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European Journal of Pharmacology 372 (1999) 25–30

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Differential involvement of μ-opioid receptor subtypes in endomorphin-1- and -2-induced antinociception

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Received 8 March 1999; accepted 12 March 1999

Abstract

We investigated the role of μ-opioid receptor subtypes in both endomorphin-1 and endomorphin-2 induced antinociception in mice using supraspinally mediated behavior. With tail pressure as a mechanical noxious stimulus, both intracerebroventricularly (i.c.v.) and intrathecally (i.t.) injected-endomorphins produced potent and significant antinociceptive activity. Antinociception induced by i.t. and i.c.v. injection of endomorphin-1 was not reversed by pretreatment with a selective μ_1-opioid receptor antagonist, naloxonazine (35 mg/kg, s.c.). By contrast, antinociception induced by i.t. and i.c.v. endomorphin-2 was significantly decreased by μ_1-opioid receptor antagonist. Antinociception of both i.t. and i.c.v. endomorphin-1 and -2 was completely reversed by pretreatment with β-funaltrexamine (40 mg/kg, s.c.). The results indicate that endomorphins may produce antinociception through the distinct μ_1 and μ_2 subtypes of μ-opioid receptor.

Keywords: Endomorphin-1; Endomorphin-2; Tail pressure test; Naloxonazine; β-Funaltrexamine

1. Introduction

Endomorphin-1 and -2 are tetrapeptide amides isolated from bovine brain, found to have high affinity and selectivity for the μ-opioid receptor, and shown to produce potent and prolonged antinociceptive activity that is reversible by naloxone and β-funaltrexamine (Zadina et al., 1997). There is biochemical and pharmacological evidence supporting the existence of μ-opioid receptor subtypes (Wolozin and Pasternak, 1981; Nishimura et al., 1984; Goodman and Pasternak, 1985). At least two μ-opioid receptor subtypes have been proposed: μ_1 and μ_2 (Pasternak and Wood, 1986). β-Funaltrexamine irreversibly antagonizes both μ_1- and μ_2-opioid receptors and inhibits both supraspinal and spinal antinociception, whereas naloxonazine selectively antagonizes the μ_1-opioid receptor. It has been suggested that these receptor subtypes have different physiological roles, with μ_1-opioid receptors mediating supraspinal antinociception, whereas μ_2-opioid receptors mediate spinal antinociception.

In most studies, the method used to measure the antinociceptive effects of opioids is the tail flick test, which depends on tail withdrawal from radiant heat as the noxious stimulus, a response that involves a spinal reflex. The tail flick test does not require the integrative action of the higher brain centers (Irwin et al., 1951). We therefore have studied antinociceptive effects of endomorphins after pretreatment with naloxonazine or β-funaltrexamine on behavior (licking or biting) that requires perception of the noxious stimulus, and integration of behavioral responses, by central (supraspinal) mechanisms. In the present study, the role of the μ-opioid receptor subtypes, μ_1 and μ_2, in the antinociceptive effects of endomorphins against the response to mechanical noxious stimuli was examined with

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00181-8

the irreversible μ_1-opioid receptor antagonist, naloxonazine and the irreversible μ_1- and μ_2-opioid receptor antagonist, β-funaltrexamine.

2. Materials and methods

Adult male ddY mice weighing 22–25 g were housed in a light- and temperature-controlled room (light on 0900–2100 h; 24°C) and had free access to food and water. Intracerebroventricular (i.c.v.) injections were administered about 2 mm caudal and 2 mm lateral to the bregma at a depth of 3 mm (Haley and McCormick, 1957). The procedure for intrathecal (i.t.) injections was adapted from the method of Hylden and Wilcox (1980) with a constant injection volume of 2 μl/mouse. For i.t. administration, a 29-gauge needle connected to a Hamilton microsyringe was inserted directly between L5 and L6, and the drug was administered at a rate of 2 μl/10 s. The antinociceptive activity of opioid peptides against the response to a mechanical stimulus was assessed by the mouse tail pressure test. The base of tail was pressed, the pressure increased at a rate of 20 mm Hg/s, and the latency to biting or struggling was recorded. A maximum pressure of 200 mm Hg was imposed to prevent tissue damage. No animal was used more than once. Only mice responding behaviorally to mechanical nociceptive stimulation (80–100 mm Hg) were selected. To prevent experimenter bias, observers were uninformed of the dose of the endomorphins and [D-Ala^2, MePhe^4, Gly(ol)^5]enkephalin (DAMGO) being injected, and were uniformed of whether naloxonazine or β-funaltrexamine was being as pretreatment when modification of each agonist-induced antinociception was investigated. After determination of pre-drug values, animals were injected with opioid peptides dissolved in sterile artificial cerebrospinal fluid (CSF) containing 7.4 g NaCl, 0.19 g KCl, 0.19 g $MgCl_2$, 0.14 g $CaCl_2$/1000 ml. β-Funaltrexamine (40 mg/kg, s.c.) and naloxonazine (35 mg/kg, s.c.) were dissolved in saline and administered in a volume of 0.1 ml/10 g body weight 24 h before opioid peptide administration. Under these conditions, β-funaltrexamine antagonizes both μ_1- and μ_2-mediated antinociception, and naloxonazine's actions are relatively selective for μ_1-receptors (Ling et al., 1986).

Antinociceptive activity for each animal was calculated with the following equation and represented as percent of maximum possible effect (%MPE): $\%MPE = (P2 - P1/200 - P1) \times 100$, where P1 and P2 are pre-drug and post-drug responsive pressures (in mm Hg), respectively. β-Funaltrexamine and naloxonazine were purchased from Research Biochemical International (Natick, MA, USA), and DAMGO was from Sigma (St. Louis, MO, USA). Endomorphin-1 and -2 were synthesized by the conventional solid phase method in our laboratory. The ED_{50} values and their 95% confidence limits (CL_{95}) were determined by the method of Litchfield and Wilcoxon (1949). Statistical significance of the data was estimated by a mixed two-factor analysis of variance (ANOVA) followed by Dunnett's test.

3. Results

3.1. Potency and time course of i.c.v. and i.t. injections of endomorphin-1 and -2

The time course of antinociceptive activity for i.c.v. endomorphin-1, -2 and DAMGO is shown in Fig. 1. Groups of mice were tested for antinociception at 1, 3, 5, 10 and 15 min after i.c.v. injection of the endomorphins. Endomorphin-1 and -2 produced dose-dependent antinociception with ED_{50} values of 1.20 (CL_{95}: 0.68–2.11) nmol, and 1.35 (CL_{95}: 0.70–2.59) nmol, respectively, with maximum effects at 5 min (Fig. 1A,B). Endomorphin-1 had a longer duration of action than endomorphin-2, but the two peptides had equal potency as seen from the ED_{50} values. The ED_{50} value for i.c.v. DAMGO was 8.0 (CL_{95}: 4.15–15.40) pmol at the 5-min peak time of antinociception (Fig. 1C).

The time course of antinociceptive activity for i.t. endomorphin-1, -2 and DAMGO is shown in Fig. 2. The i.t. endomorphin-1 and -2 at doses of 0.156–2.5 nmol produced a significant antinociceptive effect in the assay, with a peak effect at 1 min (Fig. 2A,B). ED_{50} values of endomorphin-1 and -2 were 0.33 (CL_{95}: 0.21–0.51) nmol and 0.22 (CL_{95}: 0.12–0.39) nmol, respectively.

3.2. Differential effects of endomorphin-1 and -2 induced antinociception mediated through μ-opioid receptor subtypes

The antagonistic effects of β-funaltrexamine and naloxonazine on antinociception induced by equipotent doses of i.c.v. endomorphins and DAMGO were examined (Fig. 3). Groups of mice received either β-funaltrexamine (40 mg/kg, s.c.) or naloxonazine (35 mg/kg, s.c.) 24 h before i.c.v. injection of endomorphins or DAMGO. Pretreatment with β-funaltrexamine significantly decreased the antinociceptive response in both endomorphin and DAMGO treatment groups compared with non-pretreatment groups, confirming the involvement of μ-opioid receptors in the response of the three opioid peptides. Endomorphin-1-induced antinociception was insensitive to naloxonazine (35 mg/kg, s.c.) (Fig. 3A), whereas the endomorphin-2 and DAMGO responses were significantly decreased, but not completely antagonized by the μ_1-opioid receptor antagonist (Fig. 3B,C). Antinociception induced by endomorphin-2 at a dose of 1.25 nmol was completely antagonized by pretreatment with naloxonazine, but the response to 2.5

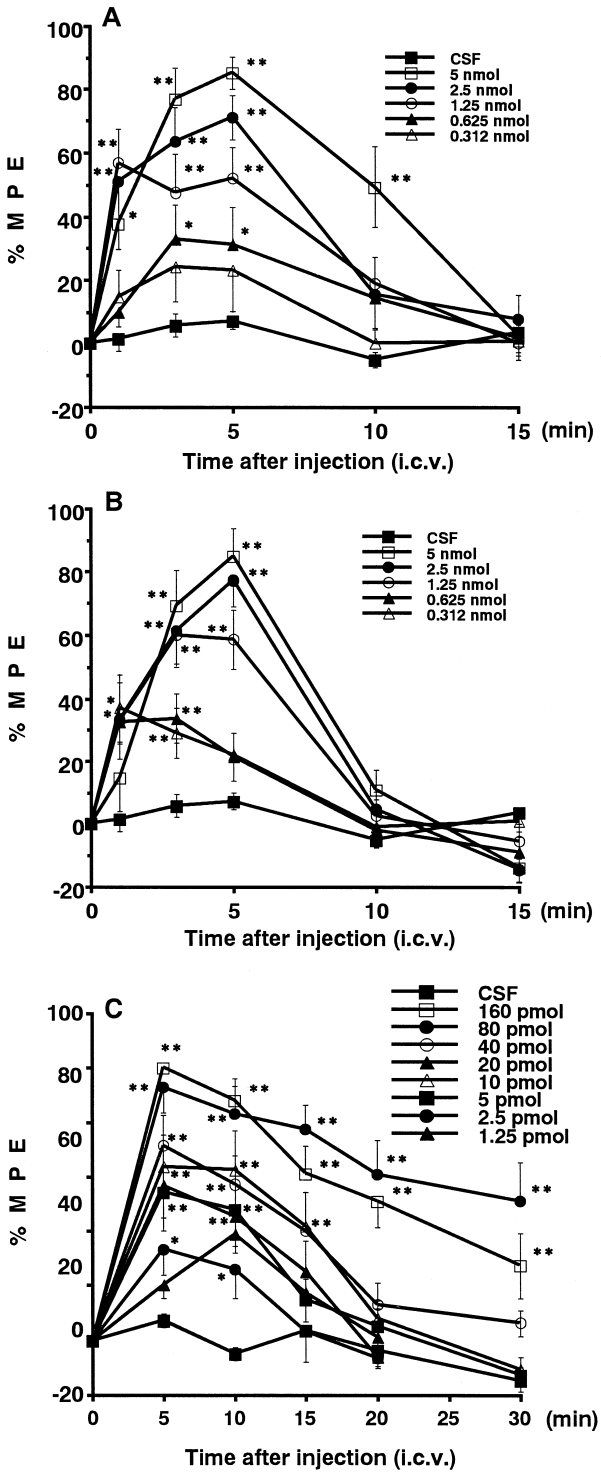

Fig. 1. Time course of effects of i.c.v. administered endomorphin-1 (A), endomorphin-2 (B) and DAMGO (C) in the mouse tail pressure test. Antinociception was expressed as percent of maximum possible effect (% MPE) = 100 × (post-drug responsive pressure − pre-drug responsive pressure)/(200 − pre-drug responsive pressure). Each data point represents the mean ± S.E.M. for 10 mice. **: $P < 0.01$ and *: $P < 0.05$, compared to the respective value in the CSF-control group.

and 5 nmol was only partially antagonized by pretreatment with naloxonazine (Fig. 3B). β-Funaltrexamine almost completely antagonized the response to all three opioid peptides (Fig. 3). Endomorphin-1-induced antinociception was not antagonized by pretreatment with naloxonazine, indicating that the endomorphin-1 response is mediated

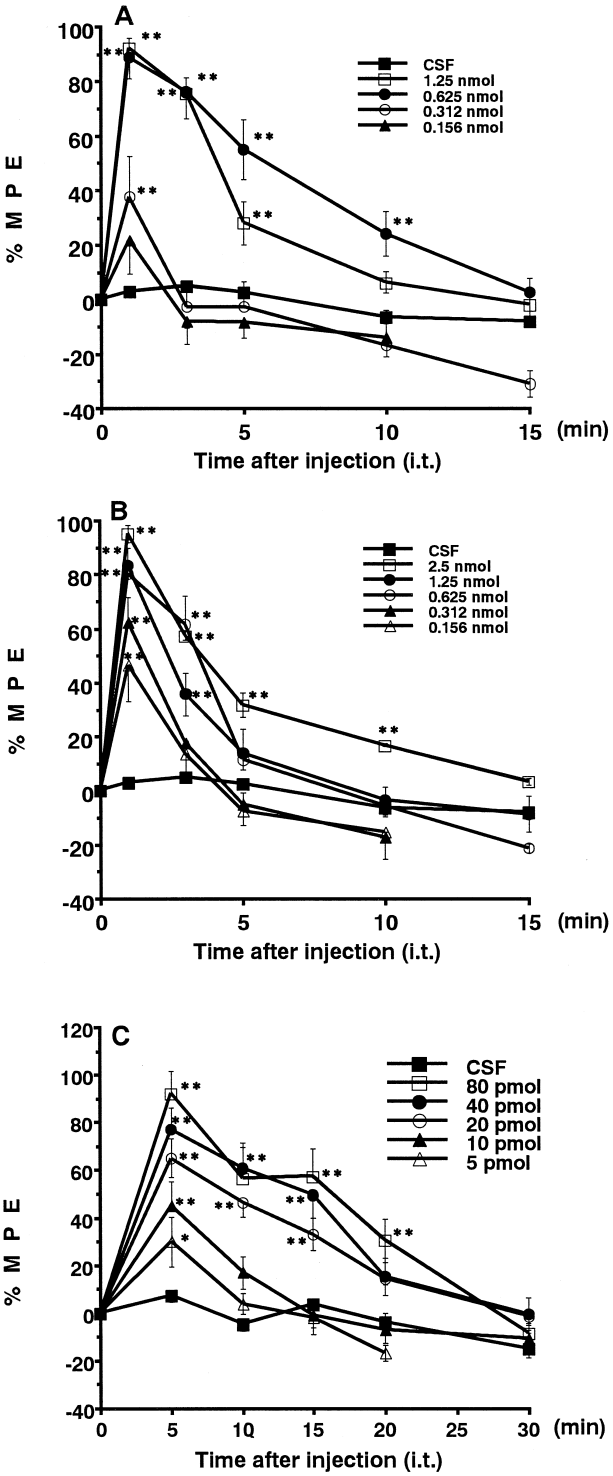

Fig. 2. Time course of effects of i.t. administered endomorphin-1 (A), endomorphin-2 (B) and DAMGO (C) in the mouse tail pressure test. Each data point represents the mean ± S.E.M. for 10 mice. **: $P < 0.01$ and *: $P < 0.05$, compared to the respective value in the CSF-control group.

through μ_2-opioid receptor subtypes. ED_{50} values for i.c.v. endomorphin-1, endomorphin-2 and DAMGO after pretreatment with naloxonazine were 1.5 (CL_{95}: 0.77–2.91) nmol, 8.6 (CL_{95}: 4.53–16.33) nmol and 70.0 (CL_{95}: 34.63–141.51) pmol, respectively. These values represent naloxonazine-induced shifts in the ED_{50} of 1.17 (endomorphin-1), 6.3 (endomorphin-2) and 8.8 (DAMGO).

For i.t. responses, pretreatment with β-funaltrexamine markedly decreased the antinociceptive response to both i.t. endomorphins and i.t. DAMGO (Fig. 4). Naloxonazine, however, was ineffective at attenuating the responses to i.t. injections of both endomorphin-1 and DAMGO (Fig. 4A,C). The antinociceptive effect of i.t. endomorphin-2 was significantly antagonized by pretreatment with naloxonazine (Fig. 4B), but the decrease was not as large as after i.c.v. administration (Fig. 3B).

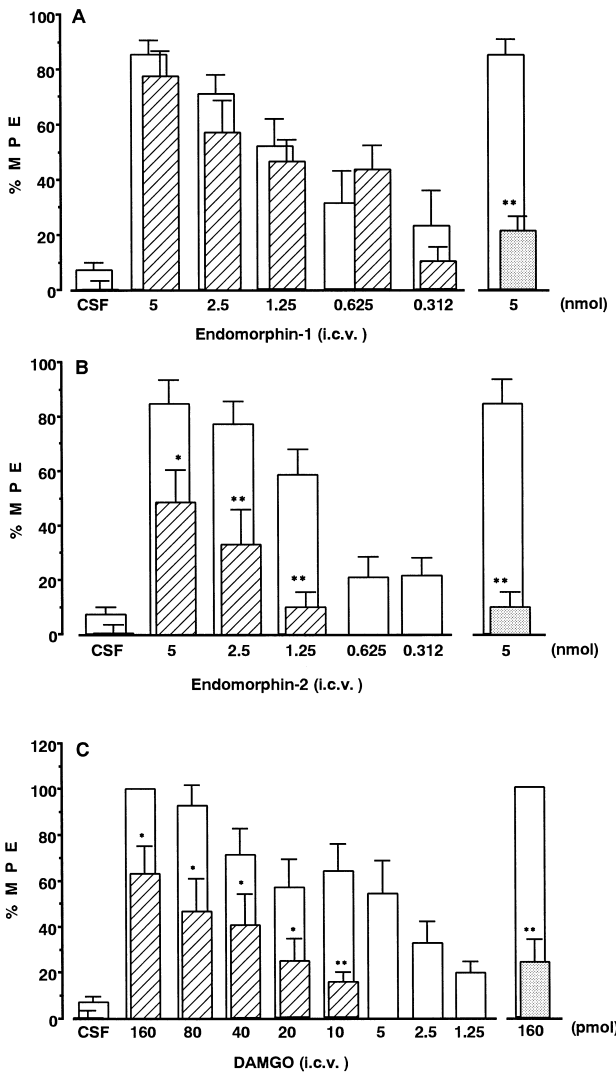

Fig. 3. Effects of naloxonazine and β-funaltrexamine on i.c.v. endomorphin-1 (A)-, endomorphin-2 (B)- and DAMGO (C)-induced antinociception in the mouse tail pressure test. Naloxonazine (35 mg/kg; hatched column) and β-funaltrexamine (40 mg/kg; dotted column) were administered s.c. 24 h before i.c.v. administration of endomorphin-1, endomorphin-2 and DAMGO. Antinociceptive effect was measured 5 min after i.c.v. administration of each μ-opioid receptor agonist. Each column represents the mean S.E.M. for 10 mice. **: $P < 0.01$ and *: $P < 0.05$, compared to each agonist alone (open column).

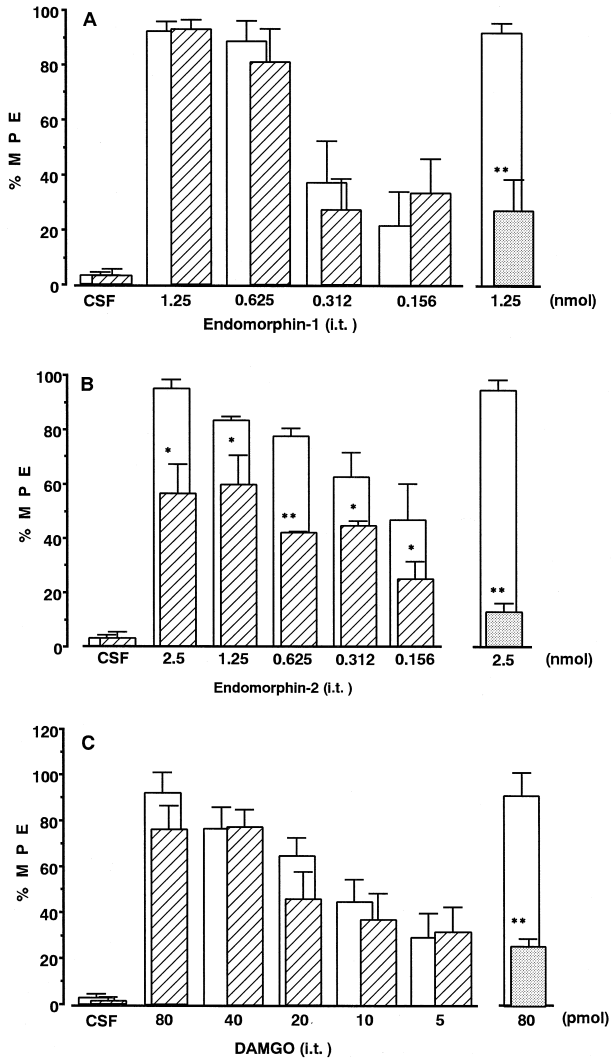

Fig. 4. Effects of naloxonazine and β-funaltrexamine on i.t. endomorphin-1 (A)-, endomorphin-2 (B)- and DAMGO (C)-induced antinociception in the mouse tail pressure test. Naloxonazine (35 mg/kg; hatched column) and β-funaltrexamine (40 mg/kg; dotted column) were administered s.c. 24 h before i.t. administration of endomorphin-1, endomorphin-2 and DAMGO. Antinociceptive effect was measured 1 min after i.t. administration of endomorphins and 5 min after i.t. administration of DAMGO. Each column represents the mean S.E.M. for 10 mice. **: $P < 0.01$ and *: $P < 0.05$, compared to each agonist alone (open column).

4. Discussion

In this study we showed that both endomorphin-1 and endomorphin-2 induced dose-dependent antinociception after central (i.c.v.) and spinal (i.t.) administration in a test involving central integration of response. The peak effects

occurred rapidly, with 5 min for i.c.v. and within 1 min for i.t. injection. The present results of i.t. injected endomorphins are in agreement with those of Stone et al. (1997) who reported that antinociception induced by endomorphin-1 and -2 is short-lasting and is absent 15 min following i.t. injection as assayed by the hot water tail flick latencies. As expected, the duration of antinociception increased with higher doses (Figs. 1 and 2, and Zadina et al., 1997). As shown previously in the tail flick (Zadina et al., 1997), the peptides were more potent in this paradigm after i.t. than after i.c.v. administration. We used the μ-opioid receptor-selective antagonists β-funaltrexamine and naloxonazine to determine the subtype of receptor involved in the antinociceptive responses to the endomorphins and DAMGO.

β-Funaltrexamine irreversibly antagonizes both μ_1- and μ_2-opioid receptors (Recht and Pasternak, 1987) and inhibits both supraspinal and spinal antinociception whereas naloxonazine selectively antagonizes μ_1-opioid receptors and supraspinal antinociception but does not antagonize spinal antinociception mediated through μ_2-opioid receptors (Ling et al., 1986; Heyman et al., 1988; Paul et al., 1989; Pick et al., 1991). These results were obtained in studies using a spinal reflex, the 'tail flick' response, which is thought to measure changes in both spinal reflexes and in the centrally modulated system involved in the spinal reflex. Central perception of noxious stimuli is not required to elicit a tail flick response.

In our experiments, the endpoint of the tail pressure test, is biting or licking. These behaviors require perception of noxious stimulus and integration of behavioral responses at the supraspinal level. As in previous reports with the tail flick test, pretreatment with the μ_1-opioid receptor-selective antagonist naloxonazine blocked the antinociceptive response of DAMGO to the mechanical stimulus after i.c.v., but not after i.t. injection. These results are consistent with a supraspinal μ_1 and a spinal μ_2 action of DAMGO. Endomorphin-1, with a Trp (W) in position 3, is structurally similar to Tyr-W-melanocyte-stimulating hormone release inhibiting factor (MIF)-1 (Tyr-Pro-Trp-Gly-NH_2), a naturally occurring, amidated tetrapeptide. Tyr-W-MIF-1 has been shown to induce antinociception after i.c.v. and i.t. injection that was antagonized by the μ-opioid receptor antagonist β-funaltrexamine but not by the μ_1-opioid receptor-selective antagonist measured with the tail flick test (Gergen et al., 1996), indicating that the inhibitory response against the thermal reflex was mediated through the μ_2-opioid receptor. Endomorphin-1 has behavioral and pharmacological similarity to Tyr-W-MIF-1 (Zadina et al., 1993, 1994, 1996) because antinociception of both i.t. and i.c.v. endomorphin-1 was markedly reversed by pretreatment with β-funaltrexamine but not with naloxonazine as measured by the tail pressure test. By contrast, effects of i.c.v. and i.t. endomorphin-2, with a Phe in position 3, were markedly antagonized by pretreatment of either β-funaltrexamine or naloxonazine. Naloxonazine did not completely abolish the action of endomorphin-2, indicating a potential residual action at μ_2-opioid receptor. Nevertheless, endomorphin-1 was relatively insensitive to antagonism by naloxonazine, whereas endomorphin-2 was relatively sensitive to the μ_1-opioid receptor antagonist. This indicates that endomorphin-1 can act as a predominantly μ_2-opioid receptor agonist and endomorphin-2 as a μ_1-opioid receptor agonist. However, the present data are inconsistent with previously reported results that naloxonazine significantly reverses the antinociceptive effect of i.c.v. injected endomorphins (Goldberg et al., 1998). The reason for these differences is unclear, but may be related to different experimental conditions, e.g., different strains of mice, different nociceptive assays and different injection routes of naloxonazine (i.c.v. vs. s.c.).

The antagonism of i.t. administered endomorphin-2 by naloxonazine was unexpected because the antinociception induced by i.t. DAMGO in this and other studies (Heyman et al., 1988; Paul et al., 1989) were not affected by the antagonist. This could reflect (a) the greater sensitivity of endomorphin-2 to the naloxonazine, (b) differences in peptide actions for which the current test paradigm (tail pressure) is particularly sensitive, or (c) the complex action of the naloxonazine on responses to spinally administered peptides (Heyman et al., 1988).

The physical basis for the putative μ_1- and μ_2-opioid receptors, and the mechanism of action of naloxonazine, are not fully understood. Studies with μ-opioid receptor knockout mice indicate that both μ_1- and μ_2-opioid receptors would arise from the known sequence of the cloned μ-opioid receptor (Matthes et al., 1996). This indicates that splice variants of this receptor [other than the MOR1b variant, (Zimprich et al., 1995)], or different physical states of the receptor, could be differentially sensitive to naloxonazine. Regardless, naloxonazine has proven to be a powerful tool in demonstrating differential effects of μ-opioid receptor agonists.

In conclusion, i.t. and i.c.v. endomorphin-1, -2 and DAMGO produced dose-related antinociceptive activity against centrally integrated responses to mechanical noxious stimuli. Pretreatment with naloxonazine significantly antagonized the antinociceptive effect of both i.c.v. and i.t. endomorphin-2 and i.c.v. DAMGO but not of i.c.v. or i.t. endomorphin-1 or i.t. DAMGO. The results indicate that endomorphin-1 and -2 may produce antinociception through differential actions at μ_1- and μ_2-opioid receptor subtypes of μ-opioid receptors, after both central and spinal administration.

Acknowledgements

The authors wish to thank Miho Nakayama for her secretarial assistance in preparing the manuscript.

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