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Effect of diabetes on bradykinin-induced thermal hyperalgesia in mice

Junzo Kamei *, Masahiro Ohsawa, Hideki Hitosugi

Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142-8501, Japan

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Abstract

To investigate the role of protein kinase C in the attenuation of bradykinin-induced thermal hyperalgesia in diabetic mice, we examined the effects of a protein kinase C activator or inhibitor on the i.t. bradykinin-induced hyperalgesia in diabetic and non-diabetic mice. Intrathecal injection of bradykinin caused a transient antinociceptive effect, which diminished within 30 min, and then produced a thermal hyperalgesia, which lasted about 120 min, in non-diabetic mice. Although the duration of the antinociceptive phase was longer in diabetic mice than in non-diabetic mice, the hyperalgesic response was not observed in diabetic mice. The bradykinin-induced hyperalgesia was dose-dependently and significantly enhanced by pretreatment with calphostin C (0.3 to 3 pmol, i.t.), a specific protein kinase C inhibitor, in diabetic mice. However, calphostin C (3 pmol, i.t.) had no significant effect on bradykinin-induced hyperalgesia in non-diabetic mice. On the other hand, pretreatment with phorbol-12, 13-dibutyrate (12.5 to 50 pmol, i.t.), a protein kinase C activator, significantly and dose-dependently reduced bradykinin-induced hyperalgesia in non-diabetic mice. However, phorbol-12, 13-dibutyrate (50 pmol, i.t.) had no significant effect on bradykinin-induced hyperalgesia in diabetic mice. These results suggest that the change in bradykinin-induced thermal hyperalgesia in diabetic mice may be due, at least in part, to the modification of nociceptive transmission in the spinal cord by the activation of protein kinase C.

Keywords: Bradykinin; Thermal hyperalgesia; Protein kinase C; Phorbol-12, 13-dibutyrate; Calphostin C; Diabetes; (Mouse)

1. Introduction

We previously demonstrated that although s.c. injection of formalin into the hindpaw produced the first phase of the nociceptive response, the second phase of the nociceptive response was barely observed in diabetic mice (Kamei et al., 1993). However, the duration of the first phase of the nociceptive response was significantly longer in diabetic mice than in non-diabetic mice (Kamei et al., 1993). When spantide, an antagonist of substance P, reduced the duration of the first phase of the nociceptive response to levels observed in non-diabetic mice, the second-phase nociceptive response appeared (Kamei et al., 1993). The first-phase response was dose-dependently and significantly reduced by pretreatment with calphostin C, a specific protein kinase C inhibitor, in diabetic mice (Ohsawa et al., 1998). The second-phase response was markedly increased when diabetic mice were pretreated with calphostin C (Ohsawa et al., 1998). On the other hand, pretreatment with phorbol-12, 13-dibutyrate, a protein kinase C activator, significantly enhanced the first-phase response and significantly reduced the second phase of the formalin-induced nociceptive response in non-diabetic mice (Ohsawa et al., 1998). Furthermore, we recently indicated that thermal allodynia and hyperalgesia in diabetic mice may be due to the enhanced substance P followed by activation of protein kinase C in the spinal cord (Ohsawa and Kamei, 1999). These results suggest that the change in the formalin-induced nociceptive response in diabetic mice may be due, at least in part, to the modification of nociceptive transmission in the spinal cord by the activation of protein kinase C (Ohsawa et al., 1998).

Formalin induces a long-lasting nociceptive response in the mouse paw, and this effect has been widely used to model persistent tonic pain of moderate intensity which

* Corresponding author. Tel.: +81-3-5498-5030; fax: +81-3-5498-5029.

E-mail address: kamei@hoshi.ac.jp (J. Kamei).

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involves chemical irritation, some tissue damage, and the formation of edema due to the release of inflammatory mediators (Hunskaar et al., 1985; Hunskaar and Hole, 1987; Murray et al., 1988). This nociceptive model usually involves two distinct phases. It has been proposed that the early phase reflects the direct stimulation of nociceptors, while the late phase may be associated with the release of inflammatory mediators (Dubuisson and Dennis, 1977; Hunskaar et al., 1985, 1986; Tjolsen et al., 1992). In addition, formalin-induced persistent pain is thought to resemble clinical pain due to its tonic nature (Dennis and Melzack, 1979; Abbott et al., 1982; Abbott and Franklin, 1986). On the other hand, there is evidence that substance P and inflammatory mediators (e.g., somatostatin, bradykinin, and prostaglandins) participate in the first and second phases of the formalin-induced nociceptive response, respectively (Shibata et al., 1989; Ohkubo et al., 1990). Recently, we demonstrated that the i.t. bradykinin-induced nociceptive response was significantly less intense in diabetic mice than in non-diabetic mice (Ohsawa et al., 1998; Kamei et al., 1999). Furthermore, the second phase, but not the first phase, of the formalin-induced nociceptive response was dose-dependently and significantly reduced by pretreatment with D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin (Hoe-140), a selective bradykinin B_2 receptor antagonist (Ohsawa et al., 1998). Based on these results, we suggested that a lower sensitivity to bradykinin in processing nociception may be responsible, at least in part, for the reduction in the second phase of the formalin-induced nociceptive response in diabetic mice (Ohsawa et al., 1998).

Thus, it is possible that increased protein kinase C activity may be responsible for the altered bradykinin-induced nociceptive response. To examine this possibility, in the present study, we assessed the effect of calphostin C, a specific protein kinase C inhibitor, on the i.t. bradykinin-induced thermal hyperalgesia in diabetic and non-diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Animal Laboratory, Tokyo, Japan), weighing about 20 g at the beginning of the experiment, were used. They had free access to solid food (MF; Oriental Yeast, Tokyo, Japan) and water in an animal room, which was maintained at 24 ± 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 M citrate buffer at pH 4.5. Age-matched control mice were injected with the vehicle alone. The experiments were conducted 2 weeks after the injection of vehicle or streptozotocin. 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, Science, Sports and Culture.

2.2. Bradykinin-induced thermal hyperalgesia

The nociceptive response was evaluated by recording the latency to withdraw the tail in response to noxious skin heating under restrained condition. Briefly, the tails of mice were exposed to a focused beam of light from a 50-W projection bulb. The voltage for a projection bulb was set to 65 V. A cut-off latency of 15 s was used to prevent injury to the tail. Intrathecal injection was performed by the method described by Hylden and Wilcox (1980). I.t. injection was performed using a 30-gauge needle directly through the intact skin between the L_5 and L_6 vertebrae. Drugs were given in a volume of 5 μl/mouse. The nociceptive response was examined before and 10, 20, 30, 60, 90, 120, 150, 180 and 360 min after i.t.

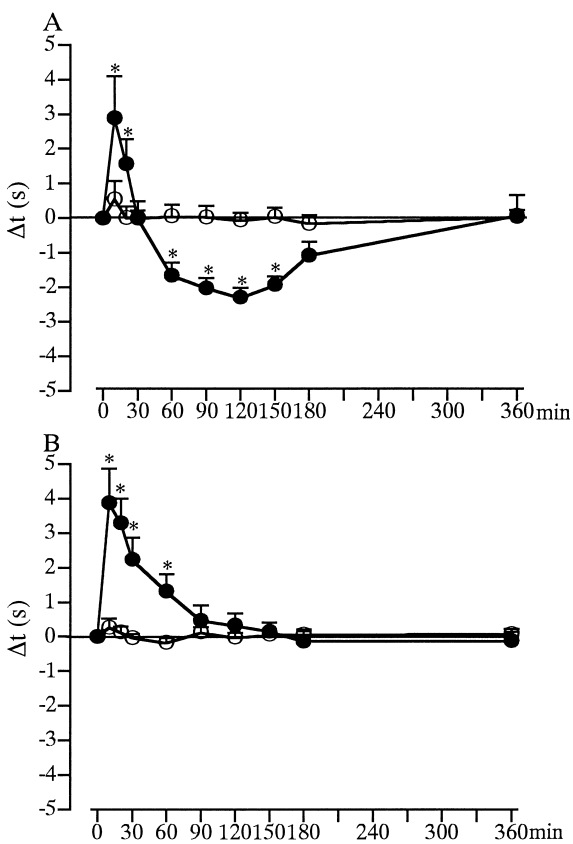

Fig. 1. Time courses of the thermal hyperalgesia induced by the intrathecal administration of bradykinin (1.0 μg, closed symbol) in non-diabetic (A) and diabetic (B) mice. Δt (s) = post-drug latency − pre-drug latency. Each point represents the mean with S.E. for 10 mice in each group. *$P < 0.05$ vs. saline-treated group (open symbol).

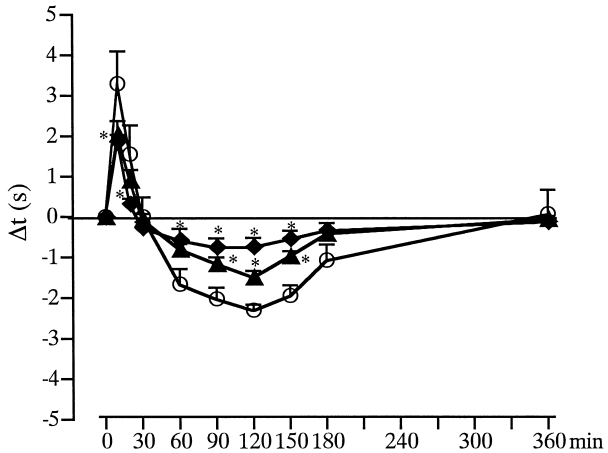

Fig. 2. Effects of Hoe-140 on bradykinin-induced thermal hyperalgesia in non-diabetic mice. Hoe-140 (30 ng, closed triangle; 100 ng, closed diamond) was injected i.t. 20 min before the injection of bradykinin (1.0 μg, i.t.). Δt (s) = post-drug latency − pre-drug latency. Each point represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ vs. vehicle + bradykinin-treated group (open circle).

administration of bradykinin (1 μg). Each animal was used only once.

2.3. Drugs

Streptozotocin was purchased from Sigma (St. Louis, MO), and calphostin C and phorbol-12, 13-dibutyrate were purchased from Calbiochem-Novabiochem International (San Diego, CA). Bradykinin and Hoe-140 were purchased from Research Biochemical International (Natick, MA). Calphostin C and phorbol-12, 13-dibutyrate were dissolved in 0.1% ethanol in saline. Hoe-140 was dissolved in saline. Calphostin C (0.3, 1 and 3 pmol) and phorbol-12, 13-dibutyrate (12.5, 25 and 50 pmol) were injected i.t. 60 min before the administration of bradykinin. Hoe-140 was injected i.t. 20 min before the administration of bradykinin. 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. Statistical analysis

Data are expressed as the means with S.E. 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. Bradykinin-induced thermal hyperalgesia

The basal tail-flick latency at a projection bulb voltage of 65 V was not different between diabetic (5.6 ± 0.1 s, $n = 80$) and non-diabetic mice (5.7 ± 0.1 s, $n = 110$). I.t. injection of higher dose of bradykinin (> 2 μg) produced marked nociceptive responses in diabetic and non-diabetic mice, which lasted more than 20 min. However, the nociceptive responses induced by 1 μg of bradykinin diminished within 10 min (data not shown). Thus, a dose of 1 μg was chosen for experiments designed to quantify the effect of bradykinin.

In non-diabetic mice, i.t. injection of bradykinin, at a dose of 1 μg, resulted in prolongation of the tail-flick latency, which diminished within 30 min after i.t. injection of bradykinin. Thereafter, the tail-flick latency was reduced. The reduction in the tail-flick latency reached its peak 120 min after injection, gradually declined and returned to the preinjection level 360 min after bradykinin injection (Fig. 1A). I.t. injection of Hoe-140 (30 and 100 ng), an antagonist of bradykinin B_2 receptors, dose-dependently and significantly antagonized the transient antinociceptive effect of i.t. bradykinin. Furthermore, the bradykinin-induced reduction in the tail-flick latency was dose-dependently and significantly antagonized when Hoe-140 (30 and 100 ng) was injected 20 min before the injection of bradykinin (Fig. 2).

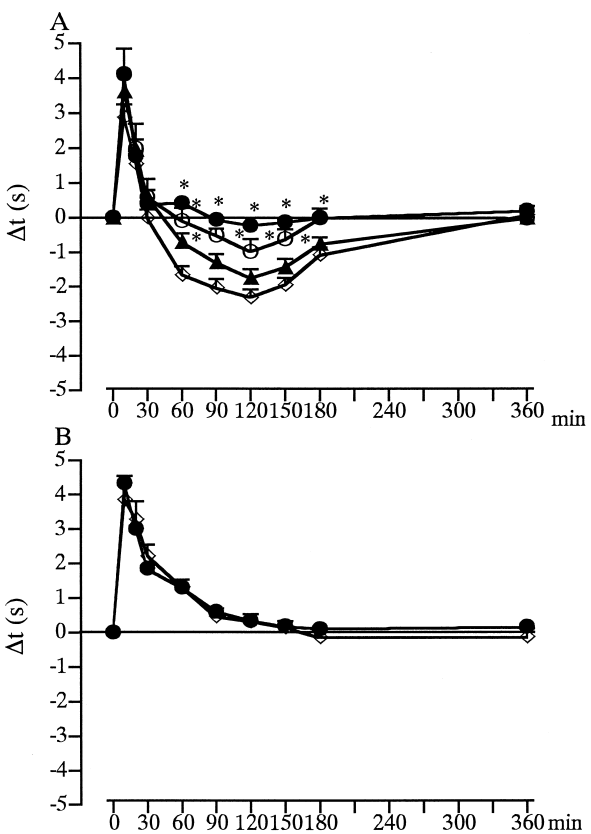

Fig. 3. Effects of phorbol-12, 13-dibutyrate on bradykinin-induced thermal hyperalgesia in non-diabetic (A) and diabetic (B) mice. Phorbol-12, 13-dibutyrate (12.5 pmol, closed triangle; 25 pmol, open circle; 50 pmol, closed circle) was injected i.t. 60 min before the injection of bradykinin (1.0 μg, i.t.). Δt (s) = post-drug latency − pre-drug latency. Each point represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ vs. vehicle + bradykinin-treated group (open diamond).

In diabetic mice, i.t. injection of bradykinin, at a dose of 1 μg, also resulted in prolongation of the tail-flick latency (Fig. 1B). The duration of the bradykinin-induced antinociceptive phase was longer in diabetic mice than in non-diabetic mice. Indeed, a significant prolongation of the tail-flick latency in diabetic mice was observed up to 60 min after i.t. injection of bradykinin, whereas the prolongation of the tail-flick latency in non-diabetic mice was diminished within 30 min after injection. However, there was no significant difference in the peak intensity of bradykinin-induced antinociception between diabetic and non-diabetic mice. On the other hand, the reduction in the tail-flick latency was not observed in diabetic mice when bradykinin was injected intrathecally (Fig. 1B).

3.2. Influence of a protein kinase C activator, phorbol-12, 13-dibutyrate, on bradykinin-induced thermal hyperalgesia

The effect of phorbol-12, 13-dibutyrate, a protein kinase C activator, on bradykinin-induced thermal hyperalgesia in diabetic and non-diabetic mice is shown in Fig. 3. In non-diabetic mice, i.t. pretreatment with phorbol-12, 13-dibutyrate (12.5, 25 and 50 pmol) 60 min before bradykinin injection dose-dependently attenuated bradykinin-induced thermal hyperalgesia. Indeed, a bradykinin-induced reduction in the tail-flick latency was not observed when mice were pretreated with phorbol-12, 13-dibutyrate (50 pmol, i.t.) (Fig. 3A). However, i.t. pretreatment with phorbol-12, 13-dibutyrate had no significant effect on the bradykinin-induced prolongation in the tail-flick latency in either non-diabetic or diabetic mice (Fig. 3A,B).

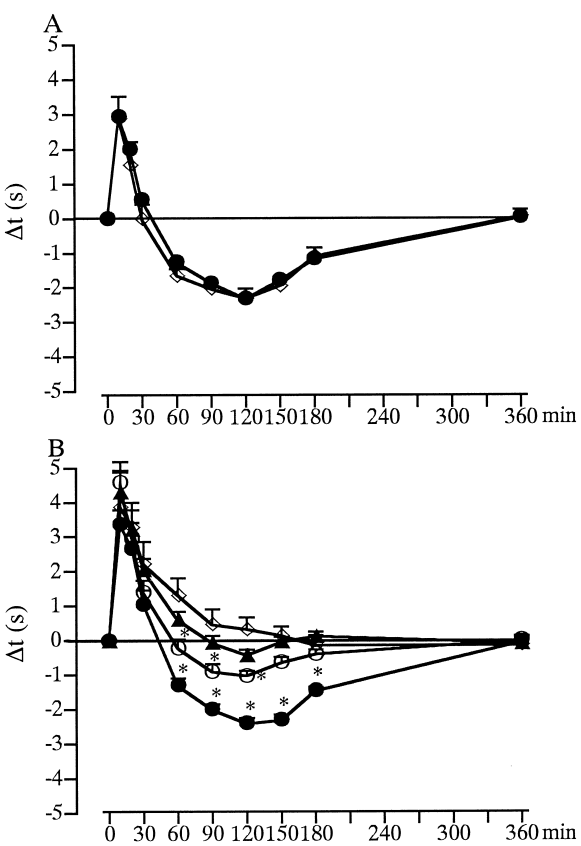

Fig. 4. Effects of calphostin C on bradykinin-induced thermal hyperalgesia in non-diabetic (A) and diabetic (B) mice. Calphostin C (0.3 pmol, closed triangle; 1 pmol, open circle; 3 pmol, closed circle) was injected i.t. 60 min before the injection of bradykinin (1.0 μg, i.t.). Δt (s) = post-drug latency − pre-drug latency. Each point represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ vs. vehicle + bradykinin-treated group (open diamond).

3.3. Influence of a protein kinase C inhibitor, calphostin C, on bradykinin-induced thermal hyperalgesia

The effect of calphostin C, a protein kinase C inhibitor, on bradykinin-induced thermal hyperalgesia in diabetic and non-diabetic mice is shown in Fig. 4. Neither bradykinin-induced antinociception nor hyperalgesia was affected by the i.t. administration of calphostin C (3 pmol) in non-diabetic mice (Fig. 4A). As shown in Fig. 4B, however, the duration, but not the peak intensity of the bradykinin-induced antinociceptive effect in diabetic mice was dose-dependently attenuated by i.t. pretreatment with calphostin C (0.3, 1 and 3 pmol). Furthermore, the tail-flick latency after bradykinin challenge was dose-dependently reduced by i.t. pretreatment with calphostin C (0.3–3 pmol) in diabetic mice (Fig. 4B). The time course and intensity of bradykinin-induced thermal hyperalgesia in calphostin C (3 pmol, i.t.)-treated diabetic mice were similar to those in non-diabetic mice.

4. Discussion

In the present study, i.t. bradykinin caused long-lasting thermal hyperalgesia in non-diabetic mice. Consistent with previous studies, the bradykinin-induced reduction in the tail-flick latency was dose-dependently and significantly antagonized by pretreatment with Hoe-140, a selective bradykinin B_2 receptor antagonist. On the other hand, bradykinin-induced thermal hyperalgesia was not observed in diabetic mice. These results are consistent with our previous finding that the bradykinin-induced nociceptive response was significantly less intense in diabetic mice than in non-diabetic mice (Ohsawa et al., 1998; Kamei et al., 1999). We recently reported that the second phase of the formalin-induced nociceptive response in non-diabetic mice was markedly reduced when non-diabetic mice were pretreated with phorbol-12, 13-dibutyrate (Ohsawa et al., 1998). Furthermore, when diabetic mice were pretreated i.t. with calphostin C, the second-phase nociceptive response was significantly increased to the level observed in non-diabetic mice (Ohsawa et al., 1998). The second phase of the formalin-induced nociceptive response may represent an enhanced response of sensitized dorsal horn neurons resulting from low-level neuronal input due to periph-

eral inflammatory insult (Hunskaar and Hole, 1987). The second phase, but not the first phase, of the formalin-induced nociceptive response was dose-dependently and significantly reduced by pretreatment with Hoe-140 (Ohsawa et al., 1998). Therefore, it seems likely that a lower sensitivity to bradykinin in processing nociception may be responsible, at least in part, for the reduction in the second phase of the formalin-induced nociceptive response in diabetic mice. Several studies have suggested that bradykinin induces strong thermal sensitization via bradykinin B_2 receptors, a phenomenon that can be attributed to protein kinase C activation (Cesare and McNaughton, 1996; Mizumura et al., 1997). 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). Thus, it is possible that the long-lasting activation of a diacylglycerol-protein kinase C cellular signal pathway occurs in diabetic animals. Cesare and McNaughton (1996) reported that application of the specific protein kinase C activator phorbol-12-myristate 13-acetate caused a long-lasting sensitization of the heat response in the dorsal root ganglion neurons, while further applications of phorbol-12-myristate 13-acetate had no effect. They also observed that staurosporine, which inhibits protein kinase C by competing for the ATP binding site, rapidly reversed the sensitization caused by phorbol-12-myristate 13-acetate. In the present study, we observed that bradykinin-induced thermal hyperalgesia in non-diabetic mice was diminished when the spinal protein kinase C was activated in readiness by i.t. pretreatment with a protein kinase C activator, phorbol-12, 13-dibutyrate. In contrast, in diabetic mice, bradykinin-induced thermal hyperalgesia appeared when mice were pretreated i.t. with a protein kinase C inhibitor, calphostin C. Thus, it is likely that the reduction in bradykinin-induced thermal hyperalgesia in diabetic and phorbol-12, 13-dibutyrate-treated non-diabetic mice results from the desensitization of sensory neurons to heat stimuli by long-lasting activation of protein kinase C.

On the other hand, i.t. injection of bradykinin resulted in prolongation of the tail-flick latency, which diminished within 30 min after i.t. injection of bradykinin in both non-diabetic mice and diabetic mice. Furthermore, this bradykinin-induced antinociception was dose-dependently and significantly antagonized by pretreatment with Hoe-140. These results were essentially equivalent to those reported by Laneuville and Couture (1987). We also observed that the duration of the bradykinin-induced antinociception in diabetic mice was significantly longer than that in non-diabetic mice. However, there was no significant difference between the intensity of the bradykinin-induced transient antinociceptive effect in non-diabetic and diabetic mice. Laneuville et al. (1989) reported that bradykinin-induced antinociception was unaffected by the prior i.t. administration of propranolol and naloxone, but was significantly potentiated by prazosin. In contrast, the response to bradykinin was significantly blocked by phentolamine, idazoxan and yohimbine as well as by 6-hydroxydopamine at a dose of 20 μg, i.t., 1 week earlier. A biochemical analysis revealed that treated with 6-hydroxydopamine reduced the noradrenaline content in the lumbar spinal cord by 60% without affecting the levels of serotonin, dopamine, adrenaline or their main metabolites (Laneuville et al., 1989). Based on these results, they suggested that bradykinin increases noradrenaline transmission in the spinal cord by a presynaptic action through a bradykinin B_2 receptor located on the terminals of bulbospinal noradrenaline-containing fibers (Laneuville et al., 1989). Furthermore, these results also indicated that noradrenaline may interact with a postsynaptic α_2-adrenoceptor (Laneuville et al., 1989). It has been reported that the release of noradrenaline is markedly suppressed in the brain and spinal cord of the diabetic mice (Kamei and Ohsawa, 1997; Bitar et al., 1999). Furthermore, Bitar et al. (1999) reported that the density and expression of mRNA encoding for α_2-adrenoceptor were also reduced in diabetic mice spinal cord. On the other hand, many investigators have indicated a direct link between noradrenaline release and protein kinase C activation (Allgaier et al., 1991; Schroeder et al., 1995; Kotsonis and Majewski, 1996; Wang and White, 1998). These results indicate that although bradykinin B_2 receptors are involved in bradykinin-induced transient antinociception, the long-lasting activation of a protein kinase C-mediated cellular signal pathway in diabetic mice does not influence this antinociception. Thus, it is possible that the enhancement of the duration of bradykinin-induced antinociception in diabetic mice may result from the secondary effect in response to the desensitization of sensory neurons to heat stimuli by the long-lasting activation of protein kinase C.

We conclude that the change in bradykinin-induced thermal hyperalgesia in diabetic mice may be due, at least in part, to the modification of nociceptive transmission in the spinal cord by the activation of protein kinase C.

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