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Short communication

The antitussive effects of endomorphin-1 and endomorphin-2 in mice

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Abstract

The antitussive effects of endomorphin-1 and endomorphin-2, endogenous μ-opioid receptor agonists, on capsaicin-induced coughs were examined in mice. Endomorphin-2, at doses of 3, 10 and 30 μg, i.c.v., dose-dependently inhibited the number of capsaicin-induced coughs. However, the same doses (3, 10 and 30 μg) of endomorphin-1 injected with i.c.v. had no significant effects on the number of capsaicin-induced coughs. The antitussive effect of endomorphin-2 was significantly reduced by β-funaltrexamine, a μ_1/μ_2-opioid receptor antagonist, but not naloxonazine, a selective μ_1-opioid receptor antagonist. Furthermore, the antitussive effect of endomorphin-2 was also partially but significantly reduced by nor-binaltorphimine, a selective κ-opioid receptor antagonist. These results indicate that the administration of the endogenous μ-opioid ligand endomorphin-2, but not endomorphin-1, into the brain produces an antitussive effect via mainly naloxonazine-insensitive μ-opioid receptors, namely μ_2-opioid receptors and partially κ-opioid receptors.

Keywords: Endomorphin-1; Endomorphin-2; Cough reflex; Antitussive effect; μ-Opioid receptor; κ-Opioid receptor

1. Introduction

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) are tetrapeptide amides that have been isolated from bovine brain. They have been shown to produce several μ-opioid receptor-mediated pharmacological responses, such as antinociception, induction of motor behavior, motivational effects (rewarding and psychomotor stimulant effects) and amnesia (impairment of short-term memory), which are mainly mediated by μ-opioid receptors (Zadina et al., 1997; Goldberg et al., 1998; Bujdoso et al., 2001; Mehta et al., 2001; Narita et al., 2001, 2002; Ukai and Lin, 2002; Zangen et al., 2002). However, it is not yet clear whether endomorphin-1 and endomorphin-2 produce antitussive effects.

The existence of two μ-opioid receptor subtypes, μ_1-opioid and μ_2-opioid receptors (μ_1-opioid receptor antagonist-insensitive μ-opioid receptors), has been proposed based on the results of receptor binding and pharmacological studies (Moskowitz and Goodman, 1985; Kamei et al., 1993a,b; Narita et al., 2001, 2002). We previously reported that naloxonazine, a selective μ_1-opioid receptor antagonist, had no effect on the antitussive effects associated with intraperitoneal morphine and i.c.v. [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin (DAMGO) (Kamei et al., 1993a). Furthermore, there was no significant difference between the morphine-induced antitussive effect in CXBK, which are deficient in μ_1-opioid receptors, and C57BL/6 mice (Kamei et al., 1993b). In addition, the antitussive effects of morphine in both CXBK and C57BL/6 mice were not antagonized by pretreatment with naltrexonazine, a selective μ_1-opioid receptor antagonist (Kamei et al., 1993b). These results indicate that μ_2- rather than μ_1-opioid receptors are involved in the antitussive effects involving μ-opioid receptors. Although endomorphins have high affinity and selectivity for the μ-opioid receptor, some pharmacological responses of endomorphins have been shown to be mediated by the stimulation of μ_2-opioid receptors (Narita et al., 2001, 2002).

Therefore, the present study was designed to investigate the antitussive effects of endomorphin-1 and endomorphin-2, and to clarify the roles of μ-opioid receptor subtypes in these antitussive effects.

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2. Materials and methods

2.1. Animals

Male ICR mice (6 weeks old; Tokyo Laboratory Animals Science, Tokyo, Japan) weighing about 30 g were used. They had free access to food and water in an animal room, which was maintained at 24 ± 1 °C with a 12-h light–dark cycle. This study was carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Antitussive assay

The cough reflex was induced as previously described (Kamei et al., 1993a,b). Briefly, animals were exposed to a nebulized solution of capsaicin (30 μmol/l) under conscious and identical conditions using a body plethysmograph. The coughs produced during a 3-min exposure period were counted. Capsaicin was dissolved in 10% ethanol and 10% Tween 80 saline solution at a concentration of 30 mg/ml, and the mixture was diluted with saline. The mice were exposed to capsaicin for 3 min beginning 30 min before the injection of drugs to determine the frequency of control coughs. The animals were again exposed to capsaicin aerosol for 30 min after the i.c.v. administration of drugs. Each animal was used only once. The number of coughs produced after drug injection (Ct) was compared with the number of control coughs (Cc). The antitussive effect was expressed as the % inhibition of the number of coughs=[(Cc − Ct)/Cc] × 100.

2.3. Drugs

Endomorphin-1, endomorphin-2, β-funaltrexamine, a μ_1/μ_2-opioid receptor antagonist, naloxonazine, a selective μ_1-opioid receptor antagonist, and nor-binaltorphimine, a selective κ-opioid receptor antagonist, were synthesized by us. All of the opioid receptor agonists and antagonists were dissolved in saline. Endomorphin-1 and endomorphin-2 were injected with i.c.v. 30 min before the antitussive assay. β-Funaltrexamine (20 mg/kg, s.c.) and naloxonazine (35 mg/kg, s.c.) were injected 24 h before testing. Nor-binaltorphimine (20 mg/kg, s.c.) was injected 3 h before testing. I.c.v. injection was performed according to the method of Haley and McCormick (1957). Briefly, endomorphin-1 or endomorphin-2 was given into the lateral cerebral ventricle of mice. The unilateral injection site was approximately 2 mm from either side of the midline on a line drawn through the anterior roots of the ears. The injection was made with a 2-mm double needle (Natsume Seisakusho, Tokyo, Japan) attached to a 25-μl Hamilton microsyringe. Drug solutions were injected in a volume of 5-μl per mouse over a period of 10 s.

2.4. Data analysis

Data are expressed as means ± S.E. The statistical significance of differences was assessed by the Mann–Whitney *U*-test to evaluate the antitussive effect. A level of probability of 0.05 or less was considered significant.

3. Results

3.1. Effects of i.c.v. administration of endomorphin-1 and endomorphin-2 on capsaicin-induced coughs

Endomorphin-2, at doses of 3, 10 and 30 μg, i.c.v., dose-dependently inhibited the number of capsaicin-induced coughs when the antitussive effect was examined 30 min after administration (Fig. 1B). However, as shown in Fig. 1A, the same doses (3, 10 and 30 μg) of endomorphin-1 injected with i.c.v. had no significant effect on the number of capsaicin-induced coughs.

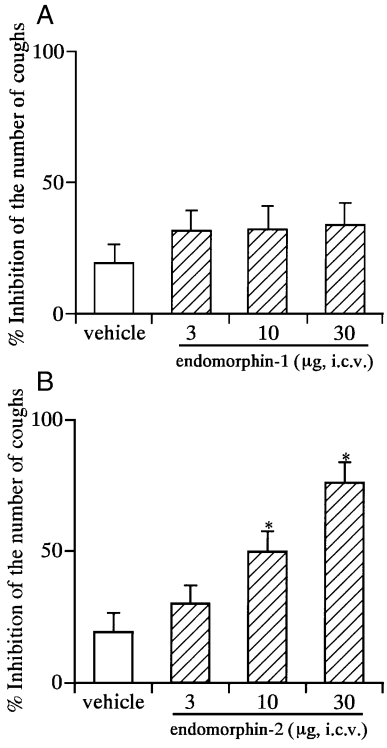

Fig. 1. Dose–response data for the antitussive effects induced by endomorphin-1 (A) and endomorphin-2 (B) in mice. The antinociceptive effects of endomorphins were assessed 30 min after i.c.v. injection. Each point or column represents the mean with S.E. for 10 mice in each group. *$P < 0.05$ vs. the value for the respective saline-treated group (open column).

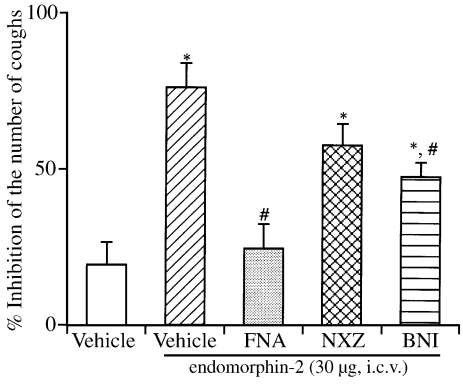

Fig. 2. Effects of β-funaltrexamine, naloxonazine and nor-binaltorphimine on the antitussive effect of endomorphin-2 in mice. β-Funaltrexamine (FNA, 20 mg/kg) and naloxonazine (NXZ, 35 mg/kg) were injected subcutaneously 24 h before testing. Nor-binaltorphimine (BNI, 20 mg/kg) was injected subcutaneously 3 h before testing. The antitussive effect was tested 30 min after i.c.v. injection of endomorphin-2 (30 μg). Each column represents the mean with S.E. for 10 mice in each group. $^{*}P<0.05$ vs. the respective saline alone-treated group (open column). $^{\#}P<0.05$ vs. saline + endomorphin-2-treated group (hatched column).

3.2. Effects of opioid receptor antagonists on the antitussive effect of endomorphin-2

The antitussive effect of endomorphin-2 (30 μg, i.c.v.) was abolished by pretreatment with the μ_1/μ_2-opioid receptor antagonist β-funaltrexamine (20 mg/kg, s.c.) (Fig. 2). However, pretreatment with the selective μ_1-opioid receptor antagonist naloxonazine (35 mg/kg, s.c.) had no significant effect on the antitussive effect of endomorphin-2 (30 μg, i.c.v.) (Fig. 2). As shown in Fig. 2, the antitussive effect of endomorphin-2 was partially but significantly reduced by pretreatment with the selective κ-opioid receptor antagonist nor-binaltorphimine.

4. Discussion

The present experiments demonstrated that i.c.v. administration of endomorphin-2 produced a dose-dependent antitussive effect in mice. The antitussive effect of endomorphin-2 was significantly reduced by pretreatment with the μ_1/μ_2-opioid receptor antagonist β-funaltrexamine, but not naloxonazine, a selective μ_1-opioid receptor antagonist. These results indicate that the naloxonazine-insensitive μ-opioid receptor, namely μ_2-opioid receptor subtype, plays an important role in mediating the antitussive effect of the selective endogenous μ-ligand endomorphin-2.

An unexpected but interesting observation in the present study was that endomorphin-1 had no effect on capsaicin-induced coughs. In this regard, Narita et al. (2001, 2002) reported that endomorphin-1 and endomorphin-2 produced μ_2-opioid receptor-mediated place preference and place aversion, respectively. Since the distinct motivational effects of endomorphin-1 and endomorphin-2 are mediated by μ_2-opioid receptors, they may consequently activate distinct μ_2-opioid receptor isoforms. Recent molecular studies have indicated that there are at least 10 exons on the μ-opioid receptor gene (Pan et al., 2000). The existence of several exons in genes encoding the μ-opioid receptor can likely provide additional diversity by virtue of splicing events. In fact, at least seven μ-opioid receptor splice variants have been identified so far (Pan et al., 2000). Recent mapping of μ-opioid receptor with antisense oligodeoxynucleotides against exons suggests that distinct μ-opioid receptor isoforms may differently mediate several opioid-induced pharmacological actions (Rossi et al., 1995). Furthermore, Narita et al. (2001) recently suggested that the place aversion produced by endomorphin-2 is regulated by the activation of μ_2-opioid receptor isoforms, which differ from the μ_2-receptor isoforms stimulated by endomorphin-1. Although further studies are needed, our results suggest that the antitussive effect also produced by endomorphin-2 may be regulated by the activation of μ_2-opioid receptor isoforms, which differ from the μ_2-receptor isoforms stimulated by endomorphin-1.

Prior studies have suggested that some pharmacological actions of endomorphin-2, such as antinociception and the place aversive effect, are associated with the stimulation of not only μ_2-opioid receptor but also κ-opioid receptors (Makulska-Nowak et al., 2001; Narita et al., 2002). The possibility that endomorphin-2 produces its pharmacological action via the activation of κ-opioid receptors was confirmed in our present study. Indeed, we observed that pretreatment with the selective κ-opioid receptor antagonist nor-binaltorphimine partially but significantly reduced the antitussive effect of endomorphin-2.

In conclusion, the present study demonstrated that administration of endogenous μ-opioid ligand endomorphin-2, but not endomorphin-1, into the brain produced an antitussive effect via mainly naloxonazine-insensitive μ-opioid receptors (probably μ_2-opioid receptors) and partially κ-opioid receptors. Furthermore, we can speculate that the antitussive effect produced by endomorphin-2 is regulated by the activation of μ_2-opioid receptor isoforms, which differ from the μ_2-receptor isoforms stimulated by endomorphin-1.

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