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Role of cholecystokinin in the reduction of endomorphin-2-induced antinociception in diabetic mice

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Abstract

We examined the role of cholecystokinin in the reduction of endomorphin-2-induced antinociception in diabetic mice. Endomorphin-1 (1–10 μg, i.c.v.) and endomorphin-2 (3–30 μg, i.c.v.) dose dependently inhibited the tail-flick response in non-diabetic and diabetic mice. There was no significant difference between the antinociceptive effect of endomorphin-1 in non-diabetic and diabetic mice. On the other hand, the antinociceptive effect of endomorphin-2 in diabetic mice was significantly less than that in non-diabetic mice. Cholecystokinin octapeptide (CCK-8) dose dependently reduced the antinociceptive effects of endomorphin-1 and endomorphin-2 in non-diabetic mice. However, in diabetic mice, CCK-8 significantly inhibited the antinociceptive effect of endomorphin-1, but not of endomorphin-2. In non-diabetic mice, CI-988 ((*R*-[*R**,*R**])-4-([3-1*H*-indol]-3-yl)-2-methyl-1-oxo-2-([{tricyclo(3.3.1.1)dec-2-yloxy}carbonyl] amino)propylamino-1-phenyl-ethylamino-4-oxybutanoic acid) had no significant effect on either endomorphin-1- or endomorphin-2-induced antinociception. In diabetic mice, while CI-988 had no significant effect on endomorphin-1-induced antinociception, it dose dependently enhanced the antinociceptive effect of endomorphin-2. The results indicated that the reduction of endomorphin-2-induced antinociception in diabetic mice might be due, at least in part, to the activation of CCK_2 receptors. © 2001 Published by Elsevier Science B.V.

Keywords: Endomorphin; Cholecystokinin; Antinociception; CCK_2 receptor; Diabetes

1. Introduction

We previously reported that the antinociceptive effects of i.c.v. administration of μ-opioid receptor agonists such as morphine and [D-Ala^2,*N*-MePhe^4,Gly-ol^5]enkephalin (DAMGO) in diabetic mice were markedly less than those in non-diabetic mice (Kamei et al., 1992a,b). Recently, we reported that endogenous μ-opioid receptor agonists, endomorphin-1 and endomorphin-2, both dose dependently inhibited the tail-flick response in non-diabetic as well as in diabetic mice (Kamei et al., 2000). There was no significant difference between the antinociceptive effects of endomorphin-1 in non-diabetic mice and diabetic mice. However, the antinociceptive effect of endomorphin-2 in non-diabetic mice was greater than that in diabetic mice. We suggested that the antinociceptive effects of endomorphin-1 and endomorphin-2 in non-diabetic mice are mediated through the activation of μ_1-opioid receptors, whereas in diabetic mice, endomorphin-1 and endomorphin-2 may produce antinociception through different actions at δ_1- and μ_1-opioid receptors, respectively (Kamei et al., 2000).

Cholecystokinin octapeptide (CCK-8) was originally discovered as a gastrointestinal hormone, and has since been identified as a putative neurotransmitter within the central nervous system (Morley, 1982; Beinfeld, 1983; Noble et al., 1999). CCK-8 has a wide variety of physiological functions, including the modulation of nociceptive transmission (Stanfa et al., 1994). Many studies have suggested that the activation of CCK_2 receptors attenuates the antinociceptive effect of μ-opioid receptor agonists in supraspinal and spinal sites (Itoh et al., 1982; Faris et al., 1983; Wang et al., 1990; Stanfa et al., 1994). We previously reported that in non-diabetic mice, the heat intensity at bulb voltages of 65 and 80 V evoked a rapid tail-flick response, whereas 50-V evoked an intermediate tail-flick latency and, 25 and 35 V evoked no tail-flick response

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(Ohsawa and Kamei, 1999a,b). In diabetic mice, the tail-flick latency after 50 V was significantly shorter than that in non-diabetic mice, indicating that diabetic mice exhibit thermal hyperalgesia (Ohsawa and Kamei, 1999a,b). In addition, a lower bulb voltage (35 V), which did not evoke a tail-flick response in non-diabetic mice, did evoke a tail-flick response in diabetic mice, indicating that diabetic mice exhibit thermal allodynia (Ohsawa and Kamei, 1999a,b). Furthermore, we recently demonstrated that the tail-flick latencies after heating at 35 and 50 V in diabetic mice were increased by i.t. pretreatment with a CCK_2 receptor antagonist, CI-988 ((*R*-[*R**,*R**])-4-([3-1*H*-indol]-3-yl)-2-methyl-1-oxo-2-([{tricyclo(3.3.1.1)dec-2-yloxy}carbonyl] amino)propylamino-1-phenyl-ethylamino-4-oxybutanoic acid) (Kamei and Zushida, 2001). Based on these results, we suggested that CCK, by acting at CCK_2 receptors in the spinal cord, might, at least in part, play an important role in thermal allodynia and hyperalgesia in diabetic mice (Kamei and Zushida, 2001). Enhanced expression of CCK mRNA and increased CCK-immunopositive neurons have been reported in the brain of streptozotocin-induced diabetic animals (Sipols et al., 1995). Thus, it is possible that reduction of the antinociceptive effect of endomorphin-2 in diabetic mice may be due to the increased CCK contents and/or activity in diabetic mice. To test this hypothesis, we examined the effect of either an agonist (CCK-8) or an antagonist (CI-988) of CCK_2 receptors on the antinociceptive effects of endomorphin-1 and endomorphin-2 in non-diabetic and diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that was maintained at 24 ± 1°C with a 12-h light–dark cycle. The animals were rendered diabetic by an injection of streptozotocin (200 mg/kg, i.v.) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched non-diabetic mice were injected with the vehicle alone. The experiments were conducted 2 weeks after the injection of streptozotocin or vehicle. Mice with serum glucose levels above 4000 mg/l were considered diabetic. This study was carried out in accordance with the Declaration of Helsinki and 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. Antinociceptive assay

The antinociceptive response was determined by recording the latency in the tail-flick test, with radiant heat applied to the tail. The intensity of the thermal stimulus for the tail-flick test was adjusted so that the animal flicked its tail within 2–4 s. A cut-off latency of 15 s was used to prevent injury to the tail. The percentage of antinociception was calculated for each animal as %antinociception = $100 \times (\text{post-drug latency} - \text{pre-drug latency})/(15 - \text{pre-drug latency})$.

2.3. Drugs

Streptozotocin was purchased from Sigma (St. Louis, MO, USA), and cholecystokinin octapeptide (Asp-Try(SO_3H)-(Met)-Gly-Trp-Met-Asp-Phe-NH_2; CCK-8) and (*R*-[*R**,*R**])-4-([3-1*H*-indol]-3-yl)-2-methyl-1-oxo-2-([{tricyclo(3.3.1.1)dec-2-yloxy}carbonyl] amino)propyl-amino-1-phenyl-ethylamino-4-oxybutanoic acid (CI-988) were purchased from Research Biochemical International (Natic, MA). Endomorphin-1 and endomorphin-2 were synthesized by Dr. Nagase (Toray Industries, Kamakura, Japan). All drugs were dissolved in saline. Endomorphin-1 and endomorphin-2 were injected i.c.v. 10 min before the antinociceptive assay. We previously reported that the antinociception produced by endomorphin-1 and endomorphin-2 in both non-diabetic and diabetic mice reached a peak 10 min after i.c.v. administration, and then decreased (Kamei et al., 2000). Thus, a time interval of 10 min after i.c.v. administration was chosen for experiments designed to quantify the antinociceptive effects of endomorphin-1 and endomorphin-2. CCK-8 was injected i.c.v. 10 min before the administration of endomorphins. CI-988 was injected i.c.v. 10 min before the administration of endomorphins. The i.c.v. injection was performed according to the method of Haley and McCormick (1957). The animals were randomly assigned to groups of 8–17 for a given series of tests. The experiments were blinded with respect to the pretreatment. Each animal was used only once.

2.4. Data analysis

The data are expressed as means $\pm$ SE. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Bonferroni/Dunn test.

3. Results

3.1. Effects of i.c.v. administrations of endomorphin-1 and endomorphin-2 on the tail-flick response

As shown in Fig. 1A, endomorphin-1, at doses of 1–10 μg, i.c.v., produced a dose-dependent inhibition of the tail-flick response in both non-diabetic and diabetic mice. There was no significant difference between the antinoci-

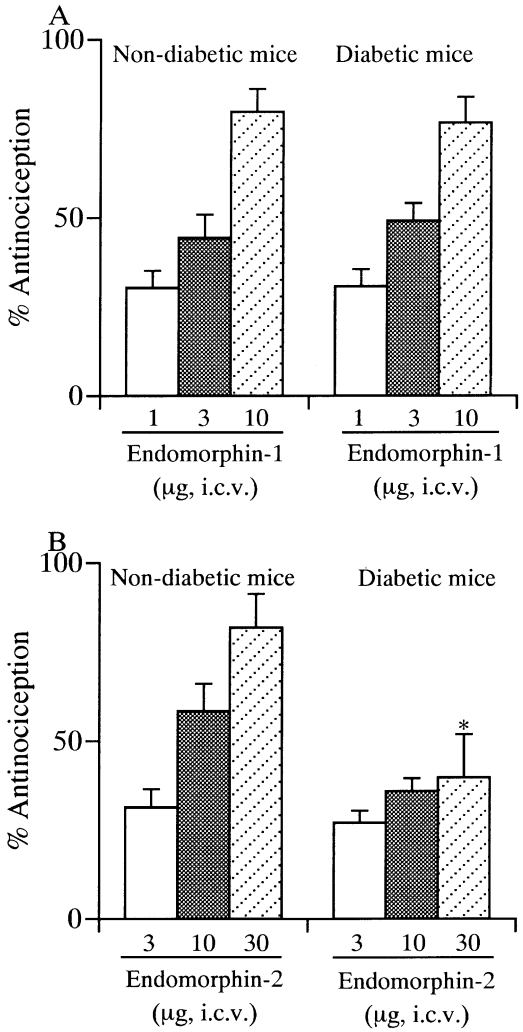

Fig. 1. Dose–response relationship of the antinociceptive effects of endomorphin-1 (A) and endomorphin-2 (B) in non-diabetic and diabetic mice. The antinociceptive effects of endomorphin-1 and endomorphin-2 were measured in the tail-flick test 10 min after injection. Each column represents the mean ± SE for 9–12 mice in each group. * $P < 0.05$ vs. respective non-diabetic mice.

ceptive effect induced by endomorphin-1 in non-diabetic and diabetic mice. Endomorphin-2, at doses of 3–30 μg, i.c.v., also produced a dose-dependent inhibition of the tail-flick response in both non-diabetic and diabetic mice but the peak antinociceptive effect of endomorphin-2 (30 μg, i.c.v.) in diabetic mice was significantly less than that in non-diabetic mice (Fig. 1B).

3.2. Effects of CCK-8 on the antinociception induced by endomorphin-1 and endomorphin-2

The effects of CCK-8 at doses of 1–10 ng, i.c.v., on the antinociceptive effects of endomorphin-1 (10 μg, i.c.v.) and endomorphin-2 (30 μg, i.c.v.) on non-diabetic and diabetic mice are shown in Fig. 2A,B. We confirmed that CCK-8 (1–10 ng, i.c.v.) itself had no significant effect on the tail-flick latency in either non-diabetic or diabetic mice (data not shown). The antinociceptive effect of endomorphin-1 in non-diabetic mice was significantly and dose dependently reduced by CCK-8. In diabetic mice, CCK-8 also dose dependently reduced the antinociceptive effect of endomorphin-1, but a significant reduction was observed only at the highest dose of CCK-8 (10 ng) (Fig. 2A).

As shown in Fig. 2B, the antinociceptive effect of endomorphin-2 in non-diabetic mice was significantly and dose dependently reduced by CCK-8. However, the antinociceptive effect of endomorphin-2 in diabetic mice was not altered by CCK-8 (Fig. 2B).

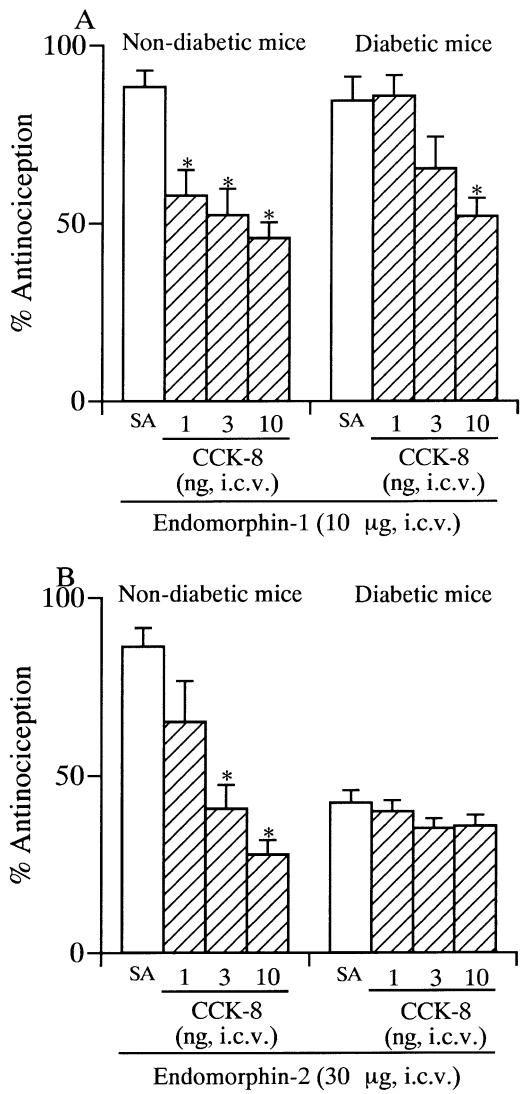

Fig. 2. Effect of CCK-8 on the antinociceptive effects of endomorphin-1 (A) and endomorphin-2 (B) in non-diabetic and diabetic mice. CCK-8 was injected 10 min before the administration of endomorphins. The antinociceptive effects of endomorphins were measured in the tail-flick test 10 min after injection. Each column represents the mean ± SE for 9–17 mice in each group. * $P < 0.05$ vs. respective saline (SA)-treated group.

3.3. Effects of CCK-B receptor antagonist on the antinociception induced by endomorphin-1 and endomorphin-2

CI-988, a selective CCK_2 receptor antagonist, at doses of 0.03–0.3 ng, i.c.v., by itself, had no significant effects on the tail-flick latency in non-diabetic or in diabetic mice (data not shown). The antinociceptive effect of endomorphin-2 (30 μg, i.c.v.) in diabetic mice was dose dependently and significantly enhanced by pretreatment with CI-988 (Fig. 3B). The highest dose of CI-988 (0.3 ng, i.c.v.) enhanced the antinociceptive effect of endomorphin-2 in diabetic mice ($78.3 \pm 10.0\%$, $n = 10$) to the level observed in non-diabetic mice ($86.3 \pm 5.2\%$, $n = 8$). However, CI-988 had no significant effect on the antinociception induced by endomorphin-2 (10 μg, i.c.v.) in non-diabetic mice (Fig. 3B). Furthermore, CI-988 also had no significant effect on the antinociceptive effects of endomorphin-1 (3 μg, i.c.v.) in either non-diabetic or diabetic mice (Fig. 3A).

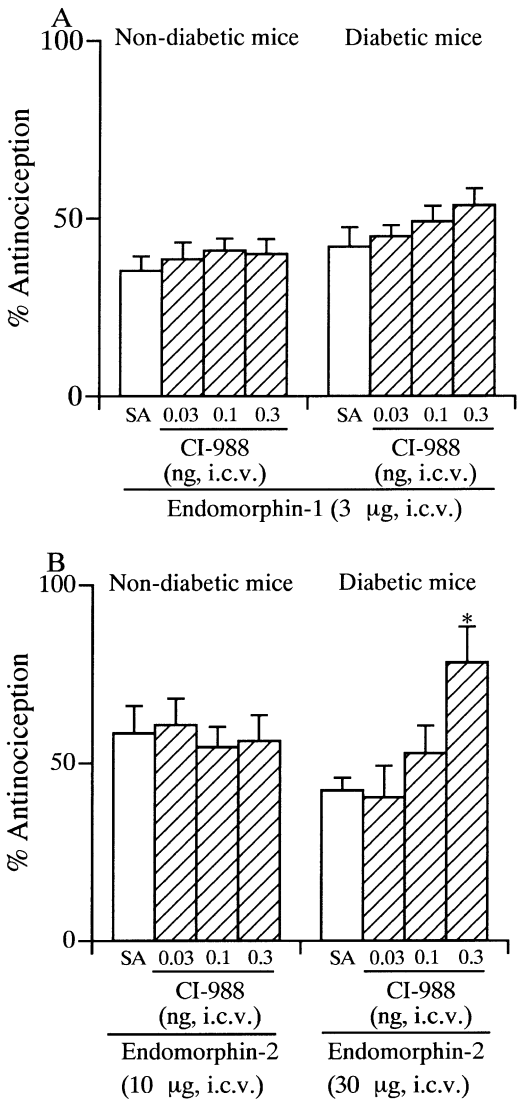

Fig. 3. Effects of CI-988 on the antinociceptive effects of endomorphin-1 (A) and endomorphin-2 (B) in non-diabetic and diabetic mice. CI-988 was injected 10 min before the administration of endomorphins. The antinociceptive effects of endomorphins were measured in the tail-flick test 10 min after injection. Each column represents the mean ± SE for 8–15 mice in each group. $^{*}P < 0.05$ vs. respective saline (SA)-treated group.

4. Discussion

Our results showed that i.c.v.-administered CCK-8 reduced the antinociceptive effects of endogenous μ-opioid receptor agonists, endomorphin-1 and endomorphin-2 in non-diabetic mice. Furthermore, the CCK-8-induced reduction of the antinociceptive effects of endomorphins was reversed by i.c.v.-administered CI-988, a CCK_2 receptor antagonist. These results agree with the suggestion of several authors that CCK may function as an endogenous opioid antagonist because small doses of CCK-8 have been reported to reduce the antinociception induced by morphine and β-endorphin (Itoh et al., 1982; Faris et al., 1983, 1984; O'Neill et al., 1989; Suh and Tseng, 1990).

In the present study, we observed that CI-988, at a dose of 0.3 ng, i.c.v., had no significant effect on the antinociceptive effects of endomorphin-1 and endomorphin-2 in non-diabetic mice. However, the antinociceptive effect of endomorphin-2 in diabetic mice was increased to the level observed in non-diabetic mice by this ineffective dose of CI-988 (0.3 ng, i.c.v.). Coudoré-Civiale et al. (2000) recently reported that morphine (0.1 mg/kg, i.v.) itself has no effect on the vocalization threshold in response to paw pressure in diabetic rats, while an appreciable antinociceptive effect is observed when it is coadministered with intrathecal CI-988 (0.1 μg). Based on these results, they suggested that spinal CCK transmission decreased opiate analgesia in the context of chronic pain, as observed in diabetic animals and humans (Coudoré-Civiale et al., 2000). On the other hand, enhanced expression of CCK mRNA and increased CCK-immunopositive neurons have been reported in the brain of streptozotocin-induced diabetic animals (Sipols et al., 1995). Considering these previous observations and our present results, it is possible that reduction of the μ-opioid receptor-mediated antinociceptive effect of endomorphin-2 in diabetic mice may be due to an excessive activation of CCK transmission, not only in the spinal cord, but also in supraspinal sites.

We previously reported that i.c.v. endomorphin-1 in diabetic mice produced its antinociception mainly through δ_1-opioid receptors, but not μ-opioid receptors (Kamei et al., 2000). In the present study, the antinociceptive effect of endomorphin-1 in diabetic mice was significantly reduced by CCK-8. These results suggest that a supraspinal CCKergic system may antagonize δ-opioid receptor-mediated antinociception in diabetic mice. However, CI-988 (0.3 ng, i.c.v.) also had no effect on the antinociceptive effect of endomorphin-1 in diabetic mice. Furthermore, the

effective dose of CCK-8 (10 ng, i.c.v.), which significantly reduced the antinociceptive effect of endomorphin-1 in diabetic mice, was relatively higher than the dose in non-diabetic mice. Thus, it is possible that δ-opioid receptor-mediated antinociception in diabetic mice may be highly resistant to the CCKergic system. However, we did not obtain any data that would directly support this hypothesis. Further studies are necessary before this possibility can be established with greater certainty.

In conclusion, our present results strongly suggest that the reduction of the antinociceptive effect of endomorphin-2 in diabetic mice may be due, at least in part, to the activation of CCK_2 receptors.

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