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

LEON F. TSENG, MINORU NARITA, CHIAKI SUGANUMA, HIROKAZU MIZOGUCHI, MASAKI 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 \(\delta_1\)-opioid antagonist 7-benzylidene naltrexamine, or the \(\delta_2\)-opioid receptor antagonist naltrexamine. 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 naltrexamine. The inhibition of the tail-flick response induced by endomorphin-2 was blocked by pretreatment with an antisera against dynorphin \(\alpha(1-17)\) 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.

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ABBREVIATIONS: \(\beta\)-FNA, \(\beta\)-funaltrexamine; NTB, naltrexamine; BNTX, 7-benzylidene naltrexamine; nor-BNI, nor-binaltorphimine; DPDPE, [\(\alpha\)-Pen\(^2\),\(\beta\)-Pen\(^3\)]enkephalin; DAMGO, [\(\alpha\)-Ala\(^2\),N-MePhe\(^4\),Gly-ol\(^5\)]-enkephalin.
tinociception induced by endomorphin-2 was potentiated by the coadministration of dipeptidyl IV inhibitor Ala-tryptidolodinyl-2-nitrile and the enzyme-resistant peptide n-Pro2-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 endomorphins is mediated by the selective stimulation of μ-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 κ-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 Antiserum. Endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂), endomorphin-2 (Tyr-Pro-Pro-Phe-NH₂; Zadina et al., 1997), naltriben (NTB; Portoghese et al., 1992), 7-benzylidene naltrexamine (BNTX; Portoghese, 1991), and β-FNA (Takemori et al., 1981) were synthesized in the laboratory of H.N. (Basic Research Laboratories, Durham, NC). [D-Ala₂]Deltorphin-II was obtained from Molecular Research Laboratories (Durham, NC). [D-Pen²,D-Pen⁵]enkephalin (DAMGO), and morphine sulfate were obtained from Mallinckrodt 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 β-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 endomorphin-1; endomorphin-2; dynorphin A(1-8); (2-5), (2-8), (6-17), or (1-6); Met-enkephalin; Leu-enkephalin; Met-enkephalin-Lys; β-endorphin; or β-neoendorphin. It showed a small degree of cross-reactivity with dynorphin A(1-9) (6.1%) and (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 β-endorphin. However, it showed 29.4% cross-reactivity with Leu-enkephalin. The antisera against Leu-enkephalin did not cross-react with β-endorphin, dynorphin A(1-13), or dynorphin A(1-17); it showed 14% cross-reactivity with Met-enkephalin. The antisera against β-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

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Endomorphin-1 and -2-induced antinociception was determined with the radiant heat source. The i.c.v. injection of endomorphin-1 or endomorphin-2 caused a dose-dependent increase in 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 endomorphin-1 appeared to be longer than that of endomorphin-2 (Fig. 1, A and B). The 5-min measurement interval after i.c.v. injection of endomorphins was therefore determined for the experiments described in the next sections. Endomorphin-1 and endomorphin-2 inhibited the tail-flick

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and hot-plate responses in a dose-dependent manner at 5 min after i.c.v. injection (Fig. 3). The AD_{50} 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 (240.7 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

![Fig. 1. Time course of the changes in i.c.v. endomorphin-1 (A)- and endomorphin-2 (B)-induced tail-flick inhibition. Groups of mice were administered an i.c.v. injection of saline or different doses of endomorphin-1 (EM-1) or endomorphin-2 (EM-2), and the tail-flick responses were measured at different times after injection. Each value represents the mean ± S.E. for 10 to 30 mice. A, saline versus 3.28 nmol EM-1 (F_{1,90} = 29.04, P < .01), saline versus 16.55 nmol EM-1 (F_{1,190} = 51.20, P < .01), saline versus 13.10 nmol EM-1 (F_{1,90} = 34.22, P < .01), and saline versus 16.38 nmol EM-1 (F_{1,90} = 67.42, P < .01). B, saline versus 8.75 nmol EM-2 (F_{1,90} = 2.43, P = NS), saline versus 1.64 nmol EM-1 (F_{1,140} = 19.82, P < .01), saline versus 17.49 nmol EM-2 (F_{1,140} = 67.42, P < .01), and saline versus 34.99 nmol EM-2 (F_{1,140} = 26.25, P < .01).

![Fig. 2. Time course of the changes in i.c.v. endomorphin-1 (A)- and endomorphin-2 (B)-induced hot-plate inhibition. Groups of mice were administered an i.c.v. injection of saline or different doses of endomorphin-1 (EM-1) or endomorphin-2 (EM-2), and the hot-plate responses were measured at different times after injection. Each value represents the mean ± S.E. for 10 to 30 mice. A, saline versus 0.82 nmol EM-1 (F_{1,90} = 24.36, P < .01), saline versus 13.10 nmol EM-1 (F_{1,90} = 24.36, P < .01), and saline versus 16.55 nmol EM-1 (F_{1,90} = 67.42, P < .01). B, saline versus 2.19 nmol EM-2 (F_{1,140} = 7.64, P < .01), saline versus 4.37 nmol EM-2 (F_{1,140} = 47.49, P < .01), saline versus 8.75 nmol EM-2 (F_{1,140} = 54.47, P < .01), and saline versus 17.49 nmol EM-2 (F_{1,140} = 64.51, P < .01).]
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. 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 [D-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 b-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 k-opioid receptors, the finding that antinociception induced by endomorphin-2 was blocked by the k-opioid receptor antagonist nor-BNI suggests that endomorphin-2 causes the release of dynorphins, which subsequently act on k-opioid receptors to produce antinociception.

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 [D-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; 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 κ-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 κ-receptors. We found in this in vivo study that the inhibition of the tail-flick and hot-plate responses induced by i.c.v. injection of 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-
The antinociception induced by either endomorphin-1 or endomorphin-2 was completely eliminated by the pretreatment with β-FNA, indicating that the predominant effect of antinociception is mediated through the stimulation of μ-opioid receptors. However, we found that the antinociception induced by endomorphin-2 was also mediated via a mechanism that was different from that of endomorphin-1. The antinociception induced by endomorphin-2 was blocked significantly by the pretreatment with the κ-opioid receptor antagonist 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 κ-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 endomorphin-2.

TABLE 2
Effects of pretreatment with β-FNA, NTB, BNTX, or nor-BNI on the tail-flick and hot-plate inhibition induced by morphine, DPDPE, [D-Ala²]Deltorphin-II, or U50,488H

<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></td>
<td></td>
<td>% MPE ± S.E.</td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td>Saline, 24 h</td>
<td>75.02 ± 5.86 (30)</td>
<td>69.63 ± 13.65 (10)</td>
</tr>
<tr>
<td>20 min</td>
<td>β-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>82.38 ± 5.42 (30)</td>
<td>65.12 ± 6.12 (30)</td>
</tr>
<tr>
<td>10 min</td>
<td>BNTX, 2 nmol, 10 min</td>
<td>28.40 ± 13.11 (10)***</td>
<td>31.14 ± 9.72 (10)**</td>
</tr>
<tr>
<td>[D-Ala²]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></td>
</tr>
<tr>
<td></td>
<td>NTB, 9.4 nmol, 10 min</td>
<td>8.34 ± 3.24 (10)***</td>
<td>39.63 ± 12.62 (10)*</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.
Nor-BNI is a slow acting but long-lasting μ-opioid receptor antagonist (Endoh et al., 1992). The blockade of nor-BNI was not verified indeed selectively blocked after 15 min of pretreatment with (15 nmol, 10 min) A/S Dyn 10 78.86 ± 8.66
(25.6 nmol, 10 min) A/S 10 63.50 ± 14.44
U50,488H NRS 20 78.86 ± 8.66
(115 nmol, 10 min) A/S Dyn 10 86.98 ± 3.95

Endomorphin-1 NRS 10 85.74 ± 10.7
(24.6 nmol, 5 min) A/S Dyn 20 90.05 ± 5.15
DAMGO NRS 10 68.20 ± 13.05
(0.04 nmol, 10 min) A/S Dyn 10 73.48 ± 14.37
Morphine NRS 10 68.45 ± 12.36
(9 nmol, 20 min) A/S Dyn 10 60.47 ± 14.62
DPDPE NRS 20 71.39 ± 9.07
(14.7 nmol, 10 min) A/S Dyn 10 79.02 ± 9.10
(40.1 nmol, 10 min) A/S Dyn 20 76.85 ± 14.30

The finding strongly indicates the involvement of κ-opioid receptors in endomorphin-2-induced antinociception. Because endomorphin-2 has a very low affinity for κ-opioid receptors in vitro ligand-binding assays, it is unlikely that the endomorphin-2-induced antinociception is mediated by the direct stimulation of κ-opioid receptors. Because dynorphin A(1-13) is an endogenous ligand for κ-opioid receptors, the possibility that the κ-ergic mechanism for the production of antinociception by endomorphin-2 is mediated by the release of dynorphin A(1-17) was then explored with the antisem to dynorphin A(1-17). The pretreatment of mice with an antisem to dynorphin A(1-17) attenuated significantly the antinociception induced by endomorphin-2. This finding indicates that endomorphin-2 releases dynorphin A(1-17), which then acts on κ-opioid receptors to induce antinociception. The finding that the pretreatment of mice with an antisem against dynorphin A(1-17) administered i.e.v. did not block the antinociception induced by U50,488H adminis-

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