Life Sciences, Vol. 61, No. 7, pp. PL 81-86, 1997

0024-3205/97 $17.00 + .00

ELSEVIER

PII S0024-3205(97)00542-0

PHARMACOLOGY LETTERS
Accelerated Communication

[MET^5]ENKEPHALIN AND δ_2-OPIOID RECEPTORS IN THE SPINAL CORD ARE INVOLVED IN THE COLD WATER SWIMMING-INDUCED ANTINOCICEPTION IN THE MOUSE

Hirokazu Mizoguchi, Minoru Narita, John P. Kampine and Leon F. Tseng

Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA

(Submitted February 4, 1997; accepted February 14, 1997;
received in final form May 21, 1997)

Abstract. Mice made cold water swimming (CWS: 4 °C, 3 min) produced an opioid-mediated antinociception. Experiments were designed to determine what types of opioid receptors and endogenous opioid peptides in the spinal cord are involved in the CWS-induced antinociception in male ICR mice. Antinociception was measured by the tail-flick test. CWS-induced antinociception was blocked by intrathecal (i.t.) pretreatment with antiserum to [Met^5]enkephalin (100 μg, 1 hr), but not by antiserum (100 μg, 1 hr) to [Leu^5]enkephalin, β-endorphin or dynorphin A (1-17). Moreover, i.t. pretreatment with δ_2-opioid receptor antagonist naltriben (NTB: 10 μg, 10 min) blocked the antinociception induced by CWS or i.t.-administered [Met^5]enkephalin (10 μg). However, the antinociception induced by CWS or i.t.-administered [Met^5]enkephalin was not blocked by i.t. pretreatment with δ_1-opioid receptor antagonist 7-benzylidene naltrexone (BNTX: 1 μg, 10 min), μ-opioid receptor antagonist D-Phe-Cys-Try-D-Try-Orn-Thr-Phe-Thr-NH_2 (CTOP: 50 ng, 10 min), or κ-opioid receptor antagonist norbinaltorphimine (norBNI: 5 μg, 24 hr). These data indicate that [Met^5]enkephalin and δ_2-opioid receptor in the spinal cord are involved in antinociception induced by CWS.

Key Words: cold water swimming, antinociception, δ_2-opioid receptors, [Met^5]enkephalin, spinal cord, mouse

Introduction

It has been well documented that some types of stress induced by environmental stimuli produce antinociception, and some of them are considered to be mediated by the activation of endogenous opioid systems (1,2). Especially, cold water swimming (CWS) in mice has been consistently shown to produce antinociception which is mediated by the activation of endogenous opioid peptides (3-5). We have previously reported that CWS-induced antinociception in the mouse is mediated by the stimulation of δ-opioid receptor in spinal cord (6), suggesting the release of endogenous opioid peptides.

The present studies were then designed to determine what endogenous opioid peptides, [Met^5]enkephalin (7), [Leu^5]enkephalin (7), dynorphin A (1-17) (8) or β-endorphin (9), are involved in the CWS-induced antinociception in the mouse spinal cord. The antisera to these endogenous opioid peptides (10), which bind the released endogenous opioid peptides, were used to identify endogenous opioid peptides. We found that [Met^5]enkephalin and the δ_2- but not δ_1-opioid receptor in the spinal cord are involved in CWS-induced antinociception in the mouse.

Corresponding Author: Dr. L.F. Tseng, Department of Anesthesiology, Medical College of Wisconsin, MEB-Room 462c, 8701 Watertown Plank Road, Milwaukee, WI 53226

Materials and Methods

Animals. Male ICR mice weighing 23 - 25 g (Sasco Inc., Omaha, NE) were used. 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 (11). For measurement of the latency of the tail-flick response, mice were gently held by hand with their tail positioned in an apparatus (Model TF6, EMDIE Instrument Co., Maidens, VA) for radiant heat stimulation on the dorsal surface of the tail. The intensity of the heat stimulus was adjusted so that the animal flicked its tail after 3 to 5 sec. The inhibition of the tail-flick response was expressed as percent maximum possible effect, % MPE, which was calculated as: $[(T_1 - T_0) / (T_2 - T_0)] \times 100$, where T_0 and T_1 were the tail-flick latencies before and after the CWS or opioid receptor agonist treatments and T_2 was the cutoff time which was set at 10 sec to avoid the injury of the tail.

Antinociception induced by CWS. A water tank (30 x 20 and 15 cm tall) filled with ice cold water (4 °C) 7.5 cm in depth was used for mice to induce swimming (6,12). After the measurement of the baseline tail-flick latency, mice were placed in the water for 3 min and dried immediately after swimming with cloth towels. The preliminary experiment indicated that the tail-flick latencies increased right after CWS, reached its peak in about 7 min, gradually declined and returned to that of the pre-swimming level in 20 - 25 min. The tail-flick response was then tested 7 min after CWS in all experiments.

Drugs. Drugs used were: δ_1-opioid receptor agonist [D-Pen^{2,5}]enkephalin (DPDPE, Bachem California, Torrance, CA); δ_2-opioid receptor agonist [D-Ala^2]deltorphin II (Molecular Research Laboratories, Durham, NC); μ-opioid receptor agonist [D-Ala^2,NHPhe^4,Gly-ol]enkephalin (DAMGO, Bachem California); κ-opioid receptor agonist U50,488H (synthesized by Dr. Hiroshi Nagase, Toray Industries Inc., Kamakura, Japan); δ_1-opioid receptor antagonist 7-benzylidene naltrexone (BNTX, synthesized by Dr. Hiroshi Nagase); δ_2-opioid receptor antagonist naltriben (NTB, synthesized by Dr. Hiroshi Nagase); μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Try-Orn-Thr-Phe-Thr-NH_2 (CTOP, Peninsula Laboratory Inc., Belmont, CA); κ-opioid receptor antagonist norbinaltorphimine (norBNI, Research Biochemicals International, Natick, MA); endogenous opioid peptide [Met^5]enkephalin (Peninsula Laboratory Inc.); aminopeptidase inhibitor bestatin (Cambridge Research Biochemicals Inc., Valley Stream, NY); carboxypeptidase inhibitor thiorphan (Sigma Chemical Co., St. Louis, MO). Antisera to [Met^5]enkephalin, [Leu^5]enkephalin, β-endorphin and dynorphin A (1-17) were produced by immunization of male New Zealand rabbits according to the method described previously and the potencies and the cross-immunireactivities of these antisera have been characterized (10). Normal rabbit serum served as control for antisera. All drugs used for i.t. injection were dissolved in sterile saline solution (0.9 % NaCl solution), and DPDPE, DAMGO, [D-Ala^2]deltorphin II and CTOP were dissolved in sterile saline solution containing 0.01 % Triton X-100. To avoid the degradation of [Met^5]enkephalin, [Met^5]enkephalin was dissolved in the vehicle which contained peptidase inhibitors, bestatin (1 μg/μl) and thiorphan (1 μg/μl) in sterile saline solution (13,14). I.t. administration was made according to the procedure of Hylden and Wilcox (15) using a 25-μl Hamilton syringe with a 30-gauge needle. Injection volume for i.t. injection was 5 μl.

Statistical analysis. The data are expressed as the mean and S.E.M. Statistical analysis of difference between groups was assessed with a one-way analysis of variance (ANOVA) following by the Newman-Keuls test.

Results

Effects of i.t. pretreatment with antisera to [Met^5]enkephalin, [Leu^5]enkephalin, dynorphin A (1-17) and β-endorphin on the CWS-induced tail-flick inhibition. Groups of mice were pretreated i.t. with antisera to [Met^5]enkephalin (1 - 100 μg), [Leu^5]enkephalin (100 μg), dynorphin A (1-17) (100 μg) or β-endorphin (100 μg) or normal rabbit serum (100 μg) 1 hr before the CWS and the tail-flick response was measured 7 min after CWS. The CWS produced a marked inhibition of the tail-flick response in mice pretreated i.t. with normal rabbit serum (62.32 ± 7.84 % MPE) (Fig. 1).

However, the tail-flick inhibition induced by CWS was significantly attenuated by the i.t. pretreatment with antiserum to [Met^5]enkephalin. The antinociception (% MPE) was significantly decreased from 62.32 % in control mice to 28.11 ± 4.80 % in mice pretreated with 100 μg of antiserum to [Met^5]enkephalin. On the other hand, i.t. pretreatment with antisera to [Leu^5]enkephalin, dynorphin A (1-17) and β-endorphin, did not affect the CWS-induced tail-flick inhibition.

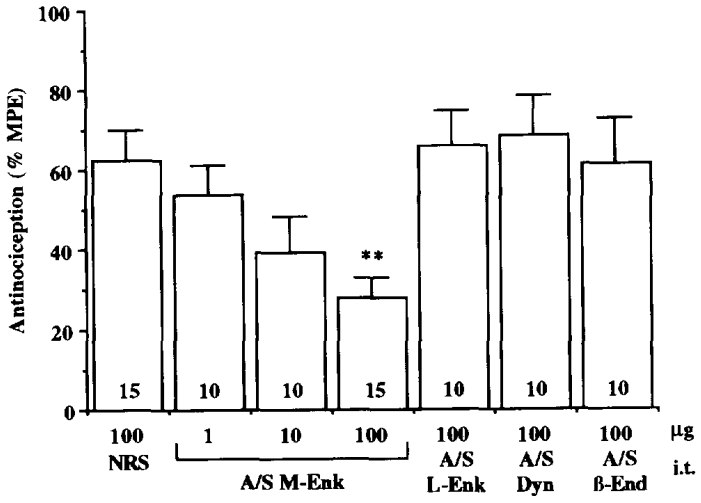

Fig. 1

Effects of i.t. pretreatment with antisera to [Met^5]enkephalin, [Leu^5]enkephalin, dynorphin A (1-17) or β-endorphin on the CWS-induced tail-flick inhibition in mice. Groups of mice were injected i.t. with normal rabbit serum (NRS: 100 μg) or antisera to [Met^5]enkephalin (A/S M-Enk: 1 - 100 μg), [Leu^5]enkephalin (A/S L-Enk: 100 μg) dynorphin A (1-17) (A/S Dyn: 100 μg) or β-endorphin (A/S β-End: 100 μg), 1 hr before the CWS (4 °C, 3 min). The tail-flick response was measured 7 min after the CWS. The numbers within the columns indicate the number of mice used and the vertical bars represent the S.E.M. **p<0.01, compared to mice pretreated with NRS.

Effects of i.t. pretreatment with δ-, μ- or κ-opioid receptor antagonist on the CWS-induced tail-flick inhibition. Groups of mice were pretreated i.t. with NTB (10 μg), BNTX (1 μg), CTOP (50 ng) or saline (5 μl) 10 min before or norBNI (5 μg) or saline 24 hr before CWS, and the tail-flick responses were measured 7 min after the CWS. As shown in Fig. 2, CWS produced a marked inhibition of the tail-flick response in mice pretreated i.t. with saline (61.14 ± 5.20 % MPE). However, the tail-flick inhibition was significantly attenuated by the i.t. pretreatment with NTB (11.36 ± 4.71 % MPE). On the other hand, i.t. pretreatment with BNTX, CTOP or norBNI did not attenuate CWS-induced tail-flick inhibition.

In order to determine if the doses of opioid receptor antagonists used in the studies of CWS-induced antinociception are sufficient to block the respective opioid receptors, the effects of i.t. pretreatment with the same doses of NTB, BNTX, CTOP and norBNI on tail-flick inhibition induced by i.t.-administered [D-Ala^2]deltorphin II, DPDPE, DAMGO or U50,488H was studied. Groups of mice were injected i.t. with saline (5 μl) or NTB (10 μg), BNTX (1 μg) or CTOP (50 ng) 10 min before or saline or norBNI (5 μg) 24 hr before i.t. injection of [D-Ala^2]deltorphin II (5 μg) DPDPE (5 μg), DAMGO (10 ng) or U50,488H (75 μg) and the tail-flick response was measured 10 min later. I.t. pretreatment with NTB, BNTX, CTOP and norBNI markedly blocked the antinociception induced by i.t.-administered [D-Ala^2]deltorphin II, DPDPE, DAMGO and U50,488H, respectively (Data not shown).

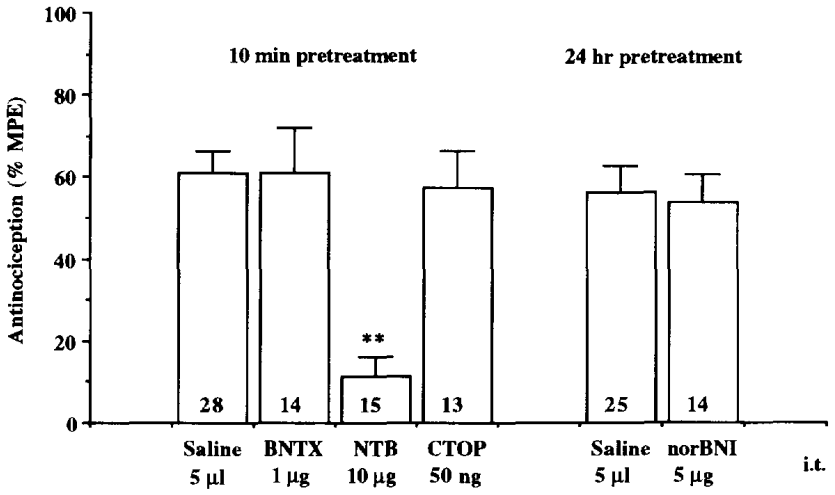

Fig. 2

Effects of i.t. pretreatment with opioid receptor antagonists on the CWS-induced tail-flick inhibition in mice. Groups of mice were injected i.t. with NTB (10 µg), BNTX (1 µg), CTOP (50 ng) or saline (5 µl) 10 min, norBNI (5 µg) or saline 24 hr before CWS (4 °C, 3 min). The tail-flick response was measured 7 min after the CWS. The numbers within the columns indicate the number of mice used and the vertical bars represent the S.E.M. **p<0.01, compared to mice pretreated with saline.

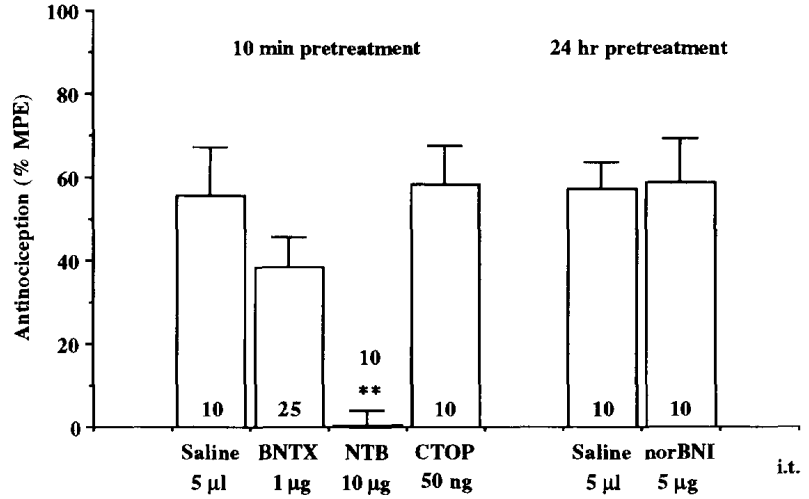

Fig. 3

Effects of i.t. pretreatment with opioid receptor antagonists on tail-flick inhibition induced by i.t.-administered [Met^5]enkephalin in mice. Groups of mice were injected i.t. with BNTX (1 µg), NTB (10 µg), CTOP (50 ng) or saline (5 µl) 10 min before or norBNI (5 µg) or saline 24 hr before the i.t. injection of [Met^5]enkephalin (10 µg). To avoid the quick degradation of [Met^5]enkephalin administered, [Met^5]enkephalin was dissolved in the vehicle which contained peptidase inhibitors, bestatin (5 µg) and thiorphan (5 µg). Mice treated i.t. with drug vehicle 10 min after i.t. pretreatment with saline served as control for [Met^5]enkephalin-induced antinociception. The tail-flick response was measured 5 min after the treatment with [Met^5]enkephalin or vehicle. The numbers within the columns indicate the number of mice used and the vertical bars represent the S.E.M. **p<0.01, compared to mice pretreated with saline before [Met^5]enkephalin treatment.

Effects of i.t. pretreatment with opioid receptor antagonists on tail-flick inhibition induced by i.t.-administered [Met^5]enkephalin. The results of experiments described above indicate that [Met^5]enkephalin is involved in CWS-induced antinociception. Thus [Met^5]enkephalin given i.t. should mimic CWS-induced antinociception by acting on δ_2-opioid receptors for producing antinociception. The experiments were designed to determine what type of opioid receptors in the spinal cord is involved in the [Met^5]enkephalin-induced antinociception. To avoid the quick degradation of [Met^5]enkephalin, [Met^5]enkephalin was dissolved in the vehicle which contained peptidase inhibitors, bestatin (5 μg) and thiorphan (5 μg) and i.t. treatment with vehicle did not produce any significant antinociception (3.77 ± 9.60 % MPE, N = 10). Groups of mice pretreated with BNTX (1 μg), NTB (10 μg), CTOP (50 ng) or saline (5 μl) 10 min before or norBNI (5 μg) or saline (5 μl) 24 hr before, were injected i.t. with [Met^5]enkephalin (10 μg) and the tail-flick response was measured 5 min after injection of [Met^5]enkephalin. As shown in Fig. 3, [Met^5]enkephalin injected i.t. produced a marked antinociception in mice pretreated i.t. with saline 10 min (57.77 ± 11.42 % MPE) or 24 hr (57.17 ± 6.33 % MPE) prior. I.t. pretreatment with NTB blocked completely the antinociception induced by i.t.-administered [Met^5]enkephalin (0.38 ± 3.59 % MPE). On the other hand, i.t. pretreatment with BNTX, CTOP or norBNI did not significantly block the antinociception induced by i.t.-injected [Met^5]enkephalin.

Discussion

We have previously reported that the antinociception induced by CWS in the mouse is mediated by the stimulation of δ-opioid receptors in the spinal cord (6). This contention is supported by the finding that the blockade of δ-opioid receptors in the spinal cord by i.t. injection of δ-opioid receptor antagonist naltrindole or depletion of δ-opioid receptors in the spinal cord by i.t. treatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (16) blocks CWS-induced antinociception (6). δ-Opioid receptors have been further classified into δ_1- and δ_2-opioid receptors (17,18), which are blocked by δ_1- and δ_2-opioid receptor blocker, BNTX and NTB, respectively. We found that δ_2-, but not δ_1-opioid receptors in the spinal cord are involved in CWS-induced antinociception, because i.t. pretreatment with a selective δ_2-opioid receptor antagonist NTB but not δ_1-opioid receptor antagonist BNTX blocked the antinociception induced by CWS. We also found that CWS-induced antinociception is not mediated by the stimulation of μ- or κ-opioid receptors, because blockade of the μ- and κ-opioid receptors in the spinal cord by i.t. pretreatment with CTOP and norBNI, respectively, did not affect CWS-induced antinociception.

[Met^5]enkephalin has been suggested to be the neurotransmitter for δ-opioid receptors (7, 19, 20). The finding that CWS-induced antinociception is mediated by the stimulation of δ-opioid receptors suggest that CWS induces the release of [Met^5]enkephalin which subsequently acts on δ-opioid receptors for the production of antinociception. We found that i.t. pretreatment with antiserum to [Met^5]enkephalin, but not [Leu^5]enkephalin, dynorphin A (1-17) and β-endorphin, attenuated inhibition of the tail-flick response induced by CWS in the mouse. Our results suggest that CWS selectively induce the release of [Met^5]enkephalin, but not [Leu^5]enkephalin, dynorphin (1-17) or β-endorphin in the spinal cord for the production of antinociception.

If the release of [Met^5]enkephalin and its subsequent stimulation of δ_2-opioid receptors in the spinal cord is involved in CWS-induced antinociception, the administration of [Met^5]enkephalin should mimic the effect of CWS by acting on δ_2-opioid receptors in the spinal cord for the production of antinociception. We found that i.t. administration of [Met^5]enkephalin in the presence of aminopeptidase inhibitor bestatin (5 μg) and carboxypeptidase inhibitor thiorphan (5 μg) to inhibit degradation of [Met^5]enkephalin (13,14) produced antinociception. The antinociception was blocked by i.t. pretreatment with NTB but not by BNTX, CTOP or norBNI indicating that only δ_2- but not δ_1, μ- or κ-opioid receptors are involved in [Met^5]enkephalin-induced antinociception.

In conclusion, present study indicates that [Met^5]enkephalin and δ_2-opioid receptor in spinal cord are involved in CWS-induced antinociception.

Acknowledgment

This work was supported by U.S. Public Health Service Grant from the National Institute on Drug Abuse (DA 03811).

References

1. D.J. MAYER and B.H. MANNING, The Pharmacology of Opioid Peptides, L.F. Tseng (Ed), 345-395, Harwood Academic Publishers, Switzerland (1995).
2. L.R. WATKINS and D.J. MAYER, Science 216 1185-1192 (1982).
3. G. TIERNEY, J. CARMODY and D. JAMIESON, Pain 46 89-95 (1991).
4. P. KILLIAN, B.B. HOLMES, A.E. TAKEMORI, P.S. PORTOGHESE and J.M. FUJIMOTO, J. Pharmacol. Exp. Ther. 274 730-734 (1995).
5. T.W. VANDERAH, K.D. WILD, A.E. TAKEMORI, M. SULTANA, P.S. PORTOGHESE, W.D. BOWEN, H.I. MOSBERG and F. PORRECA, J. Pharmacol. Exp. Ther. 262 190-197 (1992).
6. H. MIZOGUCHI, M. NARITA, H. NAGASE and L.F. TSENG, Regul. Pept. 59 255-259 (1995).
7. J. HUGHES, T.W. SMITH, H.W. KOSTERLITZ, L.A. FOTHERGILL, B.A. MORGAN and H.R. MORRIS, Nature 258 577-579 (1975).
8. A. GOLDSTEIN, W. FISCHI, L.I. LOWNEY, M. HUNKAPILLER and L. HOOD, Proc. Natl. Acad. Sci. USA 78 7219-7223 (1981).
9. B.M. COX, A. GOLDSTEIN, and C.H. LI, Proc. Natl. Acad. Sci. USA 73 1821-1823 (1976).
10. L.F. TSENG and K.A. COLLINS, J. Pharmacol. Exp. Ther. 266 1430-1438 (1992).
11. F.E. D'AMOUR and D.L. SMITH, J. Pharmacol. Exp. Ther. 72 74-79 (1941).
12. H. MIZOGUCHI, M. NARITA, J.P. KAMPINE and L.F. TSENG, Eur. J. Pharmacol. 309 37-40 (1996).
13. P. CHAILLET, H. MARCAIS-COLLADO, J. COSTENTIN, C.-C. YI, S. DE LA BAUME and J.-C. SCHWARTZ, Eur. J. Pharmacol. 86 329-336 (1983).
14. B.P. ROQUES, M.C. FOURNIE-ZALUSKI, E. SOROCA, J.M. LECOMTE, B. MALFROY, C. LLORENS and J.-C. SCHWARTZ, Nature 288 286-288 (1980).
15. J.L.K. HYLDEN and G.L. WILCOX, .Eur. J. Pharmacol. 167 313-316 (1980).
16. M. NARITA and L.F. TSENG, Eur. J. Pharmacol. 284 185-189 (1995).
17. A. MATTIA, S.C. FARMER, A.E. TAKAMORI, M. SULTANA, P.S. PORTOGHESE, H.I. MOSBERG, W.D. BOWEN and F. PORRECA, J. Pharmacol. Exp. Ther. 260 518-525 (1992).
18. P.S. PORTOGHESE, M. SULTANA, H. NAGASE and A.E. TAKEMORI, Eur. J. Pharmacol. 218 195-196 (1992).
19. R.J. KNAPP, L.K. VAUGHN and H.I. YAMAMURA, The Pharmacology of Opioid Peptides, L.F. Tseng (Ed), 1-28, Harwood Academic Publishers, Switzerland (1995).
20. M. NARITA, H. MIZOGUCHI, H. NAGASE and L.F. TSENG, Psychopharmacology (in press).