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Spinal analgesic action of endomorphins in acute, inflammatory and neuropathic pain in rats

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Abstract

We studied spinal analgesic and antiallodynic effects of endomorphin-1 and endomorphin-2 administered i.t. in comparison with Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) or morphine, during acute, inflammatory and neuropathic pain in rats chronically implanted with intrathecal cannulas. Endomorphin-1 and endomorphin-2 (2.5, 5, 10 μg i.t.) increased the tail-flick latency and, to the lesser extent, the paw pressure latency. The range of potencies in both those models of acute pain was as follows: DAMGO > morphine = endomorphin-1 > endomorphin-2. In a model of inflammatory pain, the number of formalin-induced flinching episodes was decreased by endomorphin-1. The effect of endomorphin-2 was much less pronounced. Both DAMGO and morphine significantly inhibited the pain-related behavior evoked by formalin. In a neuropathic pain model (sciatic nerve crushing in rats), endomorphin-1 and -2 (5 μg i.t.) had a statistically significant effect on the tail-flick latency and on the cold-water tail flick latency. Morphine, 5 μg, was found to be ineffective. Endomorphin-1 and -2 (2.5 and 5 μg i.t.) dose-dependently antagonized allodynia. Those effects of endomorphins were antagonized in acute (30 μg), inflammatory (30 μg) and neuropathic pain models (60 μg) by cyprodime, a selective μ-opioid receptor antagonist. In conclusion, our results show a strong analgesic action of endomorphins at the spinal cord level. The most interesting finding is a strong, stronger than in the case of morphine, antiallodynic effect of endomorphins in rats subjected to sciatic nerve crushing, which suggests a possible use of these compounds in a very difficult therapy of neuropathic pain.

Keywords: Endomorphin-1; Endomorphin-2; Morphine; DAMGO ([D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin); Pain, neuropathic; Pain, acute; Formalin model

1. Introduction

Two potent endogenous opioid peptides, endomorphin-1 and -2, which are selective against the μ-opioid receptor, have been recently isolated from bovine (Zadina et al., 1997) and human brain (Hackler et al., 1997). These endomorphins have been shown to produce analgesia and inhibit the electrically induced contraction of guinea pig ileum, which is consistent with the action of μ receptor agonists (Zadina et al., 1997). Immunoreactivity of endomorphin-2 was found in the bovine central nervous system, in regions rich in μ-opioid receptors, e.g., thalamus, hypothalamus, cortex and striatum of guinea pig (Zadina et al., 1997), and another study suggested that the peptide could be synthesized in ganglia of primary sensory neurons and then transported to superficial layers of the dorsal horn of the spinal cord in rat and monkey (Martin-Schild et al., 1997; Pierce et al., 1998). Furthermore, both the peptides bound with high affinity to the μ receptor (Zadina et al., 1997) and displayed no activity in μ-opioid receptor knock-out mice, which suggests that these peptides induce antinociception via a μ-opioid receptor-specific mechanism. Interestingly, endomorphin-1 is a partial agonist for G protein activation at the μ-opioid receptor in rat (Sim et al., 1998) and human brain (Hosohata et al., 1998)

When administered intraspinally to mice, both these peptides produced naloxone-sensitive antinociception in a

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tail-flick test. Interestingly, the study suggests that peptides display also antiallodynic properties in the mechanical allodynia induced by intrathecal administration of dynorphin to mice (Stone et al., 1997).

It is well established that morphine and μ receptor-selective opiates are poor analgesics in neuropathic pain in humans and in neuropathic pain models in animals. It has been demonstrated that the effect of morphine is markedly reduced by peripheral axotomy or spinal cord injury (Ossipov et al., 1995a,b). In the present study we studied effects of endomorphin-1 and endomorphin-2 administered i.t. in acute, inflammatory, and neuropathic pain in rats. The study has shown that endomorphins possess antinociceptive properties at the spinal cord level and display profound antiallodynia in neuropathic pain in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–350 g) were housed in single cages lined with sawdust, on a standard 12–12 h light–dark cycle (the light on at 08:00 h), with food and water ad libitum.

The rats were chronically implanted with i.t. catheters under pentobarbital anaesthesia. They were placed on the David Kopf stereotaxic table, and an incision was made in the atlanto occipital membrane. A catheter (PE 10, Clay Adams, Sparks, MD) was carefully introduced to the subarachnoid space at a rostral level of the spinal cord enlargement according to Yaksh and Rudy (1976). Intrathecal injection studies were carried out 7–12 days after the surgery. Drugs were dissolved in distilled water and were injected in a volume of 5 μl, followed by an injection of 10 μl of distilled water to flush the catheter. Control animals were injected i.t. with distilled water and were tested according to the same time schedule as described for the experimental groups. After completing the experiment, the animals were killed with an overdose of pentobarbital (i.p.). The experiments had the approval of the Institute's Animal Research Committee and were carried out according to the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Acute pain–nociceptive threshold.

Antinociceptive effects after acute thermal and mechanical stimuli were evaluated using a tail-flick and a paw pressure tests. The tail-flick test was carried out using an Analgesia Meter apparatus (Mod 33, IITC, USA). An animal was gently restrained by hand, and radiant heat was directed onto its tail. The cut-off time was 9 s. The paw pressure threshold (the Randall–Sellito test), necessary to elicit paw withdrawal, was determined using an automatic gauge (Ugo Basile, Italy). The animal was gently restrained, and an incremental pressure was applied via a piston onto the dorsal surface of the hind paw. The cut-off pressure was 500 g. Tail-flick and paw pressure measurements were taken 3 times at 15-s intervals, and their mean was used for calculations.

Effects of i.t. administration of endomorphin-1 and endomorphin-2, morphine and Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) on the nociceptive threshold were estimated in a time-course study. Measurements of tail-flick and paw pressure were taken before administration of the peptides and again at 15, 30, 60, 120 min after injection. The experiments were also designed to find out whether the selective antagonist of μ-opioid receptors cyprodime, administered i.t., antagonized the antinociceptive effect of endomorphin-1 and endomorphin-2. Cyprodime (30 μg) was injected 10 min before the investigated substances, and tail-flick and paw pressure were measured 15 min after injection of the drugs.

2.3. Inflammatory pain–formalin model

The rats were lightly anesthetized with ether, and 100 μl of a 10% formalin solution was injected subcutaneously (s.c.) into the dorsal surface of the left hind paw. The rat was then placed in a wire cage for observation of the formalin-injected paw. Pain-related behavior was quantified by counting the incidence of spontaneous flinching, shaking and jerking of the injected paw. Flinching episodes were counted continuously for 60 min for each animal and were finally scored for two characteristic time points: 0–5 (first phase) and 20–40 min (second phase) after formalin administration. The rats were injected i.t. with endomorphin-1 (2.5, 5, 10 and 20 μg i.t.) and endomorphin-2 (2.5, 5, 10 and 20 μg i.t.) 15 min before formalin administration. Cyprodime (30 μg) was injected 10 min before the peptides, and after another 10 min formalin was injected. Pain-related behavior was assessed after formalin administration. Control animals were injected i.t. and intraplantarily with distilled water and were tested according to the same time schedule as were experimental groups.

2.4. Neuropathic pain–sciatic nerve crushing

Crush lesioning was performed under pentobarbital anaesthesia, 7–12 days after i.t. implantation of cannulas. The right sciatic nerve was crushed for 30 s, at a position 27 mm distal to the sciatic notch using hemostatic forceps. The lesioning procedure was described in detail by De Koning et al., 1986.

Three behavioral tests were use to evaluate antinociceptive and antiallodynic effects. The antinociceptive effects were estimated using the tail-flick test described above, and by a 0–2°C cold water tail-flick test. The tail-flick and

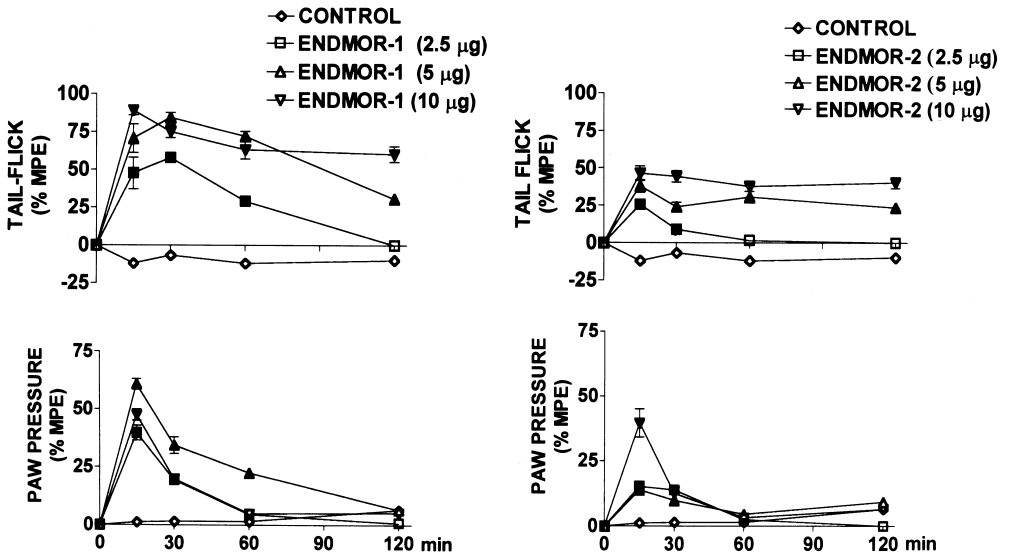

Fig. 1. The spinal antinociceptive effect of endomorphin-1 (ENDMOR-1, 2.5, 5 and 10 μg i.t.) and endomorphin-2 (ENDMOR-2, 2.5, 5 and 10 μg i.t.) on the nociceptive threshold measured by tail-flick (TF) and paw pressure (PP) tests in rat. The results are presented as % of the maximal positive effect (MPE). Solid points represent significant results ($P < 0.05$) in comparison with the control.

cold water tail-flick tests were applied 35 or 55 min after i.t. drug administration, respectively. Cyprodime (60 μg) was injected 10 min before the peptides.

The cold water allodynia test was previously described in detail (Hunter et al., 1997). Each animal was placed on a metal stage submerged to a depth of 2.5 cm in ice-cold water (0°C). The cut-off latency for the test was 40 s. An animal responded by lifting a paw on the crush side out of the water. The animals were tested 20, 40, 60 min after i.t. drug administration.

2.5. Drugs

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) were synthetized by Geza Toth (Biological Research Center of Hungarian Academy of Sciences, Szeged, Hungary); morphine was obtained from Polfa, DAMGO from Sigma, cyprodime was generously presented by Dr. Shmidhammer (Institute of Pharmaceutical Chemistry, University of Insbruck, Austria).

Endomorphin-1 and the endomorphin-2 were administered i.t. in doses of 2.5, 5, 10 and 20 μg/5 μl. DAMGO (0.25 and 0.5 μg/5 μl) and morphine (5, 25 and 50 μg/5 μl) were administered i.t. The μ-opioid receptor antagonist, cyprodime, was administered i.t. in a dose of 30 (acute and inflammatory pain) or 60 μg/5 μl (neuropathic pain).

2.6. Data analysis

The results were statistically assessed by an analysis of variance (ANOVA). Inter-group differences were analysed by Duncan's multiple-range test.

3. Results

3.1. The antinociceptive effect of endomorphin-1, endomorphin-2, DAMGO and morphine in tail-flick and paw pressure test

Endomorphin-1 (2.5, 5, 10 μg i.t.) dose-dependently increased the tail-flick latency. The most potent effect was observed at 15 and 30 min after the injection, but it was significant up to 120 min after two higher doses. The paw pressure latency was significantly increased at 15 and 30

Table 1
The effect of the μ-opioid receptor antagonist cyprodime (CP, 30 μg, i.t.) on the antinociceptive action of endomorphin-1 (EM-1) and endomorphin-2 (EM-2) in tail-flick and paw pressure tests

15 min after i.t. injection		
	Tail flick	Paw pressure
C	4.35 ± 0.07	198 ± 22
C + CP	4.5 ± 0.1	198 ± 4
EM-1, 5 μg	7.6 ± 1.03	382 ± 14.6
CP + EM-1, 5 μg	3.8 ± 0.14[a]	180 ± 6.8[a]
EM-1, 10 μg	8.5 ± 0.3	342 ± 0.9
CP + EM-1, 10 μg	4.7 ± 0.59	180 ± 6.8[a]
EM-2, 5 μg	6.1 ± 0.6	240 ± 10
CP + EM-2, 5 μg	4.2 ± 0.3[a]	208 ± 10[a]
EM-2, 10 μg	6.5 ± 0.7	318 ± 44
CP + EM-2, 10 μg	4.5 ± 0.4	180 ± 6[a]

C = control.
The data are presented as mean ± S.E.M.
[a] Indicates a significant difference ($P < 0.5$) vs. the respective endomorphin alone.

Table 2
The effect of the μ-opioid receptor antagonist cyprodime (CP, 30 μg, i.t.) on the antinociceptive action of endomorphin-1 (EM-1) and endomorphin-2 (EM-2) in formalin-injected rats

	0–10 min	10–15 min	25–30 min	45–50 min	70–75 min
C	61.4 ± 3.2	23.6 ± 2.5	45.04 ± 2.5	44.5 ± 2.3	34.3 ± 2.9
C + CP	53.2 ± 7.1	48 ± 7.1	46.5 ± 4.2	43.8 ± 3.8	47.8 ± 4.7
EM-1, 5 μg	26.2 ± 6.5	18.5 ± 5.3	21 ± 5.5	21.8 ± 5.4	22.8 ± 8.8
CP + EM-1, 5 μg	47.5 ± 10.1[a]	33 ± 3.8[a]	36 ± 5.3	45.2 ± 4.6[a]	47.2 ± 3.1[a]
EM-1, 10 μg	30.5 ± 3.8	26.6 ± 2.3	32.2 ± 4.7	26.5 ± 4.4	22.4 ± 3
CP + EM-1, 10 μg	59.7 ± 4.5[a]	29.5 ± 2.1	44.2 ± 5.9	47 ± 4.6[a]	39 ± 1.3[a]
EM-2, 5 μg	28.5 ± 5.5	19 ± 4.1	29.6 ± 2.8	21.8 ± 4.4	29 ± 3.4
CP + EM-2, 5 μg	46 ± 4.9[a]	26.7 ± 3.0	34.7 ± 7.3	42 ± 6.8[a]	39.2 ± 6.8
EM-2, 10 μg	36.5 ± 2.4	27.5 ± 2.6	29.5 ± 4.0	31.5 ± 4.3	33.8 ± 5.6
CP + EM-2, 10 μg	46.7 ± 3.0	28.6 ± 2.6	40.3 ± 4.0[a]	47.5 ± 1.6[a]	49.5 ± 4.4

C = control.
The rats were lightly anesthetized with ether, and 100 μl of a 10% formalin solution was injected subcutaneously (s.c.) into the dorsal surface of the left hind paw.
The data are presented as mean ± S.E.M.
[a] Indicates a significant difference ($P < 0.5$) vs. the respective endomorphin alone.

min after all the doses used, but at 60 min after the highest dose only.

Endomorphin-2 (2.5, 5, 10 μg i.t.) dose-dependently increased the tail-flick latency. An about 50% increase

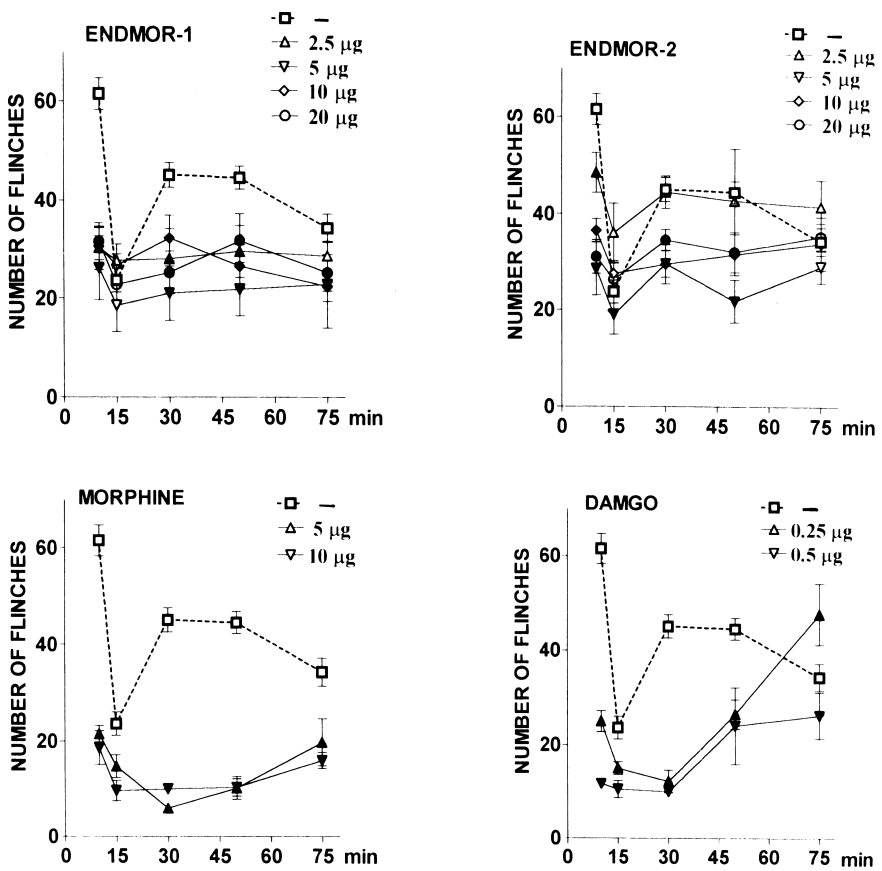

Fig. 2. The time-course of paw flinching inhibition after i.t. administration of endomorphin-1 (ENDMOR-1), endomorphin-2 (ENDMOR-2), DAMGO and morphine in a formalin model of pain. The rats were injected intraplantarily with formalin at 10 min after drug administration. Each point represents the mean ± S.E.M. number of paw flinching episodes during a 5-min observation period in the first (0–5 min) and second phase (20–40 min) of formalin-induced behavior.

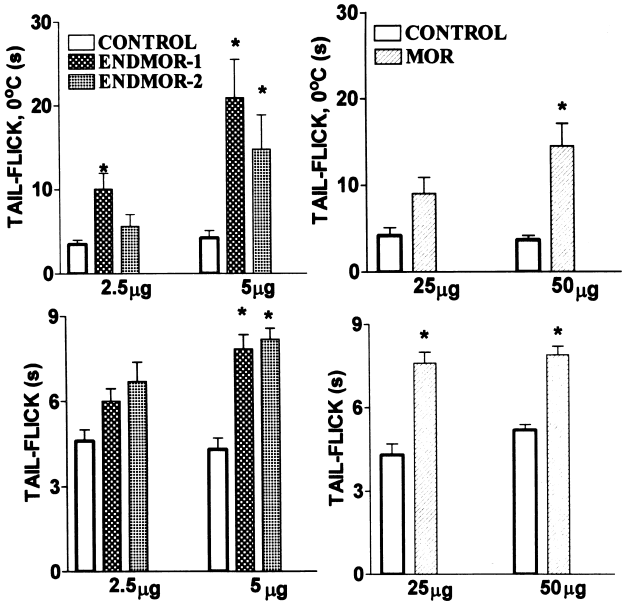

Fig. 3. The spinal antinociceptive effect of endomorphin-1 (2.5; 5 μg/5 μl i.t.), endomorphin-2 (2.5; 5 μg/5 μl i.t.) and morphine (25; 50 μg/5 μl i.t.), evaluated by the cold tail flick (CTF) and tail flick (TF) tests in rats with a sciatic nerve injury. The cut-off latency of the stimulus was 40 s and 9 s, respectively. Control animals were injected i.t. with distilled water and were tested acccording to the same time schedule as the experimental group. The data are presented as mean ± S.E.M. * Indicates a significant difference ($P < 0.5$) between endomorphin-1, -2, morphine and control.

after the highest dose persisted up to 120 min. The paw pressure latency was significant only up to 30 min after endomorphin-2. (Fig. 1). The antinociceptive effect of endomorphins was antagonized by cyprodime (30 μg i.t.) (Table 1)

3.2. The antinociceptive effect of endomorphin-1, endomorphin-2, DAMGO and morphine in a formalin test.

The number of formalin-induced flinching episodes was significantly, but not dose-dependently, reduced by endomorphin-1 in both phases of pain-related behavior. The effect evoked by endomorphin-2 was much less pronounced, but it was not significant, after the lowest dose only. The antinociceptive effect of endomorphins was antagonized by cyprodime (30 μg i.t.) (Table 2)

Both DAMGO and morphine significantly inhibited the pain-related behavior after formalin (Fig. 2).

3.3. The effect of morphine, endomorphin-1 and -2 in a tail-flick test in rats with sciatic nerve injury

Endomorphin-1 and endomorphin-2, administered i.t. in a dose of 2.5 μg each, produced a weak antinociceptive effect; however, their higher dose of 5 μg had a statistically significant effect in the tail-flick test. Morphine, 5 μg, was ineffective, but its higher doses of 25 and 50 μg had a significant effect on the normal sensory nociceptive function in the tail-flick test (Fig. 3, lower panel).

3.4. The effect of morphine, endomorphin-1 and -2 in a cold water tail-flick test in rats with sciatic nerve injury

Endomorphin-1 and endomorphin-2, administered i.t. in increasing doses of 2.5 and 5 μg each, dose-dependently increased the tail-flick latency in a cold water tail-flick test. That effect was significant at either dose of endomorphin-1, but only at the higher dose of endomorphin-2 and at the higher dose of morphine. The increase in the tail-flick latency was antagonized cyprodime, 60 μg (Fig. 3, upper panel, Table 3).

3.5. The effect of morphine, endomorphin-1 and -2 in a cold water allodynia test in rats with sciatic nerve injury

All animals with a crush injury to the sciatic nerve developed allodynia 2 days after the surgery. Endomorphin-1 and endomorphin-2, administered i.t. in increasing doses of 2.5 and 5 μg each, dose-dependently antagonized that allodynia. A significant increase in the latency of paw withdrawal from cold water was observed after 40 and 60 min. The antiallodynic effect of the endomorphins was antagonized by cyprodime, 60 μg, that action being more effective at 40 min after drug administration (Table 3). The effect of cyprodime was less pronounced after 20 min.

Morphine administered i.t. in increasing doses of 5, 25 and 50 μg produced an antiallodynic effect only after the highest dose, at all the time points measured (Fig. 4). The

Table 3
The effect of the μ-opioid receptor antagonist cyprodime (CP, 60 μg/5 μl, i.t.) on the antinociceptive and antiallodynic action of morphine (MOR, 50 μg/5 μl), endomorphin-1 (EM-1, 5 μg/5 μl i.t.) and endomorphin-2 (EM-2, 5 μg/5 μl i.t.) in a sciatic nerve injury model of neuropathic pain

	CA	CTF	TF
C	5.2 ± 2.0	3.7 ± 0.5	5.2 ± 0.2
C + CP	4.2 ± 1.5	2.6 ± 0.3	4.7 ± 0.2
EM-1	31.3 ± 4.5[a]	20.9 ± 4.7[a]	7.8 ± 0.5[a]
EM-1 + CP	14.1 ± 3.6[b]	5.4 ± 0.8[b]	5.5 ± 0.4[b]
EM-2	27.3 ± 4.5[a]	14.8 ± 4.1[a]	8.2 ± 0.4[a]
EM-2 + CP	12.3 ± 1.9[a]	3.9 ± 0.9[a]	6.9 ± 0.4
MOR	26.1 ± 4.4[b]	14.5 ± 4.0[b]	7.9 ± 0.3[b]
MOR + CP	10.7 ± 3.2[a]	4.9 ± 0.7[a]	6.6 ± 0.5

The data are presented as mean ± S.E.M.
[a] Indicates a significant difference ($P < 0.5$) vs. control.
[b] Indicates a significant difference ($P < 0.5$) vs. respective group without cyprodime.
C = control, CA—cold allodynia, CTF—cold tail-flick, TF—tail-flick.

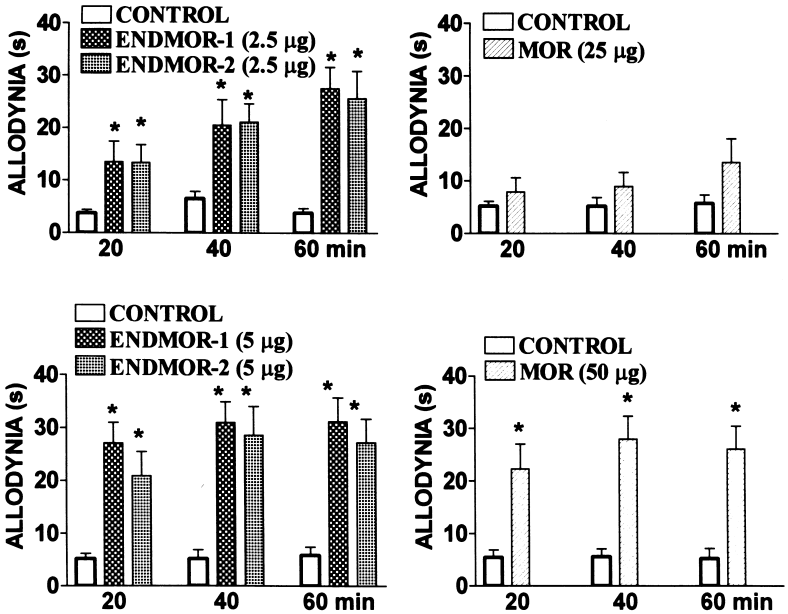

Fig. 4. The spinal antiallodynic effect of endomorphin-1 (2.5; 5 μg/5 μl i.t.), endomorphin-2 (2.5; 5 μg/5 μl i.t.) and morphine (25; 50 μg/5 μl i.t.) in rats with a sciatic nerve injury. Control animals were injected i.t. with distilled water and were tested acccording to the same time schedule as the experimental group. The animals with a crush injury to the sciatic nerve displayed signs of allodynia by lifting only the injured leg out of the ice-cold (0°C) water (mean ±S.E.M.). The cut-off latency was 40 s. ∗Indicates a significant difference ($P < 0.5$) between endomorphin-1, -2, morphine and control.

latter effect was antagonized by cyprodime, 60 μg (Table 3).

4. Discussion

Our results indicate a spinal analgesic activity of the newly described endogenous ligands of the μ-opioid receptor, endomorphin-1 and -2. Both endomorphin-1 and -2 showed a potent, dose- and time-dependent antinociceptive effect after their intrathecal injection to rats, in the tail flick and paw pressure tests. The antinociceptive effect of endomorphins was observed after acute thermal and, to a lesser extent, mechanical stimuli. The response to the thermal stimuli was long-lasting, while that to mechanical stimuli—substantially shorter. A spinal antinociceptive effect of endomorphins was recently described in mice using a tail-flick test (Stone et al., 1997). However, in the latter study endomorphins produced short-term antinociception, a maximum effect being observed at 2 min post-injection, but disappearing completely at 10–15 min post-injection. In contrast, in the present study a prolonged antinociceptive effect was found in rats. The maximum was reached after ca. 15 min, and the effect lasted for longer than 2 h. The reason for such a profound difference observed in the spinal antinociceptive activity in mice and rats is presently unknown; however, it may be due to differences in the metabolism of the peptides in both those species.

In our experiment, the endomorphin-2-induced antinociception was less potent in comparison with endomorphin-1 in both the tests used. The antinociceptive effect of the endomorphins in acute pain models in rats was weaker than that of DAMGO and comparable to the effect of morphine.

Several authors described a cardiovascular effect of endomorphins (Champion et al., 1997a,b). The peripheral vascular effect of endomorphins may influence tail skin temperature, and thus also the response to noxious heat (Hole and Tjolsen, 1993). The cardiovascular effect of endomorphins may appear after systemic penetration of the drug from the site of administration (i.t.). However, the peripheral effect of endomorphins on vascular bed is relatively short-lasting (ca. 2 min), whereas the antinociceptive effect of endomorphins in the tail-flick test is considerably longer (ca. 2 h). Moreover, the potency of endomorphins to evoke cardiovascular effects is fairly similar, while that of these two peptides differs in nociceptive tests. Further, endomorphins exhibit antinociceptive effects also in other, such as a paw pressure test in which thermal stimulus was not used. Summing up, the observed effect is not likely to stem from changes in the cardiovascular system after endomorphin administration, but further detailed studies are necessary to prove such a hypothesis.

Interestingly, endomorphins appear to be less effective compared to morphine and DAMGO in antagonizing the formalin-induced flinching episodes. It may thus be concluded that endomorphin-1, and especially endomorphin -2, are not involved in inflammatory pain, in contrast to DAMGO and morphine, other μ-opioid receptor agonists.

The most interesting of the present study are the results obtained with rats subjected to a sciatic nerve crush injury

—an animal model of neuropathic pain. It is known that nerve injury induces significant plastic changes in the dorsal root ganglia and dorsal horn of the spinal cord. Application of a unilateral sciatic nerve crush yields a pronounced, time-dependent thermal hyperesthesia. The present results demonstrate that endomorphin-1 and endomorphin-2 produce a potent antinociceptive effect and have a pronounced anti-allodynic action in a crush injury model. In contrast, the cold water allodynia was partly inhibited by a very high dose of i.t. morphine. Hence the effect of endomorphins was several times stronger than that observed after morphine. This observation is in contrast to acute nociception, in which the potency of endomorphins is similar to that of morphine. Interestingly enough, endomorphins appear to be particularly effective in the neuropathic pain resistant to other μ receptor selective opioids. It is well known that the antinociceptive efficacy of i.t. administered morphine is decreased in rats with nerve-injury (Ossipov et al., 1995a,b; Yaksh et al., 1995; Nichols et al., 1997). Bian et al., 1995 confirmed that i.t. morphine failed to alleviate mechanical allodynia even when it was used in doses up to 100 mg in a L5/L6 ligation model of neuropathic pain. Also, clinical studies generally indicate that neuropathic pain is somewhat resistant to morphine-induced alleviation (Twycross, 1982; Arner and Myerson, 1988). It has been suggested that ineffectiveness of morphine in models of neuropathic pain is due to a reduced number of presynaptic opioid receptors as a result of degeneration of primary afferent neurons, subsequent to nerve damage (Ossipov et al., 1995a,b). Such reduction in the number of μ-opioid receptors may, in fact, be an important factor in diminishing the efficacy of morphine in neuropathic pain. However, endomorphins produce a potent effect also via μ-opioid receptors, which is antagonized by cyprodime, a highly selective antagonist of this receptors (Schmidhammer et al., 1989). The reason for such a discrepancy is presently unknown, since both morphine and endomorphins appear to act via the same μ-opioid receptors. In fact, the majority of recent study have pointed to great similarity in the molecular effects of morphine and endomorphins. However, the effects of endomorphins differ from morphine, since these peptides (but not morphine) cause internalization of the μ-opioid receptor (Burford et al., 1998). Furthermore, a preliminary study of Spanish researchers demonstrated that naloxonazine, a selective μ1 receptor antagonist, reduced the analgesia evoked by morphine, but not by endomorphin-1 (Schanchez-Blasques, personal communication).

Summing up, different μ-opioid receptor subtypes may mediate effects of morphine and endomorphins in the neuropathic pain. Identification of the differences involved in this phenomenon may be of great importance to the understanding of the molecular mechanism of neuropathic pain, as well as to the development of better and more effective drugs for the treatment of neuropathic pain in humans.

In conclusion, the results of the present study show that endomorphin-1 and -2, novel endogenous ligands of the μ-opioid receptor, are more effective than morphine in this model of nerve injury. The discovery of these selective μ-opioid receptor agonists is of great importance, since it may lead to development of analgesic agents with less addictive properties and a much more potent action in neuropathic pain than morphine.

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