (35 nmol; Fig. 8). Pretreatment with nor-BNI (1.3–6.6 nmol) did not affect the inhibition of either the tail-flick or hot-plate response induced by endomorphin-1 (24.6 nmol; Fig. 7). The same pretreatment with nor-BNI (6.6 nmol) was found to effectively block the inhibition of the tail-flick and hot-plate responses induced by U50,488H (Table 2). Pretreatment with BNTX (2.0 and 9.8 nmol in the tail-flick and hot-plate tests, respectively) or NTB (9.4 nmol in both tests) did not block the inhibition of the tail-flick and hot-plate responses induced by endomorphin-1 (24.6 and 6.6 nmol in the tail-flick and hot-plate tests, respectively) and endomorphin-2 (35.0 and 17.5 nmol in the tail-flick and hot-plate tests, respectively; Figs. 5 and 6). However, the same pretreatment with BNTX and NTB markedly blocked the respective inhibition of the tail-flick and hot-plate responses induced by DPDPE (15.5 nmol in both tests) and &Ala2&deltorphin-II (25.6 and 12.8 nmol in the tail-flick and hot-plate tests, respectively) (Table 2).
tail-flick inhibition induced by endomorphin-1 (24.6 nmol), DAMGO (0.04 nmol), morphine (9.9 nmol), DPDPE (14.7 nmol), [D-Ala²]deltorphin-II (25.6 nmol), or U50,488H (115 nmol; Table 3). The tail-flick inhibition induced by endomorphin-2 (35 nmol) was not affected by the pretreatment with an antiserum against Met-enkephalin (100 μg), Leu-enkephalin (100 μg), or β-endorphin (100 μg; Fig. 9).

Discussion

The original description of the two endomorphins reveals that both compounds have a profound μ-selectivity (Zadina et al., 1997). In this initial study, both endomorphins compete for μ-binding sites more than 1000-fold more effectively than for either δ- or κ₁-receptors (Zadina et al., 1997). This finding is further confirmed by Goldberg et al. (1998). They report that both endomorphin-1 and endomorphin-2 compete for both μ₁- and μ₂-receptors sites quite potently but have no appreciable affinity for either δ- or κ₁-receptors. We found in this in vivo study that the inhibition of the tail-flick and hot-plate responses induced by either endomorphin-1 or endomorphin-2 was blocked by the selective μ-opioid receptor antagonist β-FNA but not by the δ₁-opioid receptor antagonist BNTX or the δ₂-receptor antagonist NTB. The findings are consistent with the view that these two endomorphins are selective μ-opioid receptor ligands and that the antinociception induced by endomorphin-1 and endomorphin-2 is mediated by the selective stimulation of μ-receptors but not δ₁- or δ₂-opioid receptors.

Endomorphin-1 was found to be about 3.3- and 2.4-fold more potent than endomorphin-2 in inhibiting the tail-flick and hot-plate responses, respectively. Our AD₅₀ values for endomorphin-1 and endomorphin-2 using the tail-flick test are 6.16 (4.42–8.57) and 20.17 (16.07–25.57) nmol, respec-
antinociception by these two endomorphins. However, the pretreatment with nor-BNI even at high doses only partially, not completely, blocked the antinociception induced by endomorphin-2, but not endomorphin-1, producing its antinociception in part through the stimulation of \( \kappa \)-opioid receptors by i.c.v. pretreatment with nor-BNI or nor-BNI, whereas the antinociception induced by endomorphin-1 was not blocked by the pretreatment with nor-BNI, indicating that the endomorphin-2, but not endomorphin-1, produces its antinociception in part through the stimulation of \( \kappa \)-opioid receptors. In addition, the slope of the dose-response line for endomorphin-2-induced tail-flick inhibition was much steeper than that for endomorphin-1, also suggesting that different mechanisms may be involved in producing antinociception by these two endomorphins. However, the pretreatment with nor-BNI even at high doses only partially, not completely, blocked the antinociception induced by endomor-

### Table 2

<table>
<thead>
<tr>
<th>Opioid Challenge</th>
<th>Antagonist Pretreatment</th>
<th>Tail-Flick Inhibition</th>
<th>Hot-Plate Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>Saline, 24 h</td>
<td>97.92 ± 5.68 (10)</td>
<td>69.63 ± 13.65 (10)</td>
</tr>
<tr>
<td>20 min</td>
<td>( \beta )-FNA, 2 nmol, 24 h</td>
<td>14.98 ± 9.33 (10)***</td>
<td>18.51 ± 10.22 (10)***</td>
</tr>
<tr>
<td>U50,488H</td>
<td>Saline, 24 h</td>
<td>70.47 ± 13.41 (10)</td>
<td>77.52 ± 14.12 (10)</td>
</tr>
<tr>
<td>10 min</td>
<td>Nor-BNI, 6.6 nmol, 24 h</td>
<td>10.60 ± 6.20 (10)***</td>
<td>11.76 ± 16.60 (10)***</td>
</tr>
<tr>
<td>DPDPE</td>
<td>Saline, 10 min</td>
<td>82.35 ± 5.42 (10)</td>
<td>68.12 ± 6.12 (30)</td>
</tr>
<tr>
<td>10 min</td>
<td>BNTX, 2 nmol, 10 min</td>
<td>28.40 ± 11.11 (10)***</td>
<td>31.14 ± 9.72 (10)***</td>
</tr>
<tr>
<td>([\text{D-Ala}^2\text{Deltorphin-II}])</td>
<td>Saline, 10 min</td>
<td>70.46 ± 7.47 (10)</td>
<td>78.89 ± 11.09 (10)</td>
</tr>
<tr>
<td>10 min</td>
<td>NTB, 1.9 nmol, 10 min</td>
<td>24.96 ± 9.29 (10)**</td>
<td>39.63 ± 12.62 (10)*</td>
</tr>
<tr>
<td></td>
<td>NTB, 9.4 nmol, 10 min</td>
<td>8.34 ± 3.24 (10)***</td>
<td></td>
</tr>
</tbody>
</table>

* \( P < .05 \) compared with saline-injected control.

** \( P < .01 \) compared with saline-injected control.

*** \( P < .001 \) compared with saline-injected control.
in their study. Nor-BNI is a slow acting but long-lasting 10 mg/kg nor-BNI administered systemically was not verified indeed selectively blocked after 15 min of pretreatment with the study of Goldberg et al (1998). Endoh et al. (1992) report lasts for more than 4 days. At 0.5 to 1 h after the injection of 4 h after the systemic injection of nor-BNI 5 to 20 mg/kg and antiserum against dynorphin A(1-17) administered i.c.v. did not block the antinociception induced by U50,488H administration. The finding that the pretreatment of mice with an antiserum against dynorphin A(1-17) (A/S Dyn; 100 nmol, 10 min) A/S Dyn 10 86.98 ± 8.95

Table 3

Lack of effects of the pretreatment with antiserum against dynorphin A(1-17) on the tail-flick inhibition induced by endomorphin-1, DAMGO, morphine, DPDPE, [d-Ala²]deltorphin-II, or U50,488H

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Pretreatment</th>
<th>n</th>
<th>Tail-Flick Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endomorphin-1</td>
<td>NRS</td>
<td>10</td>
<td>85.74 ± 10.7</td>
</tr>
<tr>
<td>(24.6 nmol, 5 min)</td>
<td>A/S Dyn</td>
<td>20</td>
<td>90.05 ± 5.15</td>
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<tr>
<td>DAMGO</td>
<td>NRS</td>
<td>10</td>
<td>68.20 ± 13.05</td>
</tr>
<tr>
<td>(0.04 nmol, 10 min)</td>
<td>A/S Dyn</td>
<td>10</td>
<td>73.48 ± 14.37</td>
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<tr>
<td>Morphine</td>
<td>NRS</td>
<td>10</td>
<td>68.45 ± 12.36</td>
</tr>
<tr>
<td>(9 nmol, 20 min)</td>
<td>A/S Dyn</td>
<td>10</td>
<td>60.47 ± 14.62</td>
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<tr>
<td>DPDPE</td>
<td>NRS</td>
<td>20</td>
<td>71.39 ± 9.07</td>
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<tr>
<td>(14.7 nmol, 10 min)</td>
<td>A/S Dyn</td>
<td>10</td>
<td>79.92 ± 9.10</td>
</tr>
<tr>
<td>[d-Ala²]Deltorphin-II</td>
<td>NRS</td>
<td>10</td>
<td>68.10 ± 13.77</td>
</tr>
<tr>
<td>(25.6 nmol, 10 min)</td>
<td>A/S</td>
<td>10</td>
<td>63.50 ± 14.44</td>
</tr>
<tr>
<td>U50,488H</td>
<td>NRS</td>
<td>20</td>
<td>78.86 ± 8.86</td>
</tr>
<tr>
<td>(115 nmol, 10 min)</td>
<td>A/S Dyn</td>
<td>10</td>
<td>86.98 ± 8.95</td>
</tr>
</tbody>
</table>

Nor-BNI was significantly blocked by the pretreatment of mice with an antiserum against dynorphin A(1-17), which then acts on k-opioid receptors to induce antinociception. This finding strongly indicates the involvement of k-opioid receptors in endorphin-2-induced antinociception.

Because endorphin-2 has a very low affinity for k-opioid receptors in vitro ligand-binding assays, it is unlikely that the endorphin-2-induced antinociception is mediated by the direct stimulation of k-opioid receptors. Because dynorphin A(1-17) is an endogenous ligand for k-opioid receptors, the possibility that the k-ergic mechanism for the production of antinociception by endorphin-2 is mediated by the release of dynorphin A(1-17) was then explored with the antiserum to dynorphin A(1-17). The pretreatment of mice with an antiserum to dynorphin A(1-17) attenuated significantly the antinociception induced by endorphin-2. This finding indicates that endorphin-2 releases dynorphin A(1-17), which then acts on k-opioid receptors to induce antinociception. The finding that the pretreatment of mice with an antiserum against dynorphin A(1-17) administered i.c.v. did not block the antinociception induced by U50,488H administered i.c.v. excludes that the possibility that the release of dynorphin A(1-17) by endorphin-2 is due to direct stimulation of k-opioid receptors by this peptide.

It is concluded that both endorphin-1 and endorphin-2 produce their antinociception through the stimulation of k-opioid receptors. However, the antinociception induced by endorphin-2 contains an additional component, which is mediated by the release of dynorphin A(1-17) acting on k-opioid receptors. The exact mechanism of this component is not clear at this time. It is possible that endorphin-2 stimulates a subtype of k-opioid receptor that induces the release of dynorphins. More studies are needed to support or refute this possibility.

References


Send reprint requests to: Leon F. Tseng, Ph.D., Department of Anesthesiology, MEB-462c, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226. E-mail: ltseng@mcw.edu