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European Journal of Pharmacology 362 (1998) 47–51

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Short communication

Possible involvement of protein kinase C in the attenuation of the morphine-induced Straub tail reaction in diabetic mice

Masahiro Ohsawa, Junzo Kamei *

Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-Chome, Shinagawa-ku, Tokyo, 142, Japan

Received 25 September 1998; revised 16 October 1998; accepted 20 October 1998

Abstract

To investigate the role of protein kinase C in the attenuation of the morphine-induced Straub tail reaction in diabetic mice, we examined the effects of protein kinase C activator or inhibitor on the i.c.v. morphine-induced Straub tail reaction in mice. This reaction was less in diabetic mice than in normal mice. Intracerebroventricular pretreatment with phorbol 12,13-dibutyrate (50 pmol), a potent protein kinase C activator, attenuated the morphine-induced Straub tail reaction in normal mice, but not in diabetic mice. I.c.v. pretreatment with calphostin C (10 pmol), a selective protein kinase C inhibitor, enhanced the reaction in diabetic mice, but not in normal mice. The dose-response curve for the morphine-induced Straub tail reaction in normal mice, but not in diabetic mice, was shifted to the right by i.c.v. pretreatment with phorbol 12,13-dibutyrate (50 pmol). Furthermore, i.c.v. pretreatment with calphostin C (3 pmol) shifted the dose-response curve to the left in diabetic mice, but not in normal mice. These results indicate that activation of protein kinase C reduces the morphine-induced Straub tail reaction in normal mice. Also, the attenuation of the morphine-induced Straub tail reaction in diabetic mice may be due in part to increased protein kinase C activity.

Keywords: Straub tail reaction; Morphine; Diabetes; Protein kinase C; Supraspinal; Desensitization

1. Introduction

Three opioid receptors, 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 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. There is accumulating evidence that the activation of protein kinase C regulates several cellular functions through phosphorylation of proteins, including some receptors, whose function is then down-regulated or up-regulated (Moran and Dascal, 1989). Activation of protein kinase C by treatment with phorbol ester potentiates the desensitization of the μ-opioid receptor-induced K^+ current (Chen and Yu, 1994). 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., 1997, Ohsawa and Kamei, 1997). These results suggest that protein kinase C may be involved in the desensitization of μ-opioid receptor-mediated pharmacological actions in mice.

Morphine contracts the sacrococcygenus muscle in mice, which results in erection of the tail (Straub tail reaction) (Bilbey et al., 1960). Previous studies have suggested that the morphine-induced Straub tail reaction is evoked through the activation of μ-opioid receptors (Kameyama et al., 1978; Narita et al., 1994; Kamei et al., 1994b). Murray and Cowan (1990) have suggested that δ-opioid receptors are not involved in the opioid agonist-induced Straub tail reaction. It was also reported that activation of the κ-opioid receptor reduces the morphine-induced Straub tail reaction (Narita et al., 1994). These results clearly indicated that the morphine-induced Straub tail reaction in mice is mediated through the activation of μ-opioid receptors. Thus, the morphine-induced Straub tail reaction is a useful model, for testing the influence of a protein kinase C modulator on a μ-opioid receptor agonist-induced pharmacological action, without the influence of another opioid receptor.

* Corresponding author. Tel.: +81-3-5498-5030; Fax: +81-3-5498-5029; E-mail: kamei@hoshi.ac.jp

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00765-1

We have demonstrated that diabetes affects the sensitivity of animals to μ-opioid receptors, including antinociception and Straub tail reaction (Kamei et al., 1992a,b, 1994a,b). We 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, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO), were markedly greater in normal mice than in diabetic mice (Kamei et al., 1992a,b, 1994a). Furthermore, we reported that the morphine-induced Straub tail reaction in normal mice was stronger than that in diabetic mice (Kamei et al., 1994b). Many investigators have reported that hyperglycemia or elevated glucose levels can increase diacylglycerol levels and activate protein kinase C in vascular tissue, cardiac tissues or cultured cells (Craven and De Rubertis, 1989; King et al., 1990; Tanaka et al., 1991; Inoguchi et al., 1992). Activation of the diacylglycerol–protein kinase C cellular signal pathway is linked to vasculature dysfunction in diabetes (Craven and De Rubertis, 1989; Wolf et al., 1990; Shiba et al., 1993). We recently reported that calphostin C, a protein kinase C inhibitor, reverses the attenuation of DAMGO-induced antinociception in diabetic mice to the level in normal mice (Ohsawa and Kamei, 1997). These results suggest that increased protein kinase C activity in diabetic mice might alter μ-opioid receptor function in the central nervous system. Thus, the first aim of the present study was to investigate the effects of a protein kinase C activator and inhibitor on the i.c.v. morphine-induced Straub tail reaction in mice. An additional aim was to investigate the role of protein kinase C in the attenuation of the morphine-induced Straub tail reaction 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 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 normal mice were injected with 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/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, Sports and Culture.

2.2. Measurement of the Straub tail reaction

The Straub tail reaction was graded using a minor modification of the numerical scoring system of Kameyama et al. (1978) as follows; 0 = 0°, 0.5 = 1–30°, 1.0 = 31–45°, 1.5 = 46–60°, 2.0 = 61–90°, 2.5 = more than 90°. The angle was measured above the horizontal plane of the table. Morphine was injected intracerebroventricularly (i.c.v.) as described by Haley and McCormick (1957) using a 50-μl Hamilton syringe. The Straub tail reaction was observed 20 min after the i.c.v. administration of morphine.

2.3. Drugs

The drugs used were streptozotocin (Sigma, St. Louis, MO) and morphine hydrochloride (Sankyo, Tokyo, Japan). Calphostin C and phorbol-12,13-dibutyrate were purchased from Calbiochem-Novabiochem International (San Diego, CA), and were injected 1h before the i.c.v. injection of morphine. The dose and schedule for calphostin C and phorbol-12,13-dibutyrate in this study were determined as described previously (Narita et al., 1997; Ohsawa and Kamei, 1997).

2.4. Data analysis

The data are expressed as means ± S.E. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Bonferroni test. The potency ratio for normal mice and diabetic mice was calculated using Program 11 of the Pharmacological Calculation system of Tallarida and Murray (1987).

3. Results

3.1. Effects of a protein kinase C activator, phorbol 12,13-dibutyrate, on the morphine-induced Straub tail reaction in diabetic and normal mice

As shown in Fig. 1A, i.c.v. administration of morphine, 15 μg, induced a Straub tail reaction in both diabetic and normal mice. Mice with diabetes showed significantly less sensitivity in the i.c.v. morphine-induced Straub tail reaction. Intracerebroventricular pretreatment with phorbol-12,13-dibutyrate (10 and 50 pmol) 60 min prior to an i.c.v. challenge with morphine (15 μg) attenuated the morphine-induced Straub tail reaction in normal mice. In diabetic mice, however, phorbol-12,13-dibutyrate had no significant effect on the morphine (15 μg)-induced Straub tail reaction (Fig. 1A). As shown in Fig. 1B, i.c.v. pretreatment with calphostin C (10 pmol) 60 min prior to an i.c.v. injection of morphine did not affect the morphine-induced Straub tail reaction in normal mice. In diabetic mice, i.c.v.

pretreatment with calphostin C, at doses of 3 and 10 pmol, progressively enhanced the morphine-induced Straub tail reaction (Fig. 1B).

The i.c.v.-administered morphine-induced Straub tail reaction in diabetic mice was less than that in normal mice, as evidenced by a 2.3-fold rightward shift in the dose–response curve (Fig. 2). As shown in Fig. 2, i.c.v. pretreatment with a protein kinase C activator, phorbol-12,13-dibutyrate, at a dose of 50 pmol attenuated the i.c.v. morphine-induced Straub tail reaction in normal mice; the dose response curve for the morphine-induced Straub tail reaction was markedly shifted to the right 2.1-fold. The potency ratio (95% CL) of the morphine-induced Straub tail reaction in phorbol-12,13-dibutyrate-treated normal mice versus that in vehicle-treated normal mice was 2.1 (1.4-4.3)

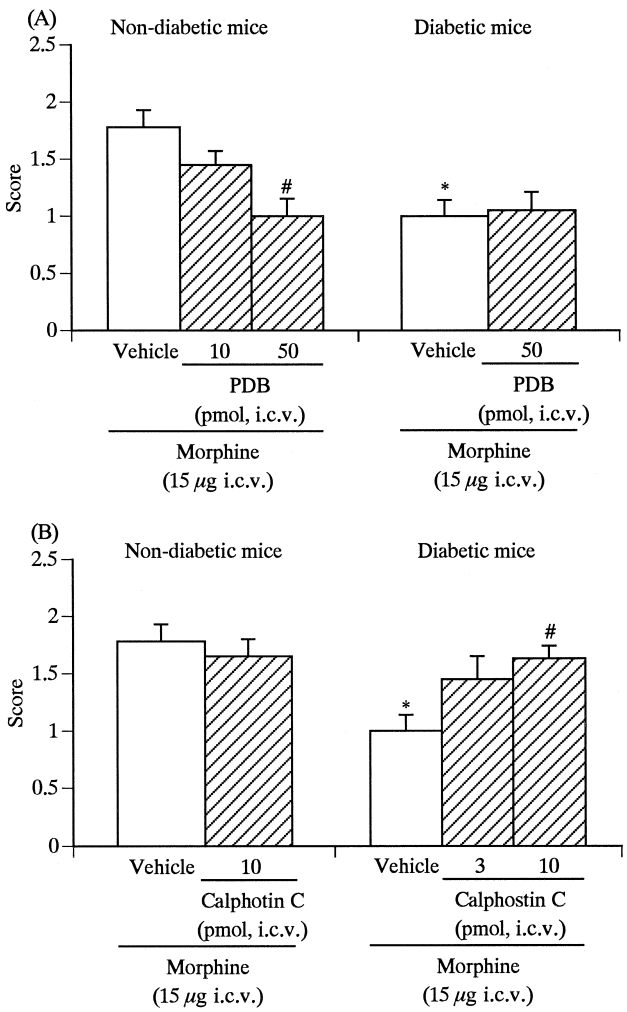

Fig. 1. Effects of i.c.v. pretreatment with phorbol 12,13-dibutyrate (PDB; A) and calphostin C (B) on the i.c.v. morphine-induced Straub tail reaction in diabetic and normal mice. PDB (10 and 50 pmol) or calphostin C (1 or 3 pmol) was injected i.c.v. 60 min before the administration of morphine (15 μg, i.c.v.). The Straub tail reaction was graded using numerical scores, and was observed 20 min after the i.c.v. administration of morphine. Each column represents the mean with S.E. for 9–15 mice in each group. $*P < 0.05$ vs. normal mice. $\#P < 0.05$ vs. respective vehicle-treated group.

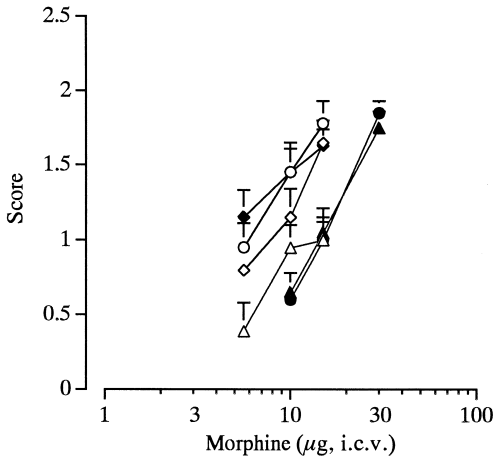

Fig. 2. Effects of i.c.v. pretreatment with phorbol 12,13-dibutyrate (diamond) and calphostin C (triangle) on the dose–response curve for the i.c.v. morphine-induced Straub tail reaction in diabetic (closed symbol) and normal mice (open symbol). Phorbol 12,13-dibutyrate (50 pmol) or calphostin C (3 pmol) was injected i.c.v. 60 min before the administration of morphine. The Straub tail reaction was graded using numerical scores, and was observed 20 min after the i.c.v. administration of morphine. Each point represents the mean with S.E. for 9–15 mice in each group.

(Fig. 2). In contrast, the i.c.v. morphine-induced Straub tail reaction in diabetic mice was not affected by i.c.v. pretreatment with phorbol-12,13-dibutyrate (Fig. 2). Intracerebroventricular pretreatment with a protein kinase C inhibitor, calphostin C, did not affect the morphine-induced Straub tail reaction in normal mice (Fig. 2), but enhanced it in diabetic mice. The dose–response curve for the morphine-induced Straub tail reaction in the latter mice was markedly shifted to the left 2.8-fold. The potency ratio (95% CL) of the morphine-induced Straub tail reaction in calphostin C-treated diabetic mice versus that in vehicle-treated diabetic mice was 2.8 (2.3-3.5) (Fig. 2). There was no significant difference in the potency of the morphine-induced Straub tail reaction between calphostin C-treated diabetic mice and naive normal mice. The potency ratio (95% CL) of the morphine-induced Straub tail reaction in calphostin C-treated diabetic mice versus that in naive normal mice was 1.0 (0.98–1.07).

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 phosphorylation of proteins, including some receptors. The results of the present study demonstrated that the i.c.v. morphine-induced Straub tail-reaction in normal mice

is attenuated by i.c.v. pretreatment with phorbol-12,13-dibutyrate (50 pmol), which stimulates protein kinase C. Many investigators have proposed that the phosphorylation of receptors by protein kinase C may be a possible mechanism for the development of desensitization (Shearman et al., 1989). Activation of protein kinase C by phorbol ester attenuates the opioid-induced inhibition of adenylyl cyclase activity in neuroblastoma × glioma NG108-15 hybrid cells (Louie et al., 1990). Furthermore, activation of protein kinase C by phorbol ester potentiates the desensitization of the μ-opioid receptor-induced K^+ current (Chen and Yu, 1994; Zhang et al., 1996). We previously indicated that i.c.v. pretreatment with phorbol-12,13-dibutyrate attenuates DAMGO- and morphine-induced antinociception (Narita et al., 1997; Ohsawa and Kamei, 1997). Thus, these previous results and the present data suggest that the activation of protein kinase C by phorbol ester attenuates the pharmacological action of μ-opioid receptor agonists.

In contrast, phorbol-12,13-dibutyrate had no significant effect on the morphine-induced Straub tail reaction in diabetic mice. Furthermore, i.c.v. pretreatment with calphostin C (10 pmol), which had no significant effect on the morphine-induced Straub tail reaction in normal mice, significantly and dose dependently reversed the attenuation of the morphine-induced Straub tail reaction in diabetic mice. There was no significant difference in the potency of the morphine-induced Straub tail reaction between calphostin C-treated diabetic mice and naive normal mice. Recently, we reported that the attenuation of DAMGO-induced antinociception in diabetic mice was increased by i.c.v. pretreatment with calphostin C (Ohsawa and Kamei, 1997). Furthermore, calphostin C inhibits the binding of diacylglycerol to the regulatory domain of protein kinase C (Kobayashi et al., 1989). Thus, it is likely that the attenuation of several of morphine's pharmacological actions, i.e. antinociception and the Straub tail reaction, in diabetic mice may be due, in part, to the phosphorylation of μ-opioid receptors increased by the activation of protein kinase C.

The morphine-induced Straub tail reaction involves both central and peripheral components of the nervous system. We recently reported that the systemic morphine-induced Straub tail reaction is greater in normal mice than in diabetic mice (Kamei et al., 1994b). In the present study, the i.c.v. morphine-induced Straub tail reaction was greater in normal mice than in diabetic mice, as seen with the systemic administration of morphine (Kamei et al., 1994b). Many investigators have indicated that central administration of morphine induces the Straub tail reaction in mice (Narita et al., 1994; Nath et al., 1994). Diabetes mellitus causes various complications, including dysfunction of skeletal muscles and peripheral nerves (Pain and Garlick, 1974). It has been reported that the activation of protein kinase C induces dysfunction of skeletal muscle in streptozotocin-induced diabetic mice (Nojima et al., 1995). Thus, it is possible that the dysfunction of peripheral neurons and/or muscle causes the attenuation of the systemic morphine-induced Straub tail reaction in diabetic mice. However, the i.c.v. administration of morphine induced the Straub tail reaction in both diabetic and normal mice. Furthermore, i.c.v. pretreatment with calphostin C reverses the attenuation of the i.c.v. morphine-induced Straub tail reaction in diabetic mice. These results indicate that the attenuation of the morphine-induced Straub tail reaction in diabetic mice may be due to the desensitization of supraspinal μ-opioid receptors by the activation of protein kinase C but not to the dysfunction of peripheral nerves and muscles.

Acknowledgements

We thank Mr. M. Yamaguchi, Mr. K. Zushida, Ms. N. Osada and Ms. R. Maruyama for their helpful technical assistance.

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