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Analgesic tolerance and cross-tolerance to i.c.v. endomorphin-1, endomorphin-2, and morphine in mice

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Abstract

The present study examined the development of analgesic tolerance to endomorphin-1 (EM1), endomorphin-2 (EM2), and morphine, and cross-tolerance among these drugs. Male Swiss Webster mice were injected i.c.v. with EM1, EM2, morphine, or vehicle once daily for 5 days, and tested for analgesia in the tail flick test. To determine the extent of cross-tolerance, on the sixth day mice from each of the above groups received i.c.v. injections of EM1, EM2, morphine, or vehicle before analgesic testing. The development of tolerance to EM1 and EM2 closely resembled that of morphine. Complete, symmetrical cross-tolerance was observed between all drugs in the study. These results demonstrate a time-course and extent of tolerance similar to morphine, and support a common mechanism of action through the mu-opioid receptor.

Keywords: Antinociception; Analgesia; Pain; Hot-plate; Opioid; Peptides

Zadina et al. [25] recently isolated from neural tissue two heretofore unknown endogenous opioid peptides, which they named endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2). These two peptides display high affinity and selectivity for the mu-opioid receptor and are known to produce many of the same effects as the prototypic mu-opioid receptor agonist, morphine, including analgesia [25].

Differences between these peptides and morphine have been described. Endomorphin-1 (EM1)- and endomorphin-2 (EM2)-induced antinociception appears more rapidly and is of a shorter duration than morphine-induced antinociception [5,21]. Of particular clinical interest are the findings that EM1 shows less potential for cardiorespiratory side effects than morphine [2] and in contrast to morphine, the analgesic effects of EM1 can be separated from its rewarding effects [23].

An additional, important question about the clinical potential of these two peptides involves tolerance. Tolerance can be defined as the need for escalating doses of the drug to maintain a stable effect [1]. It has been shown that tolerance develops to the antinociceptive effects of EM1 and EM2 in both acute models with a single pretreatment [6,20,24] and chronic administration models [8,15].

The development of cross-tolerance among EM1, EM2, and morphine is less clear. It has been shown that although pre-treatment with EM2 markedly reduces the analgesic potency of test doses of EM1, pretreatment with EM1 can have only a minimal effect on EM2-induced antinociception [6,24]. Others have found significant EM1- [13] or EM2-induced analgesia [8] in rats made tolerant to morphine. The research reported here was designed to further elucidate the time course of tolerance development to chronic doses EM1 and EM2 relative to morphine and the degree of cross-tolerance among these drugs.

Male Swiss Webster mice were maintained on a 12 h/12 h light/dark cycle (onset at 06:00 h) and given free access to food and water. All procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [13].

The mice were anesthetized with i.p. ketamine (200 mg/kg) and xylazine (10 mg/kg) and implanted unilaterally with

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a 23-gauge outer cannula aimed at the lateral ventricle (anterior/posterior: 0.2 mm, medial/lateral: 0.10 mm, dorsal/ventral: 0.25 mm). Each cannula was anchored in place with dental cement poured around the outer cannula and the heads of three jeweler's screws placed in the skull. A stainless steel stylet (size 00) extending just beyond the tip of the cannula was inserted during surgery and left in place until time of testing. The animals were allowed to recover for 7–10 days before analgesic testing.

Morphine sulfate (National Institute of Drug Abuse), EM1, and EM2 (custom synthesized (JEZ) by American Peptide Company, Sunnyvale, CA, USA) were dissolved in a 0.001 M acetic acid solution. Drugs were administered i.c.v. in a volume of 1 μl through a 30 gauge inner cannula that extended 0.25 mm beyond the tip of the outer cannula. The injection cannula was left in place for 60 s to allow for diffusion of the drugs into the ventricles. Equipotent doses approximating four times the ED_{50} determined in earlier studies [25] were used as follows: 10 μg (30 nmol) morphine, 18 μg (30 nmol) EM1 or 30 μg (60 nmol) EM2 dissolved in 1 μl of injection vehicle. Control groups received 1 μl injections of the vehicle.

Pain sensitivity was tested in the tail flick assay (Tail Flick Analgesia Meter, Columbus Instruments, Columbus, OH, USA) in which a beam of light was focused on the ventral surface of the animal's tail 2.5 cm (±2 cm) from the tip. A cutoff latency of 10 s was imposed to prevent tissue damage.

After the surgical recovery period, animals were randomly assigned to one of four groups. Each day that the tail flick test was performed, animals were exposed to the test room for a 15-min habituation period. On the day prior to the first drug administration, baseline tail flick was recorded. For the next 5 days, 10 min after i.c.v. injection of EM1, EM2, morphine, or vehicle animals were tested in the tail flick test. To determine whether analgesic cross-tolerance develops between the drugs used in this experiment, one day following the completion of tolerance testing (day 6) animals from each of the drug groups (morphine, EM1, EM2, and vehicle) were randomly assigned to four subgroups. These four subgroups were then tested for analgesia 10 min after receiving i.c.v. injections of morphine, EM1, EM2, or vehicle in the tail flick procedure as described above.

After completion of each experiment, animals were overdosed with sodium pentobarbital and injected i.c.v. with 5 μl of methylene blue dye. Ten minutes later, brains were removed and frozen. Frozen brains were dissected and only data from those animals with dye throughout the lateral ventricle ipsilateral to the cannula were included in statistical analysis.

All analyses were conducted using SPSS for Windows (Version 6.0) with the probability of a Type I error set at 0.05. The data were examined for outliers and none were found. Tolerance data were analyzed using a Drug × (Day × Subjects) mixed factorial ANOVA. Cross-tolerance data were examined using a two-way (Pretreatment Drug × Cross-tolerance Drug) between-groups factorial ANOVA.

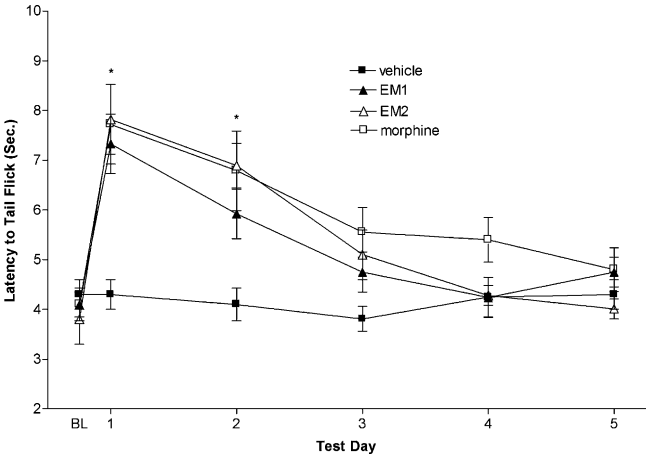

Fig. 1. Development of tolerance. Mean (±S.E.M.) tail flick latencies following i.c.v. injections of morphine, EM1, EM2, or vehicle ($n = 26$–31) over the 5 days of tolerance induction. Asterisk (∗) denotes significant difference between all drug groups and vehicle group as determined by Tukey's HSD test.

Subsequent analyses of simple effects were made using Tukey's HSD test to control for increases in the family-wise error rate.

As indicated in Fig. 1, the i.c.v. administration of EM1 and EM2 resulted in marked antinociception that was similar to morphine on days 1 and 2 (Omnibus two-way interaction: $F(11, 377) = 4.25$, $P < 0.01$, power = 0.99, partial $\eta^2 = 0.11$; day 1: $F(3, 107) = 14.27$, $P < 0.01$, power > 0.99, partial $\eta^2 = 0.29$; day 2: $F(3, 107) = 7.36$, $P < 0.01$, power = 0.98, partial $\eta^2 = 0.17$). Tolerance to the antinociceptive effects of EM1, EM2 and morphine was evident by day 3, when scores for all three agonist groups were not significantly different from controls. Cross-tolerance data are summarized in Fig. 2. In the present study, symmetrical cross-tolerance developed between all drugs used. Test doses of EM2 and morphine produced significant

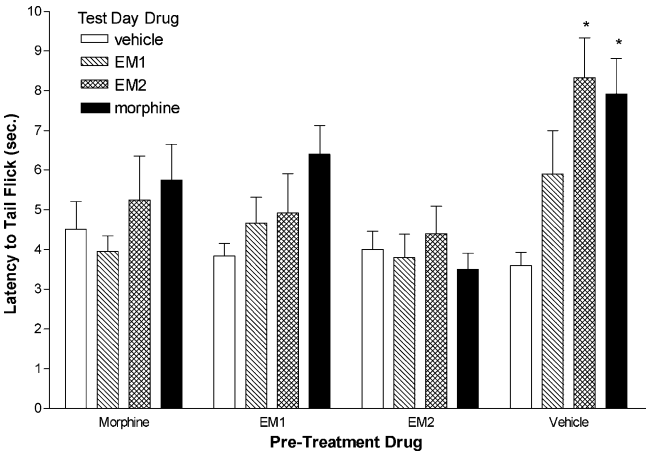

Fig. 2. Cross-tolerance. Mean (±S.E.M.) tail flick latencies following i.c.v. morphine, EM1, EM2, or vehicle injections ($n = 6$–8) after 5 days pretreatment with morphine, EM1, EM2, or vehicle. Asterisk (∗) denotes significant difference from vehicle injection as determined by Tukey's HSD test.

analgesia in the vehicle pretreated group ($F(3, 23) = 6.94$, $P < 0.01$, power > 0.96, partial $\eta^2 = 0.45$); EM1 did not significantly alter tail flick latencies in vehicle-pretreated animals. No other significant results were obtained.

The data presented here replicate previous findings that EM1 and EM2 are potent antinociceptive agents. In opioid-naive animals (day 1 of the study), i.c.v. injections of these peptides produced potent analgesia similar to the prototypic mu-opioid receptor agonist, morphine. In the present study equimolar doses of EM1 and morphine were found to produce equipotent analgesia; however, approximately twice as much EM2 is required to produce the same analgesic effect. This is consistent with dose–response relationships described earlier [6,15,24,25]. As in other studies, analgesic tolerance developed to daily i.c.v. injections of EM and morphine by day 3 [7–9]. Other studies have shown more rapid development of tolerance for the endomorphins than for morphine after chronic intrathecal (i.t.) administration to rats [8]. Several experimental variables (route of administration, injection dose and timing, species used, etc.) are likely to contribute to these differences, and indicate that the time course of tolerance is dependent on these factors.

We report here that symmetrical analgesic cross-tolerance is present among morphine, EM1, and EM2. This finding supports the hypothesis that the antinociceptive effects of the endomorphins and morphine are mediated by a common mechanism, agonist activity at the mu-opioid receptor. Our results are consistent with evidence that EM1 and EM2 are known to activate mu-opioid receptor, but not delta-opioid receptor mediated second messenger cascades in vitro and that signaling [12] and antinociception [10,11] are absent in mu-opioid receptor knockout mice.

However, these are in contrast to reported asymmetric tolerance observed in other paradigms [6,8,24]. It has been postulated that different effects of the mu agonist could be mediated by either different subtypes, splice variants, or physical states of the mu receptor, or different post-(mu)-receptor effects. For example, Sakurada et al. [16,18] demonstrated that although both EM1- and EM2-induced analgesia is completely reversed by the mu-opioid receptor specific antagonist β-funaltrexamine, EM2- but not EM1-induced antinociception is sensitive to antagonism by the mu_1-opioid receptor specific antagonist naloxonazine and to a novel morphine-6β-glucuronide receptor antagonist, 3-methoxynaltrexone. Furthermore, although both EM1- and EM2-induced antinociception are reversed by β-funaltrexamine, EM2-induced antinociception can be attenuated by the kappa-opioid receptor specific antagonist, nor-binaltorphimine, and by antiserum against dynorphin A(1–17), suggesting the downstream involvement of kappa-opioid receptors in EM2's effects [14,17,22]. It is possible, therefore, that EM2 is active at a discrete population of mu-opioid receptors, which subsequently engage dynorphin-mediated systems [14,22].

The above findings of differences between the mediators of EM1 and EM2 action could account for observations that tolerance to the antinociceptive effects of EM1 [6,24], and in some cases morphine [8], does not attenuate the expression of EM2-induced analgesia. Reasons for the discrepancy between reports of asymmetrical cross-tolerance and our findings are not clear. It is possible that the difference in the paradigms used between these studies [6,8,20,24], including the type of tolerance induced (acute versus chronic), species used (rats versus mice), and route of administration (i.t. versus i.c.v.) may account for some of the discrepancies, and make direct comparisons difficult. It should also be noted that, although not statistically significant, the pretreatment group that appeared to provide the greatest responsiveness to subsequent opioid challenge was the EM1 pretreatment group (see Fig. 2), which is consistent with earlier findings [6,24]. The cause of this difference between EM1 and the other drugs is unclear. However, the trend for EM1 to produce reduced analgesia regardless of the pretreatment drug suggests that this drug is the most sensitive to prior opiate administration.

The fact that the EM1-induced increase in latency failed to reach significance after vehicle pretreatment is intriguing. The cause of this difference between EM1 and the other drugs is unclear. However, the trend for EM1 to produce reduced analgesia regardless of the pretreatment drug (see Fig. 2) suggests that the cause may lie in an interaction between EM1 and repeated handling or testing of the animals. Handling [19], restraint [3], and repeated testing [4] have been shown to activate the endogenous opioid systems and to speed the development of tolerance to opioid analgesia. We have observed that EM1, but not EM2, is cross-tolerant with stress-induced analgesia (unpublished findings). Perhaps the failure of EM1 to produce statistically significant analgesia on day 6 of the study is indicative of its greater sensitivity to cross-tolerance with stress-induced analgesia.

The endomorphins are endogenous peptides that possess high affinity and selectivity for the mu-opioid receptor. The present study shows that repeated i.c.v. injections of EM1 and EM2 result in potent analgesia in the tail flick test and in a tolerance profile similar to that of morphine. The demonstration of symmetrical cross-tolerance among morphine and the peptides is consistent with the concept that tolerance to the three agonists in this paradigm is mediated by a common physical state of the mu-opioid receptor.

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References

[1] J. Cleary, M. Backonja, Translating opioid tolerance research, APS Bull. 6 (1996) 4–7.

[2] M.A. Czapla, D. Gozal, O.A. Alea, R.C. Beckerman, J.E. Zadina, Differential cardiorespiratory effects of endomorphin 1, endomorphin 2, DAMGO, and morphine, Am. J. Respir. Crit. Care Med. 162 (2000) 994–999.
[3] J. Dymshitz, S. Amir, Opposing effects of restraint on morphine analgesia and naloxone-induced jumping, Pharmacol. Biochem. Behav. 30 (1988) 905–910.
[4] G. Gebhart, C. Mitchell, The relative contributions of the testing cylinder and the heated plate in the hot plate procedure to the development of tolerance to morphine in rats, Eur. J. Pharmacol. 18 (1972) 56–62.
[5] G. Horvath, M. Szikszay, C. Tomboly, G. Benedek, Antinociceptive effects of intrathecal endomorphin-1 and -2, Life Sci. 65 (1999) 2635–2641.
[6] K.-C. Hung, H.-E. Wu, H. Mizoguchi, L.F. Tseng, Acute antinociceptive tolerance and unidirectional cross-tolerance to endomorphin-1 and endomorphin-2 given intraventricularly in the rat, Eur. J. Pharm. 448 (2002) 169–174.
[7] B. Kest, E. Hopkins, Morphine tolerance after chronic intracerebroventricular injection in male and female mice, Brain Res. 892 (2001) 208–210.
[8] D. Labuz, R. Przewlocki, B. Przewlocka, Cross-tolerance between the different mu-opioid receptor agonist endomorphin-1, endomorphin-2 and morphine at the spinal level in the rat, Neurosci. Lett. 334 (2002) 127–130.
[9] Z.H. Li, L.D. Shan, X.H. Jiang, S.Y. Guo, G.D. Yu, T. Hisamitsu, Q.Z. Yin, Analgesic effect of endomorphin-1, Acta Pharmacol. Sin. 22 (2001) 976–980.
[10] H.H. Loh, H.C. Lui, A. Cavalli, W. Yang, Y.F. Chen, L.N. Wei, mu-Opioid receptor knockout in mice: effects on ligand-induced analgesia and morphine lethality, Mol. Brain Res. 54 (1998) 321–326.
[11] H. Mizoguchi, M. Narita, D.E. Oji, C. Suganuma, H. Nagase, I. Sora, G.R. Uhl, E.Y. Cheng, L.F. Tseng, The mu-opioid receptor gene dose-dependent reductions in G-protein activation in the pons/medulla and antinociception induced by endomorphins in mu-opioid receptor knockout mice, Neuroscience 94 (1999) 203–207.
[12] K. Monory, M.-C. Bourin, M. Spetea, C. Tomboly, G. Toth, H.W. Matthes, B.L. Keiffer, J. Hanoune, A. Borsodi, Specific activation of the mu-opioid receptor (MOR) by endomorphin-1 and endomorphin-2, Eur. J. Neurosci. 12 (2000) 577–584.
[13] National Institues of Health Guide for the Care and Use of Laboratory Animals, NIH publications 80-23, revised, 1996.
[14] M. Ohsawa, H. Mizoguchi, M. Narita, H. Nagase, J.P. Kampine, L.F. Tseng, Differential antinociception induced by spinally administered endomorphin-1 and endomorphin-2 in the mouse, J. Pharm. Exp. Ther. 298 (2001) 592–597.
[15] R. Przewlocki, D. Labuz, J. Mika, B. Przewlocka, C. Tomboly, G. Toth, Pain inhibition by endomorphins, Ann. N.Y. Acad. Sci. 897 (1999) 154–164.
[16] S. Sakurada, T. Hayashi, M. Yuhki, T. Fujimura, K. Murayama, A. Yonezawa, C. Sakurada, M. Takeshita, T. Sato, J.E. Zadina, A.J. Kastin, T. Sakurada, Differential antagonism of endomorphin-1 and endomorphin-2 supraspinal antinociception by naloxonazine and 3-methylnaltrexone, Peptides 23 (2002) 895–901.
[17] S. Sakuarada, T. Hayashi, M. Yuhnki, T. Orito, J.E. Zadina, A.J. Kastin, T. Fujimura, K. Murayama, C. Sakurada, T. Sakurada, M. Narita, T. Suzuki, K. Tan-no, L.F. Tseng, Differential antinociceptive effects induced by intrathecally administered endomorphin-1 and endomorphin-2 in the mouse, Eur. J. Pharm. 427 (2001) 203–210.
[18] S. Sakurada, J.E. Zadina, A.J. Kastin, S. Katsuyama, T. Fujimura, K. Murayama, M. Yuki, H. Ueda, T. Sakurada, Differential involvement of mu-opioid receptor subtypes in endomorphin-1- and endomorphin-2-induced antinociception, Eur. J. Pharm. 372 (1999) 25–30.
[19] F. Smith, D. Brace, D. Dombrowski, W. Dewey, Endogenous opioids released by suspending mice by tail selectively enhance mu opioid analgesia, J. Pharmacol. Exp. Ther. 270 (1994) 1177–1185.
[20] L. Stone, C. Fairbanks, T. Laughlin, H. Nguyen, T. Bushy, M. Wessendorf, G. Wilcox, Spinal analgesic actions of the new endogenous opioid peptides endomorphin-1 and -2, Neuroreport 8 (2000) 3131–3135.
[21] R.D. Soignier, A. Brennan, A.L. Vaccarino, A.J. Kastin, J.E. Zadina, Analgesic effects of endomorphin-1 and endomorphin-2 in the formalin test in mice, Life Sci. 67 (2000) 907–912.
[22] L.F. Tseng, M. Narita, C. Suganuma, H. Mizoguchi, M. Ohsawa, H. Nagase, J.P. Kampine, Differential antinociceptive effects of endomorphin-1 and endomorphin-2 in the mouse, J. Pharm. Exp. Ther. 292 (2000) 576–583.
[23] A.M. Wilson, R.D. Soignier, J.E. Zadina, A.J. Kastin, W.L. Nores, R.D. Olson, G.A. Olson, Dissociation of analgesic and rewarding effects of endomorphin-1 in rats, Peptides 21 (2000) 1871–1874.
[24] H.-E. Wu, K.-C. Hung, H. Mizoguchi, J.M. Fujimoto, L.F. Tseng, Acute antinociceptive tolerance and cross-tolerance between endomorphin-1 and endomorphin-2 given intracerebroventricularly in the mouse, J. Pharm. Exp. Ther. 299 (2001) 1120–1125.
[25] J.E. Zadina, L. Hackler, L. Ge, A.J. Kastin, A potent and selective endogenous agonist for the mu-opiate receptor, Nature 386 (1997) 499–502.