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Possible involvement of spinal protein kinase C in thermal allodynia and hyperalgesia 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 142-8501, Japan

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Abstract

We examined the tail-flick response to various heat intensities in diabetic and non-diabetic mice. Heat intensities were set to one of five values by adjusting the source voltage of a 50-W projection bulb to 25, 35, 50, 65 and 80 V. These heat intensities produced surface skin heating rates of 0.1, 0.4, 0.9, 3.0 and 7.3°C/s, respectively. Tail-flick latencies at source voltages of 35 and 50 V in diabetic mice were significantly shorter than those in non-diabetic mice. However, there were no significant differences in tail-flick latencies at 25, 65 and 80 V. In non-diabetic mice, tail-flick latencies were not affected by intrathecal (i.t.) pretreatment with capsaicin 24 h before testing. Tail-flick latencies at 35 and 50 V in diabetic mice were increased by pretreatment with capsaicin. Moreover, although tail-flick latencies in non-diabetic mice were not affected by i.t. pretreatment with calphostin C, a selective protein kinase C inhibitor, those at 35 and 50 V in diabetic mice were increased. However, i.t. pretreatment with (8*R*, 9*S*, 11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2, 3, 9, 10-tetrahydro-8, 11-epoxy-1*H*, 8*H*, 11*H*-2, 7*b*, 11*a*-triazadibenzo [*a*, *g*]cycloocta[*cde*]-trinden-1-one (KT5720), a selective protein kinase A inhibitor, did not affect tail-flick latencies in either diabetic or non-diabetic mice. In non-diabetic mice, i.t. pretreatment with phorbol 12,13-dibutyrate (PDB), a protein kinase C activator, decreased tail-flick latencies at 35 and 50 V. Tail-flick latencies in diabetic mice were not affected by i.t. pretreatment with PDB 60 min before testing. Furthermore, the attenuation of tail-flick latencies induced by i.t. pretreatment with PDB in non-diabetic mice was reversed by i.t. pretreatment with capsaicin 24 h before testing. These results indicate that diabetic mice exhibit thermal allodynia and hyperalgesia. Furthermore, this thermal allodynia and hyperalgesia in diabetic mice may be due to the enhanced release of substance P followed by activation of protein kinase C in the spinal cord.

Keywords: Diabetes; Protein kinase C; Allodynia; Hyperalgesia; Substance P; (Mouse)

1. Introduction

Diabetic neuropathy accompanied by anomalies in pain perception is one of the most frequent complications of insulin-dependent diabetes in humans (Guy et al., 1985; Le Quesne and Fowler, 1986; Ziegler et al., 1988; Watkins, 1990). Many clinical and experimental studies have suggested that diabetes or hyperglycemia alters pain sensitivity (Morley et al., 1984). In humans, diabetic neuropathy can be associated with burning tactile hypersensitivity (Bays and Pfeifer, 1988). Behavioral reactions of hyperalgesia in animal models of diabetes have been described previously (Forman et al., 1986; Lee and McCarty, 1990; Kamei et al., 1991; Courteix et al., 1993). However, the etiology of these disturbances is still unknown, although metabolic factors such as hyperglycemia (Greene et al., 1987), neuronal loss (Dyck et al., 1985; Saïd et al., 1992) or neurotransmitter alteration (Bitar et al., 1985; Chu et al., 1986; Trulson et al., 1986; Masiello et al., 1987; Lackovick and Salkovic, 1990; Bellush and Reid, 1991) may be involved.

Activation of protein kinase C has been implicated in changes in pain perception. Phorbol esters, which activate protein kinase C, enhance the number of electrical impulses of knee joint afferents in response to passive joint movement (Schepelmann et al., 1993) and enhance nociceptive responses after tissue injury induced by formalin (Coderre, 1992). In addition, noxious thermal and mechanical stimuli increase the activation of protein kinase C in

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

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the dorsal horn of the spinal cord (Mao et al., 1992; Yashpal et al., 1995). Furthermore, activation of protein kinase C increases the release of substance P from rat sensory neurons (Barber and Vasko, 1996). In addition, activation of protein kinase C by phorbol esters or intrathecal injection of protein kinase C increases the release of excitatory amino acids in dorsal horn slices (Gerber et al., 1989). Thus, the protein kinase C-induced enhancement of neurotransmitter release may underlie the neuronal sensitization that produces hyperalgesia.

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 has been linked to vasculature dysfunction in diabetes (Craven and De Rubertis, 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. Moreover, we recently reported that calphostin C, a protein kinase C inhibitor, reversed the attenuation of [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO)-induced antinociception in diabetic mice to the level in non-diabetic mice (Ohsawa and Kamei, 1997). These results suggest that increased protein kinase C activity in diabetic mice might alter the transmission of pain in the spinal cord. Thus, in the present study, we investigated the role of protein kinase C in thermal hyperalgesia and allodynia in diabetic mice.

2. 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 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 N citrate buffer at pH 4.5. Age-matched non-diabetic 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 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, Sports and Culture.

2.2. Assessment of the nociceptive response

The nociceptive response was evaluated by recording the latency to withdrawal of the tail in response to several different rates of noxious skin heating. Briefly, the tails of mice were exposed to a focused beam of light from a 50-W projection bulb. The heat intensity was set to one of five values by adjusting the source voltage of the bulb from 25 to 80 V. When a withdrawal response occurred, the stimulus was terminated and the response latency was measured electronically. In the absence of a response up to a predetermined maximum latency (30 s), the trial was terminated to prevent tissue damage. All measurements were performed by an investigator who was unaware of the treatment group of individual animals. Skin temperature changes during heating were measured using a focused infrared thermometer (Horiba, Kyoto, Japan) aimed at the cutaneous receiving field.

2.3. Intrathecal (i.t.) injection

I.t. administration was performed following the method described by Hylden and Wilcox (1980). Each i.t. injection was administered using a 30-gauge needle directly through the intact skin between the L5 and L6 vertebrae. This site combines the best intervertebral accessibility with the least possibility of spinal damage. (Hylden and Wilcox, 1980). Drugs were given in a volume of 5 μl/mouse.

2.4. Drugs

Streptozotocin was purchased from Sigma (St. Louis, MO). Calphostin C, phorbol 12,13-dibutyrate (PDB) and (8*R*, 9*S*, 11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2, 3, 9, 10-tetrahydro-8, 11-epoxy-1*H*, 8*H*, 11*H*-2,

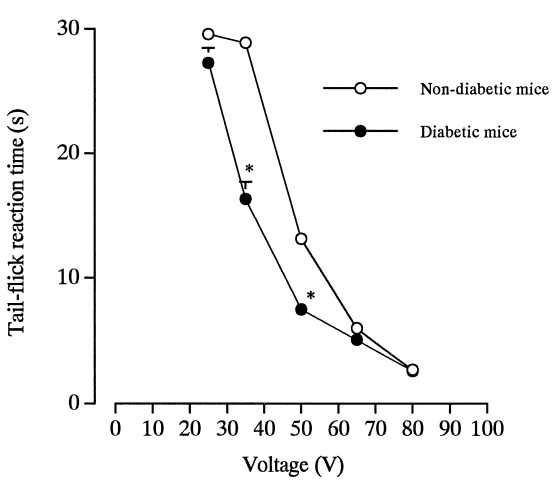

Fig. 1. Tail-flick latency at various intensities of heating in diabetic (closed circle) and non-diabetic (open circle) mice. Each point represents the mean with S.E. for 10 mice in each group. $^*P < 0.05$ compared with the respective non-diabetic mice. The error bars of the non-diabetic mice are obscured by the symbol.

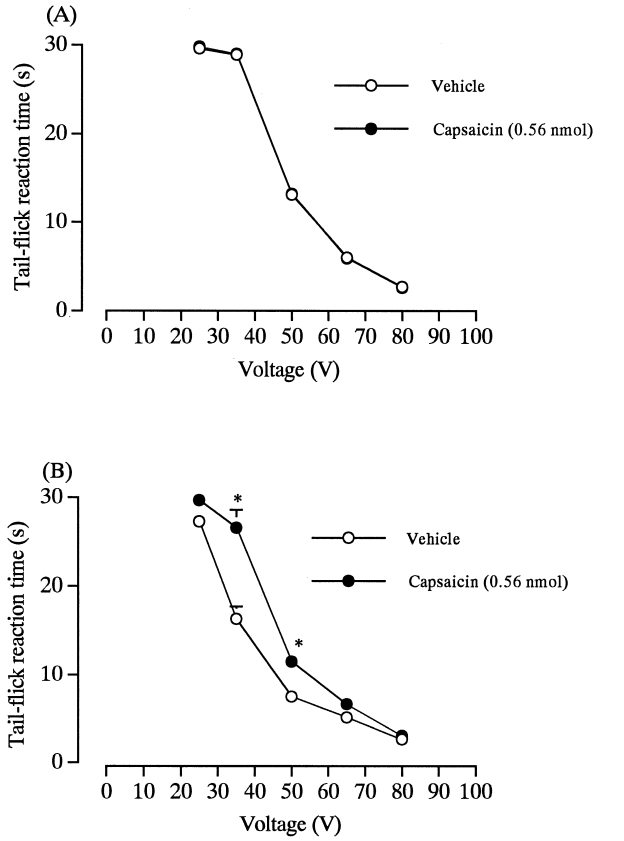

Fig. 2. Effect of capsaicin on the tail-flick latency at various intensities of heating in non-diabetic (A) and diabetic (B) mice. Capsaicin (0.56 nmol, closed circle) and its vehicle (open circle) were injected i.t. 24 h before testing. Each point represents the mean with S.E. for 10 mice in each group. In A, the mean values for the capsaicin-treated animals overlie the values for the vehicle-treated animals. The error bars of several points are obscured by the symbol. $^{*}P < 0.05$ compared with the respective vehicle-treated group.

7*b*, 11*a*-triazadibenzo [*a*, *g*]cycloocta[*cde*]-trinden-1-one (KT5720) were purchased from Calbiochem-Novabiochem International (San Diego, CA). Capsaicin was dissolved in 10% ethanol and Tween 80 in saline (0.9% NaCl). The solution was diluted with saline. Calphostin C, PDB and KT5720 were dissolved in 0.1% ethanol in saline. Capsaicin (0.56 nmol) was injected i.t. 24 h before testing. This schedule and dose for capsaicin reportedly does not affect the content of substance P in normal mice (Goettl et al., 1997). Calphostin C (1 and 3 pmol), KT5720 (10 pmol) and PDB (50 and 100 pmol) were injected i.t. 60 min before testing. The dose and schedule for calphostin C, PDB and KT5720 in this study were determined as described previously (Ohsawa and Kamei, 1997; Narita et al., 1997a,b).

2.5. Data analysis

The data are expressed as means ± 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 (comparison among multiple groups).

3. Results

3.1. Tail-flick latency at various intensities of heating in diabetic and non-diabetic mice

As shown in Fig. 1, in non-diabetic mice, voltages of 25 and 35 V did not cause a tail-flick response within the 30-s limit. However, when the voltage of the bulb was increased to 50 V, the mean tail-flick latency was significantly less than 30 s. Furthermore, when the voltage was increased to 65 and 80 V, the mean tail-flick latency decreased to approximately 3 and 2.2 s, respectively. In diabetic mice, tail-flick latencies were shorter than those in non-diabetic mice at bulb voltages of 35 and 50 V (Fig. 1). However, there were no differences in the tail-flick latencies at 25, 60 and 80 V between diabetic and non-diabetic mice (Fig. 1). The heat intensities at 25, 35, 50, 65 and 80 V produced surface skin heating rates of 0.1, 0.4, 0.9, 3.0 and 7.3°C/s, respectively.

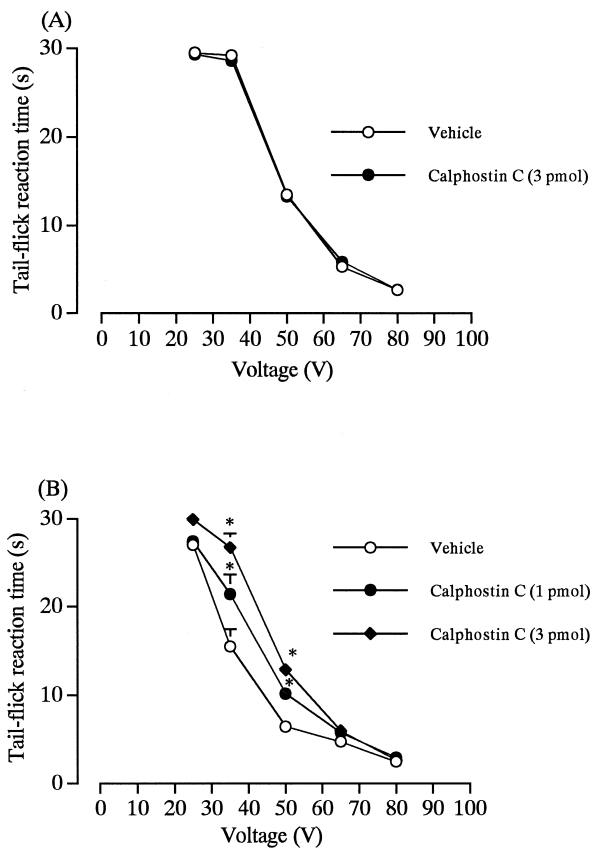

Fig. 3. Effect of calphostin C on the tail-flick latency at various intensities of heating in non-diabetic (A) and diabetic (B) mice. Calphostin C (1 or 3 pmol, closed symbol) and its vehicle (open circle) were injected i.t. 60 min before testing. Each point represents the mean with S.E. for 10 mice in each group. The error bars of several points are obscured by the symbol. $^{*}P < 0.05$ compared with the respective vehicle-treated group.

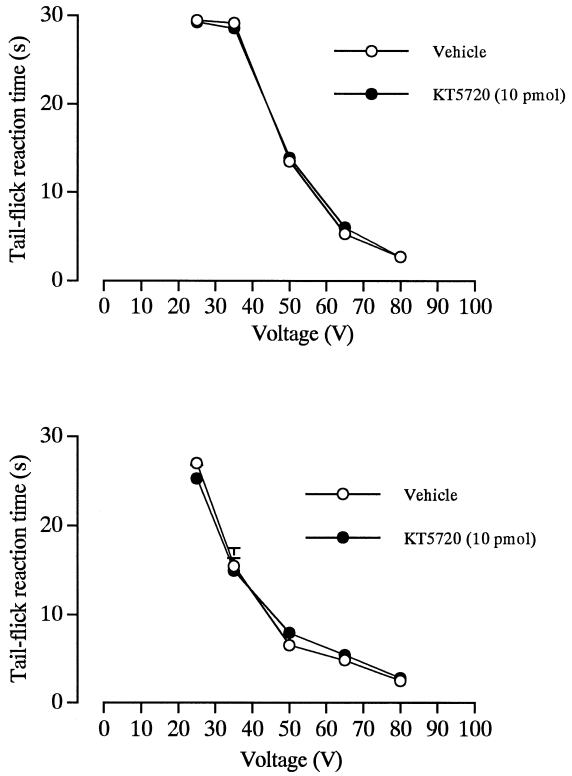

Fig. 4. Effect of KT5720 on the tail-flick latency at various intensities of heating in non-diabetic (A) and diabetic (B) mice. KT5720 (10 pmol, closed circle) and its vehicle (open circle) were administered i.t. 60 min before testing. Each point represents the mean with S.E. for 10 mice in each group. The error bars of several points are obscured by the symbol.

Pretreatment with glucose (30 mmol/kg, i.p.) increased the serum glucose levels of non-diabetic mice (493.7 ± 20.2 mg/dl, $n = 25$) to the level of diabetic mice. However, tail-flick latencies at various intensities of skin heating in non-diabetic mice were not affected by pretreatment with glucose (without glucose; 25 V, 29.34 ± 0.42 s; 35 V, 29.30 ± 0.51; 50 V, 14.58 ± 0.82 s; 65 V, 7.57 ± 0.65 s; 80 V, 3.01 ± 0.46 s; with glucose; 25 V, 29.80 ± 0.20 s; 35 V, 29.88 ± 0.12; 50 V, 14.58 ± 0.87 s; 65 V, 7.53 ± 1.70 s; 80 V, 3.28 ± 0.71 s).

3.2. Effects of capsaicin on the tail-flick latency at various intensities of heating in diabetic and non-diabetic mice

The effects of capsaicin on the tail-flick latency in diabetic and non-diabetic mice are shown in Fig. 2. Pretreatment with vehicle did not affect the tail-flick latencies at various rates of skin heating in non-diabetic mice. Moreover, pretreatment with vehicle also did not affect the tail-flick latencies at various rates of skin heating in diabetic mice. In non-diabetic mice, tail-flick latencies were not affected by pretreatment with capsaicin 24 h beforehand (Fig. 2A). However, as shown in Fig. 2B, tail-flick latencies at 35 and 50 V in diabetic mice significantly increased when capsaicin (0.56 nmol, i.t.) was administered 24 h before testing.

3.3. Effects of a protein kinase C inhibitor, calphostin C and a protein kinase A inhibitor, KT5720, on the tail-flick latency at various intensities of heating in diabetic and non-diabetic mice

The effects of a protein kinase C inhibitor, calphostin C, on the tail-flick latency in diabetic and non-diabetic mice is shown in Fig. 3. I.t. pretreatment with vehicle did not affect tail-flick latencies at various rates of skin heating in non-diabetic mice. Moreover, i.t. pretreatment with vehicle also did not affect the tail-flick latencies of various rates of skin heating in diabetic mice. I.t. pretreatment with calphostin C (3 pmol) did not affect the tail-flick latencies at various intensities of heating in non-diabetic mice (Fig. 3A). In contrast, as shown in Fig. 3B, tail-flick latencies at 35 and 50 V in diabetic mice were dose dependently increased by i.t. pretreatment with calphostin C (1 and 3 pmol).

As shown in Fig. 4A and B, i.t. pretreatment with KT5720 (10 pmol), a selective protein kinase A inhibitor, did not affect the voltage-dependence of tail-flick latencies in either diabetic or non-diabetic mice.

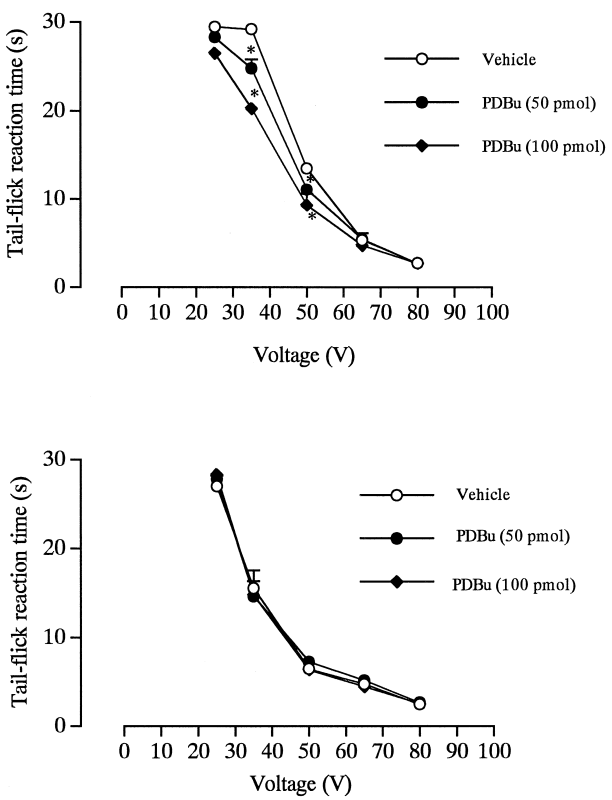

Fig. 5. Effect of PDB on the tail-flick latency at various *intensities* of heating in non-diabetic (A) and diabetic (B) mice. PDB (50 or 100 pmol, closed symbol) and its vehicle (open circle) were injected i.t. 60 min before testing. Each point represents the mean with S.E. for 10 mice in each group. The error bars of several points are obscured by the symbol. $^{*}P < 0.05$ compared with each vehicle-treated group.

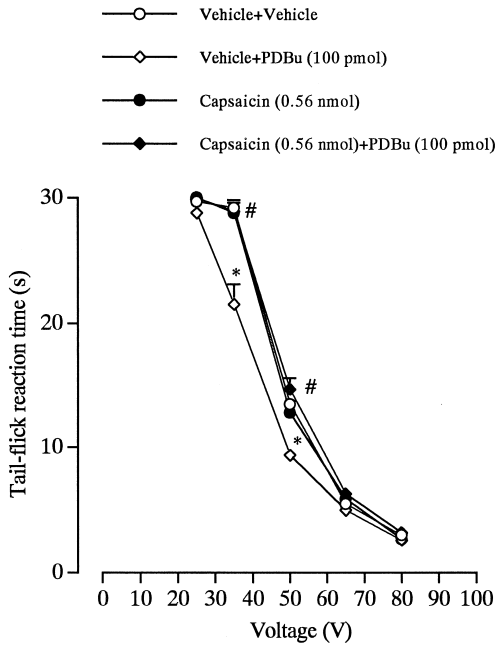

Fig. 6. Effect of capsaicin on the PDB-induced attenuation of the tail-flick latency at various intensities of heating in non-diabetic mice. Capsaicin (0.56 nmol, closed symbol) and its vehicle (open symbol) were injected i.t. 24 h before testing. PDB (100 pmol, diamond) and its vehicle were (circle) injected i.t. 60 min before testing. Each point represents the mean with S.E. for 10 mice in each group. The error bars of several points are obscured by the symbol. $^{*}P < 0.05$ compared with the vehicle-pretreated vehicle (open circle) group. The sharp denotes the significant differences between vehicle pretreated PDB (open diamond) and capsaicin pretreated PDB (closed diamond) groups ($\#P < 0.05$).

3.4. Effects of a protein kinase C activator, PDB on the tail-flick latency at various intensities of heating in diabetic and non-diabetic mice

The effects of a protein kinase C activator, PDB on the tail-flick latency in diabetic and non-diabetic mice are shown in Fig. 5. In non-diabetic mice, i.t. pretreatment with PDB (50 and 100 pmol) dose dependently reduced the tail-flick latencies at 35 and 50 V (Fig. 5A). However, the tail-flick latencies in diabetic mice were not affected by i.t. pretreatment with PDB (50 and 100 pmol; Fig. 5B).

Furthermore, the PDB-induced reduction in the tail-flick latency at 35 and 50 V in non-diabetic mice was reversed when capsaicin (0.56 nmol, i.t.) was administered 24 h before testing (Fig. 6).

4. Discussion

In the present study, diabetic mice showed thermal hyperalgesia and allodynia in the tail-flick test. In non-diabetic mice, the heat intensity at bulb voltages of 65 and 80 V evoked a rapid tail-flick response, whereas that at 50 V evoked an intermediate tail-flick latency, and those at 25 and 35 V evoked no tail-flick response. In diabetic mice, the tail-flick latency after heating the tail at 50 V was significantly shorter than that in non-diabetic mice, indicating that diabetic mice exhibit thermal hyperalgesia. In addition, a lower voltage bulb (35 V), which did not evoke a tail-flick response in non-diabetic mice, did evoke a tail-flick response in diabetic mice. It has been reported that low rates of skin heating in the range 1–2°C/s activate C-fiber nociceptors, but rarely activate A-fiber nociceptors in the rat saphenous nerve (Kenins, 1982; Steen et al., 1992). Furthermore, Yeomans and Proudfit (1996) and Yeomans et al. (1996) have reported that heating the hind paw skin of the rat at a relatively high rate of 6.5°C/s activates Aδ-fibers, whereas heating at a low rate of 0.9°C/s activates C-fibers. In the present study, the heat intensities at source voltages of 25, 35, 50, 65 and 80 V produced surface skin heating rates of 0.1, 0.4, 0.9, 3.0 and 7.3°C/s, respectively. Thus, it is possible that the highest two voltages, i.e., 65 and 80 V, are associated with rapid temperature increases that more selectively and synchronously activate Aδ-fibers while the middle two voltages, i.e., 35 and 50 V, may more selectively activate C-fibers. Therefore, it has been suggested that diabetic mice show a selective change in C-fibers, but not in Aδ-fibers (Kamei et al., 1991). In the present study, the heat intensity at a source voltage of 25 V did not produce a tail-flick response in either diabetic or non-diabetic mice. Indeed, the tail surface temperature at a bulb voltage of 25 V was 34°C, whereas that needed to produce a tail-flick response was above 43.6 and 35.4°C in non-diabetic and diabetic mice, respectively. Therefore, it is likely that the lowest voltage is lower than the stimulus strength needed to produce a tail-flick response in diabetic and non-diabetic mice. Furthermore, the heat intensity at a bulb voltage of 35 V, which did not produce a tail-flick response in non-diabetic mice, produced a tail-flick response in diabetic mice. The tail surface temperature at a bulb voltage of 35 V was 38.8°C, which can produce a tail-flick response in diabetic mice, but not in non-diabetic mice. Thus, it is possible that diabetic mice exhibit thermal allodynia. It has been reported that experimental insulin-dependent diabetes mellitus in rats (streptozotocin-induced diabetic rats) disturbs the responses to both nociceptive (thermal, mechanical and chemical) and non-nociceptive (thermal and mechanical) stimuli (Courteix et al., 1993). Furthermore, we previously reported that streptozotocin-induced diabetic mice show a selective change in the nociceptive threshold with regard to noxious mechanical stimuli (Kamei et al., 1991). These studies and the present data suggest that diabetes mellitus in animals is associated with thermal hyperalgesia and allodynia.

In the present study, streptozotocin-induced diabetic mice showed a decrease in the thermal nociceptive threshold, as determined by the application of noxious and non-noxious thermal stimuli. Furthermore, pretreatment with capsaicin (0.56 nmol, i.t.) 24 h beforehand, which did not increase the tail-flick latency in non-diabetic mice,

increased the tail-flick latency in diabetic mice. It has been reported that pretreatment with capsaicin decreases the content and release of substance P from primary afferent fibers (Gamse, 1982; Goettl et al., 1997). We previously reported that streptozotocin-induced diabetic mice show a selective change in their neuronal system that involves substance P in the spinal cord (Kamei et al., 1991). Moreover, we suggested that the release of excessive amounts of substance P from the spinal cord may be associated with abnormalities in nociceptive transmission in mice with diabetes (Kamei et al., 1991). It has also been suggested that the release of substance P from primary afferent terminals may mediate nociception evoked by low-rate heating but not high-rate heating (Zachariou et al., 1997). Therefore, it seems likely that the thermal allodynia and hyperalgesia seen in diabetic mice may be due to enhancement of the release of substance P from primary afferent fibers in the spinal cord.

In the present study, tail-flick latencies after heating at 35 and 50 V in diabetic mice were increased by i.t. pretreatment with a protein kinase C inhibitor, calphostin C, but not with a protein kinase A inhibitor, KT5720. Furthermore, in non-diabetic mice, tail-flick latencies were not affected by pretreatment with either a protein kinase C inhibitor or a protein kinase A inhibitor. Recently, Hayes et al. (1992) reported that treatment with GM1 gangliosides, which inhibit the translocation and activation of protein kinase C (Vaccarino et al., 1987), attenuated thermal hyperalgesia in a model of peripheral nerve injury. They also suggested that these changes may be related to an increase in spinal cord membrane-bound protein kinase C in this model of persistent pain (Hayes et al., 1992; Mao et al., 1992). It has been reported that persistent nociceptive behavior in rats induced by the subcutaneous injection of formalin is significantly reduced by i.t. pretreatment with an inhibitor of protein kinase C (1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H-7)) and is significantly enhanced by a phorbol ester (phorbol 12-myristate 13-acetate) and a stimulator of protein kinase C (*N*-(*n*-heptyl)-5-chloro-1-naphthalenesulfonamide (SC-10)), but not by a stimulator of protein kinase A (forskolin) (Coderre, 1992). It has been reported that the selectivity of KT5720 for protein kinase A is >36-fold greater than that for protein kinase C, protein kinase G and myosin light chain kinase. Furthermore, the selectivity of calphostin C for protein kinase C is >1000, >50 and >100-fold greater than that for protein kinase A, protein kinase G and myosin light chain kinase (Kobayashi et al., 1989). In the present study, tail-flick latencies at 35 and 50 V were decreased by i.t. pretreatment with a protein kinase C activator, PDB, in non-diabetic mice, but not in diabetic mice. Thus, it seems likely that thermal hyperalgesia and allodynia may be due to enhancement of the activation of protein kinase C in the spinal cord. Moreover, it has been recently reported that the mechanical hyperalgesia and C-fiber hyperactivity in response to mechanical stimuli seen in streptozotocin-induced diabetic rats are reduced by agents that inhibit protein kinase C (Ahlgren and Levine, 1994). Thus, these results suggest that thermal hyperalgesia and allodynia in diabetic mice may be due to the activation of protein kinase C in the spinal cord.

In this study, thermal hyperalgesia and allodynia were caused by i.t. pretreatment with a protein kinase C activator, PDB. The neuronal mechanisms which underlie PDB-induced thermal hyperalgesia and allodynia are unclear. Schepelmann et al. (1993) demonstrated that intra-arterially applied PDB increases the response of knee joint afferents to mechanical stimuli. In this study, we observed that the PDB-induced attenuation of tail-flick latencies at voltages of 35 and 50 V was reversed by i.t. pretreatment with capsaicin. Recently, it was reported that exposing rat sensory neurons in culture to PDB significantly increased substance P release (Barber and Vasko, 1996). Therefore, it seems likely that PDB-induced thermal hyperalgesia and allodynia may be mediated by the activation of substance P-containing neurons. It has recently been reported that capsaicin decreases the content and release of substance P from primary afferent fibers (Goettl et al., 1997). In addition to decreasing the content and release of substance P, capsaicin may also affect the release of excitatory amino acids. Capsaicin has been shown to induce an immediate release of glutamate and aspartate into the extracellular fluid of the dorsal spinal cord of free-moving unanesthetized rats (Skilling et al., 1990; Smullin et al., 1990). Moreover, it has been suggested that long-term treatment with capsaicin may decrease the release or action of excitatory amino acids (Goettl et al., 1997). Gerber et al. (1989) demonstrated that phorbol esters enhance the depolarization-evoked release of excitatory amino acids and increase the amplitude and duration of the depolarization produced by *N*-methyl-D-aspartate (NMDA) and glutamate. It has been reported that protein kinase C can increase the effectiveness of NMDA receptors by altering the magnesium block (Chen and Huang, 1992). Therefore, these results suggest that PDB-induced thermal hyperalgesia and allodynia may be due to the enhancement of NMDA receptors, following the activation of protein kinase C. However, Barber and Vasko (1996) reported that exposing rat sensory neurons in culture to PDB significantly increased substance P release. Moreover, the NMDA receptor is present in axon terminals in the spinal cord dorsal horn (Liu et al., 1994). Furthermore, Liu et al. (1997) indicated that tachykinin NK_1 receptor antagonists or neonatal capsaicin treatment, which severely damages C-fibers, blocked the pain behavior induced by the i.t. administration of NMDA. This block, however, was only partial ($\sim 50\%$). Electron microscopic and light microscopic analysis of substance P receptor-immunoreactive fibers showed that substance P-induced internalization of the receptor, which is indicative of substance P release, was prevented by selective substance P antagonists or by neonatal capsaicin treatment. Therefore, they suggested

that if NMDA receptor activation occurred only postsynaptically, there would not be any substance P component, unless a synaptic feedback loop existed or, alternatively, if NMDA directly excited primary afferent C-fibers (Liu et al., 1997). Thus, it seems likely that nociceptive transmission may be influenced by the activation of either pre- or post-synaptic NMDA receptors at the first central synapse for primary afferents in the dorsal horn. In the present study, PDB-induced thermal hyperalgesia and allodynia were blocked by i.t. pretreatment with capsaicin. Furthermore, we previously reported that the release of excessive amounts of substance P from the spinal cord was associated with the abnormalities in nociceptive transmission in mice with diabetes (Kamei et al., 1991). In light of these results, it seems likely that PDB-induced thermal hyperalgesia and allodynia may be due to the excessive release of substance P from the spinal cord. In addition, the excessive release of substance P is mediated by the activation of protein kinase C.

In conclusion, activation of protein kinase C in the spinal cord caused thermal hyperalgesia and allodynia in diabetic mice. Furthermore, the activation of protein kinase C leads to increased substance P release, which leads to thermal hyperalgesia and allodynia.

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