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Possible involvement of protein kinase C in the attenuation of [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin-induced antinociception in diabetic mice

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Abstract

The effects of pretreatment with a highly selective protein kinase C inhibitor, calphostin C, on the antinociception induced by the intracerebroventricular (i.c.v.) administration of the μ-opioid receptor agonist [D-Ala^2,NMePhe^4,Gly-ol^5]enkephalin (DAMGO) were studied in diabetic and non-diabetic mice. The antinociceptive potency of i.c.v. DAMGO in diabetic mice was lower than that in non-diabetic mice. I.c.v. pretreatment with phorbol 12,13-dibutyrate (50 pmol), a protein kinase C activator, for 60 min attenuated the antinociception induced by i.c.v. DAMGO in non-diabetic mice, but not in diabetic mice. I.c.v. pretreatment with calphostin C (3 pmol) for 60, 120 and 240 min, but not 10 min, increased the antinociceptive effect of DAMGO (10 ng) in diabetic mice, but not in non-diabetic mice. The dose–response curve for DAMGO-induced antinociception in diabetic mice, but not in non-diabetic mice, was shifted to the left by i.c.v. pretreatment with calphostin C (3 pmol) for 60 min. In non-diabetic mice, i.c.v. pretreatment with a high dose of calphostin C (10 pmol) for 60 and 120 min potentiated DAMGO-induced antinociception. These results indicate that protein kinase C may be involved in DAMGO-induced antinociception in mice. Furthermore, these results suggest that the attenuation of DAMGO-induced antinociception in diabetic mice may be due in part to increased protein kinase C activity. © 1997 Elsevier Science B.V.

Keywords: Antinociception; DAMGO ([D-Ala^2,NMePhe^4,Gly-ol^5]enkephalin); Diabetes; Protein kinase C; Calphostin C; (Mouse)

1. Introduction

Three opioid receptors, the μ-, δ-, and κ-opioid receptors, have recently been cloned (Evans et al., 1992; Kieffer et al., 1992; Chen et al., 1993; Yasuda et al., 1993; Liang et al., 1995). These three opioid receptors contain several potential phosphorylation sites in the first and third loops and in the C-terminus of intracellular domains (Miotto et al., 1995). It has been suggested that phosphorylation of these three opioid receptors is involved in desensitization. Narita et al. (1995) reported that intrathecal (i.t.) pretreatment with calphostin C, a specific protein kinase C inhibitor, blocks the development of acute antinociceptive tolerance to i.t. DAMGO in the mouse. Furthermore, we recently reported that activation of protein kinase C by phorbol 12,13-dibutyrate leads to desensitization of μ-opioid receptor-mediated antinociception (Narita et al., 1996). These results suggest that protein kinase C may be involved in the desensitization of μ-opioid receptor-mediated antinociception in the mice.

It has been reported that the antinociceptive potency of morphine is decreased in several rodent models of hyperglycemia, including a spontaneously diabetic strain of mice and rodents with streptozotocin-induced diabetes, an animal model of type I diabetes (Simon and Dewey, 1981). We previously reported that the antinociceptive effects of i.c.v., but not i.t., administration of μ-opioid receptor agonists, such as morphine and [D-Ala^2,NMePhe^4,Gly-ol^5]enkephalin (DAMGO), in non-diabetic mice were markedly greater than those in diabetic mice (Kamei et al., 1992a,b). Therefore, we suggested that diabetic mice are selectively hyporesponsive to supraspinal μ-opioid receptor-mediated antinociception, but not to that which is mediated by spinal μ-opioid receptors (Kamei et al., 1994). However, the detailed mechanisms that are responsible for this hyporesponsiveness to supraspinal μ-receptor-mediated antinociception in diabetic mice are unclear.

Many investigators have reported that hyperglycemia or elevated glucose levels can increase diacylglycerol levels and activate protein kinase C in vascular tissues, cardiac tissues, or cultured cells (Craven and DeRubertis, 1989; King et al., 1990; Tanaka et al., 1991; Inoguchi et al.,

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1992). Activation of the diacylglycerol–protein kinase C cellular signal pathway is linked to vascular dysfunction in diabetes (Craven and DeRubertis, 1989; Wolf et al., 1990; Shiba et al., 1993). Furthermore, Ahlgren and Levine (1994) reported that both the mechanical behavioral hyperalgesia and C-fiber hyperexcitability in response to mechanical stimuli seen in streptozotocin-induced diabetic rats are reduced by agents that inhibit protein kinase C. This result suggests that increased protein kinase C activity might alter the excitability of primary afferent nociceptors. Thus, the aim of our study was to investigate the role of protein kinase C in the attenuation of the antinociception induced by DAMGO in diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science, Tokyo), weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room which was maintained at 22 ± 1°C with a 12 h light-dark cycle. 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 injection of streptozotocin or vehicle. Mice with serum glucose levels above 400 mg/dl were considered diabetic. 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. Antinociceptive assay

The antinociceptive response was evaluated by recording the latency in the tail-flick test with radiant heat as a stimulus. The intensity of the thermal stimulus was adjusted so that the animal flicked its tail in 2–4 s. A cut-off latency of 30 s was used to prevent injury to the tail. Animals which did not respond within 30 s were removed and assigned a score of 30 s. The percent maximum possible effect (MPE) was calculated for each animal as $\%\mathrm{MPE} = 100 \times (\text{post drug latency} - \text{pre drug latency})/(30 - \text{pre drug latency})$.

2.3. Intracerebroventricular injection

Intracerebroventricular (i.c.v.) administration was performed following the method described by Haley and McCormick (1957), using a 50 μl Hamilton syringe. The injection site was 1.5 mm from the midline, 0 mm from the bregma and 3.0 mm from the surface of the skull. Injection volumes were 5 μl for i.c.v. administration.

2.4. Drugs

The following drugs were used: streptozotocin (Sigma Chemical, St. Louis, MO), (D-Ala^2,NMePhe^4,Gly-ol^5) enkephalin (DAMGO; Peninsula Laboratories, San Carlos, CA), phorbol 12,13-dibutyrate (PDB; Calbiochem-novabiochem, San Diego, CA) and calphostin C (Calbiochem-novabiochem, San Diego, CA). PDB and calphostin C were dissolved in ethanol 0.01% in saline (0.9% NaCl solution). DAMGO was dissolved in saline. The doses of opioid agonist, PDB and calphostin C in this study were determined as described previously (Narita et al., 1996).

2.5. Data analysis

The data are expressed as means $\pm$ S.E. The statistical significance of differences between groups was assessed with Student's *t*-test (comparison of two groups) or an analysis of variance (ANOVA) followed by the Bonferroni test (comparisons among multiple groups). The potency ratio for non-diabetic mice and diabetic mice was calculated using Program 11 of the Pharmacological Calculations system of Tallarida and Murray (1987).

3. Results

3.1. Effect of i.c.v. pretreatment with phorbol 12,13-dibutyrate (PDB) on the antinociceptive effect induced by i.c.v.-administered DAMGO in diabetic and non-diabetic mice

As shown in Fig. 1, i.c.v. administration of DAMGO (10 ng) produced an average % MPE of 84.2 ± 9.3% and 33.3 ± 8.4% in non-diabetic and diabetic mice, respectively. Diabetic mice were significantly less sensitive to i.c.v. DAMGO than non-diabetic mice, as assessed by the tail-flick test. Pretreatment with PDB (50 pmol, i.c.v.) 60 min prior to an i.c.v. challenge with DAMGO attenuated the antinociceptive effect of DAMGO (10 ng) in non-diabetic mice. The attenuation of DAMGO-induced antinociception in non-diabetic mice was reversed by concomitant pretreatment with calphostin C (3 pmol, i.c.v.) In diabetic mice, however, PDB (50 and 100 pmol) had no significant effect on the antinociceptive effect of DAMGO (10 ng).

3.2. Effects of i.c.v. pretreatment with calphostin C on DAMGO-induced antinociception in diabetic and non-diabetic mice

As shown in Fig. 2A, pretreatment with calphostin C, at a dose of 3 pmol, i.c.v., did not affect DAMGO (10 ng,

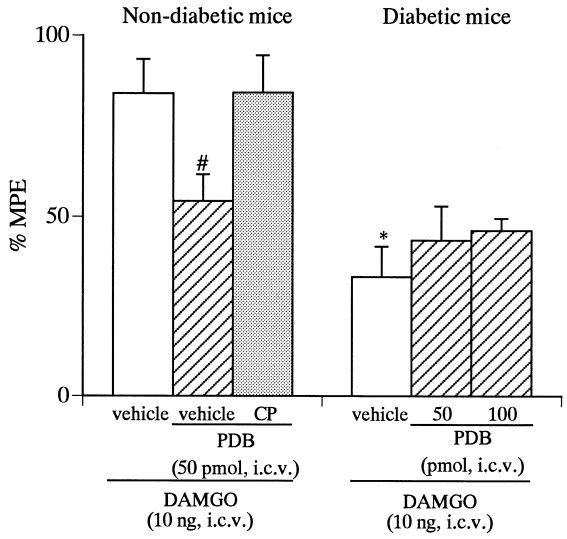

Fig. 1. Effect of i.c.v. pretreatment with phorbol 12,13-dibutyrate (PDB) on DAMGO-induced antinociception in diabetic and non-diabetic mice. PDB (50 or 100 pmol) alone or a combination of PDB (50 pmol) and calphostin C (CP; 3 pmol) was injected i.c.v. 60 min before the administration of DAMGO (10 ng, i.c.v.). Mice were tested 10 min after the injection of DAMGO in the tail-flick test. Each column represents the mean with S.E. for 10 mice in each group. $^{\#}P < 0.05$ compared with the vehicle-pretreated group. $^{*}P < 0.05$ compared with respective non-diabetic mice.

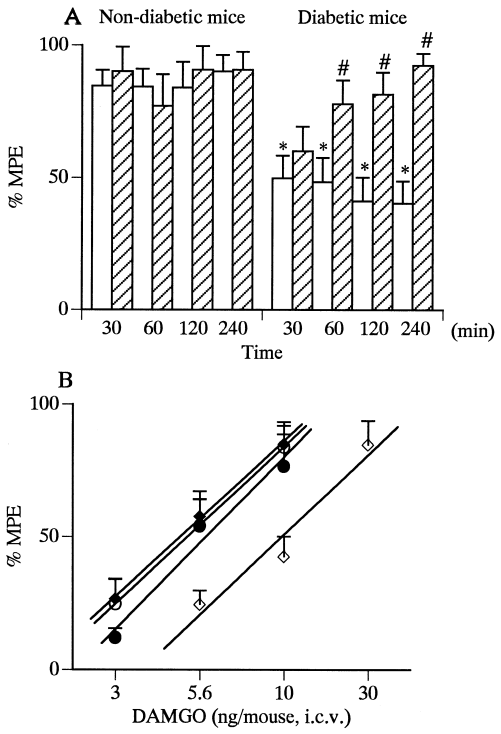

Fig. 2. (A) effect of calphostin C (3 pmol, hatched column) on DAMGO-induced antinociception after different pretreatment times in diabetic and non-diabetic mice. (B) effect of i.c.v. pretreatment with calphostin C (3 pmol, closed symbol) and its vehicle (open symbol) on the dose–response curve for DAMGO-induced antinociception in diabetic (diamond) and non-diabetic (circle) mice. Calphostin C (3 pmol) was injected i.c.v. 30, 60, 120 and 240 min (A) or 60 min (B) before the administration of DAMGO (10 ng, i.c.v.). Mice were tested 10 min after the injection of DAMGO in the tail-flick test. Each column represents the mean with S.E. for 10 mice in each group. $^{\#}P < 0.05$ compared with the vehicle-pretreated group (open column). $^{*}P < 0.05$ compared with respective non-diabetic mice.

i.c.v.) induced antinociception in non-diabetic mice. Furthermore, calphostin C, at a dose of 3 pmol, also had no effect on the antinociception induced by a lower dose (3 ng, i.c.v.) of DAMGO. However, pretreatment with a higher dose (10 pmol, i.c.v.) of calphostin C for 60 and 120 min significantly increased DAMGO (3 ng, i.c.v.) induced antinociception in non-diabetic mice (Fig. 3).

In diabetic mice, pretreatment with calphostin C (3 pmol, i.c.v.) for 60, 120 and 240 min, but not for 30 min, progressively increased DAMGO-induced antinociception. DAMGO produced dose-dependent antinociception at 5.6–30 ng i.c.v. in diabetic mice and at 3–10 ng i.c.v. in non-diabetic mice. The antinociceptive potency of i.c.v. DAMGO in diabetic mice was less than that in non-diabetic mice, as evidenced by a 2.3-fold rightward shift in the dose–response curve for DAMGO-induced antinociception (Fig. 2B). Fig. 2B shows that pretreatment with calphostin C (3 pmol, i.c.v.) for 60 min can prevent the rightward shift in the DAMGO dose–response curve, which is indicative of a decreased potency of DAMGO in diabetic mice. However, pretreatment with calphostin C (3 pmol) for 60 min had no effect on the dose–response curve for DAMGO-induced antinociception in non-diabetic mice.

The effects of various doses of calphostin C on the antinociceptive effect of DAMGO in both diabetic and non-diabetic mice are shown in Fig. 4. Pretreatment with calphostin C for 60 min, at doses of 1–20 pmol, did not have any effect on the antinociception induced by DAMGO (10 ng). In diabetic mice, pretreatment with calphostin C

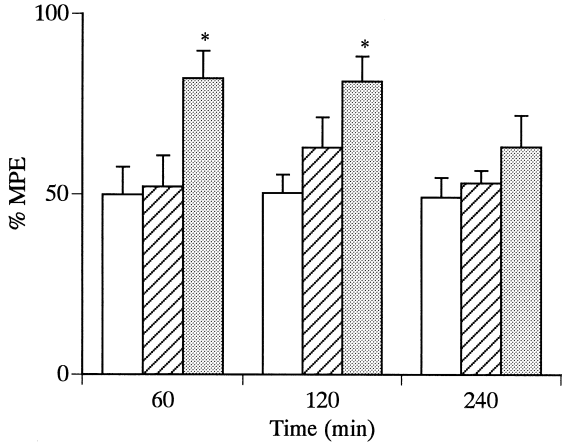

Fig. 3. The effect of calphostin C on DAMGO-induced antinociception in non-diabetic mice after different pretreatment times. Calphostin C (3 pmol, hatched column; 10 pmol, closed dotted column) was injected i.c.v. 60, 120 and 240 min before the administration of DAMGO (5.6 ng, i.c.v.). Mice were tested 10 min after the injection of DAMGO in the tail-flick test. Each column represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ compared with the vehicle-pretreated group (open column).

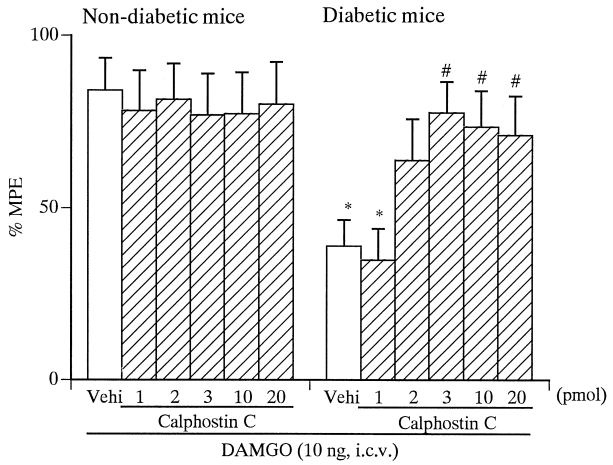

Fig. 4. Dose–response effect of i.c.v. pretreatment with calphostin C on DAMGO-induced antinociception in diabetic and non-diabetic mice. Calphostin C and vehicle (Vehi) were injected i.c.v. 60 min before the administration of DAMGO. Mice were tested 10 min after the injection of DAMGO in the tail-flick test. Each column represents the mean with S.E. for 10 mice in each group. $^{\#}P < 0.05$ compared with the vehicle-pretreated group. $^{*}P < 0.05$ compared with respective non-diabetic mice.

dose dependently increased the antinociceptive effect of DAMGO (10 ng).

4. Discussion

There have been several suggestions regarding the possible functions of protein kinase C, including involvement in secretion and exocytosis, modulation of ion conductance, regulation of receptor interaction with components of the signal transduction apparatus, smooth muscle contraction, gene expression and cell proliferation (Nishizuka, 1988). Protein kinase C regulates several cellular functions through the phosphorylation of proteins, including some receptors. Protein kinase C can be stimulated by tumor-promoting phorbol esters such as PDB, which presumably bind to the same site in the regulation domain as diacylglycerol. The results of this study demonstrated that i.c.v. pretreatment with PDB (50 pmol) attenuated the inhibition of the tail-flick response induced by i.c.v. DAMGO in non-diabetic mice. Furthermore, the attenuation of i.c.v. DAMGO-induced antinociception by PDB was reversed by concomitant i.c.v. pretreatment with calphostin C, a selective protein kinase C inhibitor. These results are consistent with our previous observation and support the suggestion that the attenuation by PDB of μ-opioid receptor-mediated antinociception is specifically mediated by the activation of protein kinase C (Narita et al., 1996).

In contrast, PDB, by itself, had no significant effect on DAMGO-induced antinociception in diabetic mice. Furthermore, pretreatment with calphostin C (3 pmol, i.c.v.), which had no significant effect on DAMGO-induced antinociception in non-diabetic mice, significantly and dose dependently increased DAMGO-induced antinociception in diabetic mice. Indeed, there was no significant difference in the potency of DAMGO-induced antinociception between calphostin C-pretreated diabetic mice and naive non-diabetic mice. In the present study, moreover, we found that pretreatment with a higher dose of calphostin C (10 pmol) potentiated low-dose (3 ng, i.c.v.) DAMGO-induced antinociception in non-diabetic mice. Calphostin C specifically inhibits the binding of diacylglycerol to the regulatory domain of protein kinase C (Kobayashi et al., 1989), and therefore is a more selective inhibitor than staurosporine or 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H-7), which interact with the ATP-binding site of protein kinase C, a site that shares substantial homology with that of other protein kinases. The phosphorylation of receptors by protein kinase C has been proposed to be a possible mechanism for the development of tolerance or desensitization (Shearman et al., 1989). According to recent cloning studies, several potential phosphorylation sites for protein kinases are present in cloned opioid receptors, including μ-opioid receptors (Miotto et al., 1995). Previously, we reported that i.c.v. pretreatment with PDB produced a calphostin C-sensitive attenuation of DAMGO-induced antinociception (Narita et al., 1996). These results suggest that μ-opioid receptors can be phosphorylated by the activation of protein kinase C, and this receptor phosphorylation by protein kinase C leads to desensitization of μ-opioid receptor-mediated responses. Several studies have suggested that protein kinase C systems may be up-regulated in diabetes (Craven and DeRubertis, 1989; Wolf et al., 1990; Shiba et al., 1993). We recently reported that at least 30–60 min of pretreatment is required for PDB to desensitize μ-opioid receptor-mediated responses (Narita et al., 1996). In the present study, at least 60 min of pretreatment was required for calphostin C to increase DAMGO-induced antinociception in diabetic mice. Thus, it is possible that the attenuation of the antinociceptive effect of DAMGO in diabetic mice may be due, in part, to the increased phosphorylation of μ-opioid receptors by the activation of protein kinase C.

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