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Antinociceptive effect of U-50488H, a κ-opioid agonist, in streptozotocin-induced diabetic mice

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Abstract

We compared the antinociceptive activity of a κ-opioid agonist, U-50488H, in streptozotocin-induced diabetic mice with that in non-diabetic mice. Subcutaneously administered U-50488H (3 and 10 mg kg^{-1}) showed a more potent antinociceptive effect, as evaluated by the tail-pressure method, in diabetic mice than in non-diabetic mice. Increased antinociceptive activity of U-50488H observed in diabetic mice was also observed in mice given U-50488H intrathecally (3 and 10 μg). However, there were no differences observed between diabetic and non-diabetic mice given U-50488H intracerebroventricularly (3 and 10 μg). Although the antinociceptive effect of U-50488H (3 mg kg^{-1}, s.c.) in non-diabetic mice was increased by treatment with PD135158 (100 ng, i.c.v.), a cholecystokinin$_B$ (CCK_B) antagonist, the antinociceptive activity of U-50488H which was enhanced in diabetic mice was not influenced by PD135158. Moreover, the increased antinociceptive activity of U-50488H (3 mg kg^{-1}, s.c.) in diabetic mice diminished when desulfated octapeptide of cholecystokinin (3–100 ng, i.c.v.), a CCK_B agonist, was administered. These results suggested that diabetic mice were selectively hyper-responsive to spinal κ-opioid receptor-mediated antinociception. The function of the analgesia inhibitory system in which cholecystokinin is used as a transmitter might be diminished in diabetic mice.

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Introduction

Neuropathy is typical of diabetic complications. Diabetic neuropathy can be associated with tactile hypersensitivity or a burning sensation. Since hyperalgesia and resistance to the antinociceptive activity of morphine in streptozotocin (STZ)-induced or spontaneously diabetic rodents was recently clarified, attention is currently being focused on the experimental models of painful diabetic neuropathy (Lee & McCarty 1990; Kamei et al 1991; Simon & Dewey 1981; Ahlgren & Levine 1993; Courteix et al 1994). Animals with diabetes show a reduced threshold for pain perception, especially in response to noxious mechanical stimuli (Kamei et al 1991; Ahlgren & Levine 1993).

Evidence of altered opioid receptor function in diabetic animals has been accumulated. We had reported that the antinociceptive effects of intracerebroventricular, but not intrathecal, administration of μ-opioid-receptor agonists, such as morphine and [D-Ala^2, *N*-Me-Phe^4, Gly-ol^5] enkephalin (DAMGO), in STZ-induced diabetic mice were markedly less than those in non-diabetic mice (Kamei et al 1992a). These findings indicate that diabetic mice are selectively hypo-responsive to supraspinal μ_1-opioid-receptor-mediated antinociception due to certain factor(s) derived from spleen cells (Kamei et al 1992b, 1994a, b). We also

demonstrated that the supraspinal δ_1-opioid-receptor-mediated antinociceptive system is enhanced in diabetic mice, compared with that in non-diabetic mice (Kamei et al 1994c). Recently, we found that an antinociceptive effect of processed *Aconiti* tuber, a crude drug that induces analgesia by releasing dynorphin, an endogenous κ-opioid ligand, in the spinal cord increased in diabetic mice (Suzuki et al 1999). Thus, in this study, to clarify the possibility of alteration in κ-opioid receptor function in diabetes, the antinociceptive activity of U-50488H, a κ-opioid agonist, in STZ-induced diabetic mice was compared with that in non-diabetic mice.

Materials and Methods

Induction of diabetes mellitus

Male ddY mice (Japan SLC, Shizuoka) were used, initially weighing 20–25 g. Mice had free access to solid food and water in an animal room maintained at 22 ± 2°C with a 12-h light–dark cycle. Evaluation using diabetic mice was initiated 2 weeks after intravenous administration of STZ (150 mg kg^{-1}), which was dissolved in 33.3 mM citrate buffer solution (pH 4.5). Age-matched control mice were given only vehicle. Mice with blood glucose levels above 400 mg dL^{-1} were considered diabetic.

Drugs

U-50488H (Research Biochemicals International; RBI, Natick, MA) and DAMGO (Sigma Chemical, St Louis, MO), [D-Pen^{2,5}]enkephalin (DPDPE; RBI) and nor-binaltorphimine (nor-BNI; RBI) were dissolved in saline. The subcutaneous injections of U-50488H or saline were given in a volume equivalent to 10 mL kg^{-1}. Nor-BNI was injected subcutaneously 2 h before treatment with U-50488H. The intracerebroventricular and intrathecal injections were performed according to the methods of Haley & McCormick (1957) and Hylden & Wilcox (1980), respectively. U-50488H, DAMGO, DPDPE or saline was administered in a volume of 5 μL for both injections. PD135158 (RBI) and desulfated octapeptide of cholecystokinin (des-CCK8; Sigma) were dissolved in 5 μL saline and injected intracerebroventricularly 20 and 15 min after the U-50488H treatment, respectively.

Measurement of antinociceptive response

The antinociceptive response was evaluated by the tail-pressure method. Noxious mechanical stimulation was applied to the root of the tail using an analgesimeter (Ugo Basile, Milan, Italy) with a wedge-shaped piston at a loading rate of 16 g s^{-1}. The antinociceptive activity was evaluated every 0.5 h up to 3 h (except 2.5 h) after the subcutaneous injections of U-50488H. The nociceptive threshold after the intrathecal or intracerebroventricular injections of opioid agonists was evaluated every 10 min up to 30 min and every 15 min up to 1 h.

Data analysis

The antinociceptive effect was expressed as the area under the time–response curve (AUC) calculated by plotting the increase in threshold ($\triangle$g) from the value at 0 min on the ordinate and time interval (h) on the abscissa. The results are expressed as means $\pm$ s.e.m. Significance was determined using one-way analysis of variance followed by Student's *t*-test or Dunnett's *t*-test. In all cases, differences of $P < 0.05$ were considered significant.

Results

Antinociceptive effect of opioid agonists

Diabetic mice had lower nociceptive threshold values than non-diabetic mice in the tail-pressure test (non-diabetic mice, 104 ± 0.3 g, n = 116; diabetic mice, 72.9 ± 0.2 g, n = 120; $P < 0.01$). U-50488H (3 and 10 mg kg^{-1}, s.c.) showed more prominent antinociceptive activity in STZ-induced diabetic mice than in non-diabetic mice (Figure 1). The antinociceptive effects observed after administering U-50488H, DAMGO or DPDPE by intracerebroventricular or intrathecal injection in diabetic and non-diabetic mice are shown in Figure 2. When U-50488H (3 and 10 μg) was administered by intracerebroventricular injection, antinociceptive activity was not increased in diabetic mice. In contrast, U-50488H administered by intrathecal injection showed a more potent antinociceptive effect in diabetic mice than in non-diabetic mice. The antinociceptive effect of U-50488H at the high dose in each experiment was completely eradicated by pretreatment with nor-BNI (10 mg kg^{-1}, s.c.), a highly selective κ-opioid antagonist (data not shown).

Decreased antinociceptive activity of a μ-opioid agonist, DAMGO (0.1 μg, i.c.v.), and increased antinociceptive activity of a δ-opioid agonist, DPDPE (1 μg, i.c.v.), were observed in the tail-pressure test in diabetic mice. However, there were no differences in the antinociceptive effects of DAMGO (2 μg) or DPDPE (0.1 μg) administered by intrathecal injection between diabetic and non-diabetic mice.

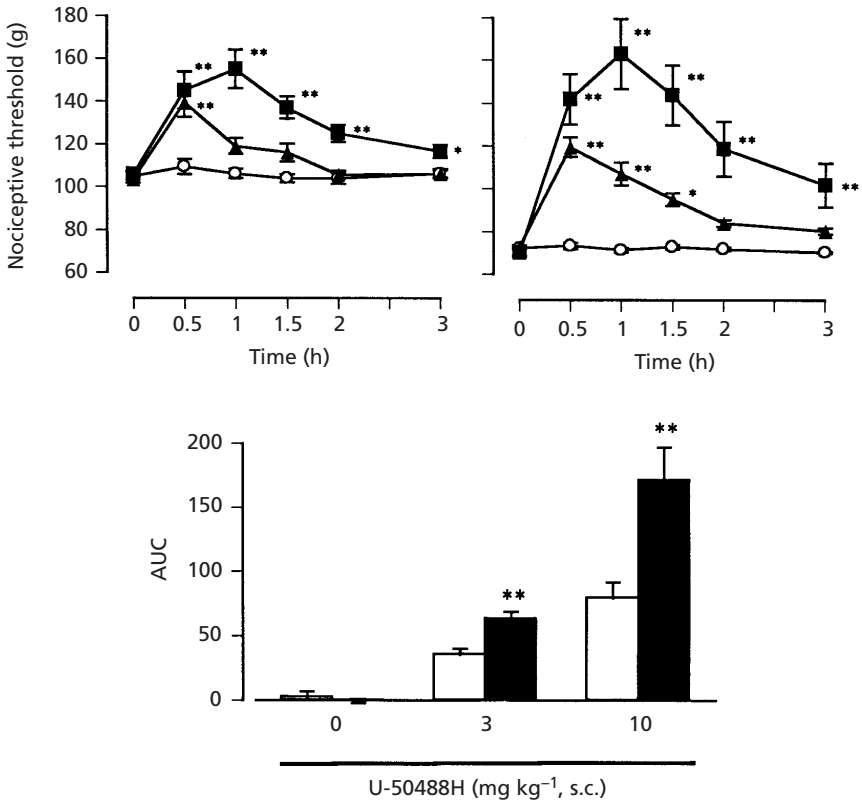

Figure 1 Upper panels: time-course of the effect of U-50488H on nociceptive threshold in non-diabetic (upper-left panel) and diabetic (upper-right panel) mice. The nociceptive threshold was determined by the tail-pressure test. ○, saline (10 mL kg^{-1}, s.c.); ▲, U-50488H (3 mg kg^{-1}, s.c.); ■, U-50488H (10 mg kg^{-1}, s.c.). Each point and vertical bar represents the mean ± s.e.m. of 8–10 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with saline-treated animals (Dunnett's *t*-test). Lower panel: antinociceptive activity of U-50488H in non-diabetic (open column) and diabetic (closed column) mice. The results are indicated as the area under the time–response curve (AUC). Each column and vertical bar represents the mean ± s.e.m. of results from 8–10 mice. $^{**}P < 0.01$ compared with non-diabetic mice (Student's *t*-test).

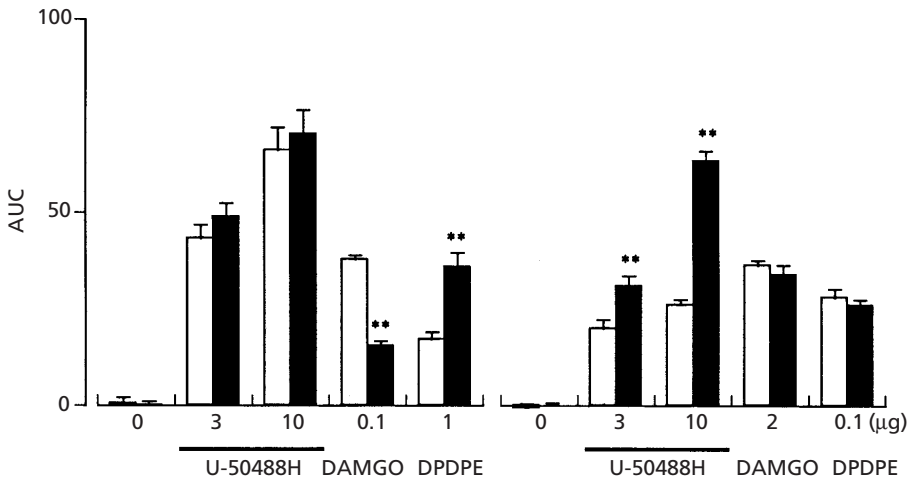

Figure 2 Effects of U-50488H, DAMGO and DPDPE on the tail-pressure response in non-diabetic (open column) and diabetic (closed column) mice. The drugs were injected intracerebroventricularly (left panel) or intrathecally (right panel). The results are indicated as the area under the time–response curve (AUC). Each column and vertical bar represents the mean ± s.e.m. of results from 7–9 mice. $^{**}P < 0.01$ compared with non-diabetic mice (Student's *t*-test).

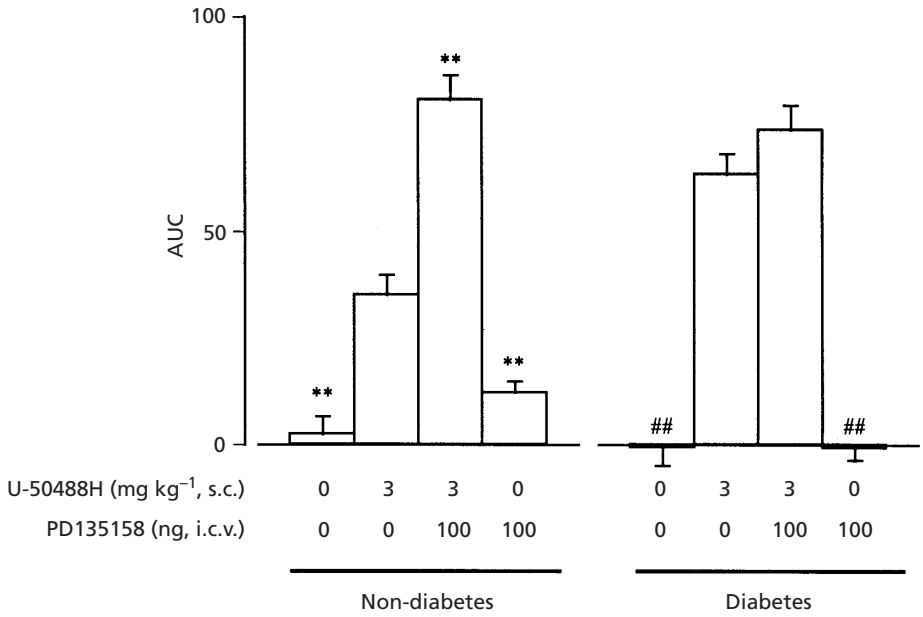

Figure 3 Effect of PD135158 on U-50488H-induced antinociception in non-diabetic and diabetic mice. The results are indicated as the area under the time–response curve (AUC). Each column and vertical bar represents the mean ± s.e.m. of results from 8–10 mice. $^{**}P < 0.01$ compared with U-50488H- and saline (5 μL, i.c.v.)-treated non-diabetic mice (Dunnett's *t*-test). $^{\#\#}P < 0.01$ compared with U-50488H- and saline-treated diabetic mice (Dunnett's *t*-test).

Effect of cholecystokinin$_B$ receptor agonist or antagonist on U-50488H-induced antinociception in diabetic mice

The antinociceptive effect of U-50488H (3 mg kg^{-1}, s.c.) in non-diabetic mice was increased by co-administration of PD135158 (100 ng, i.c.v.), a cholecystokinin$_B$ (CCK_B) antagonist. Meanwhile, the antinociceptive activity of U-50488H observed in diabetic mice was not influenced by PD135158 (Figure 3).

Treatment with des-CCK8 (3–100 ng, i.c.v.), a CCK_B agonist, did not influence the antinociceptive effect of U-50488H (3 mg kg^{-1}, s.c.) in non-diabetic mice, although it diminished the antinociceptive activity of U-50488H (3 mg kg^{-1}, s.c.) which was enhanced in diabetic mice (Figure 4).

Discussion

The major finding of this study was hyper-responsiveness to antinociception mediated by κ-opioid receptors in STZ-induced diabetic mice. The increase in the antinociceptive activity of U-50488H injected subcutaneously observed in diabetic mice was considered to be based on an altered antinociceptive function associated with spinal κ-opioid receptors.

Several articles have reported the alteration of dynorphin (an endogenous ligand for κ-opioid receptors) and its receptors in spinal cord caused by pathophysiological stimuli (Faden et al 1985; Krumins & Faden 1986; Iadarola et al 1988; Kajander et al 1990). Desmeules et al (1993), who found an increase in the antinociceptive activity of U-69593, a κ-opioid agonist, in an experimental model of mononeuropathic pain produced by loose ligatures around the common sciatic nerve in rats, pointed out that the cause may have elevated extracellular levels of dynorphin in the spinal cord due to sciatic nerve injury. Iadarola et al (1988) demonstrated that activation of dynorphin biosynthesis in the spinal cord is a common feature in hyperalgesia and peripheral inflammation. Based on these findings, it was considered that the increased antinociceptive activity of U-50488H observed in diabetic mice might be due to the increased intraspinal concentration of dynorphin. However, in our previous evaluation using enzyme immunoassay, there were no changes in the intraspinal concentration of immunoreactive dynorphin between non-diabetic and STZ-induced diabetic rats (non-diabetic rats, 410.01 ± 19.96 pg (g tissue)$^{-1}$, n = 6; diabetic rats, 424.59 ± 41.19 pg (g tissue)$^{-1}$, n = 6).

Takeshige et al (1992) indicated that non-acupuncture analgesia is usually masked by the analgesia inhibitory system transmitted by CCK. Non-acupuncture anal-

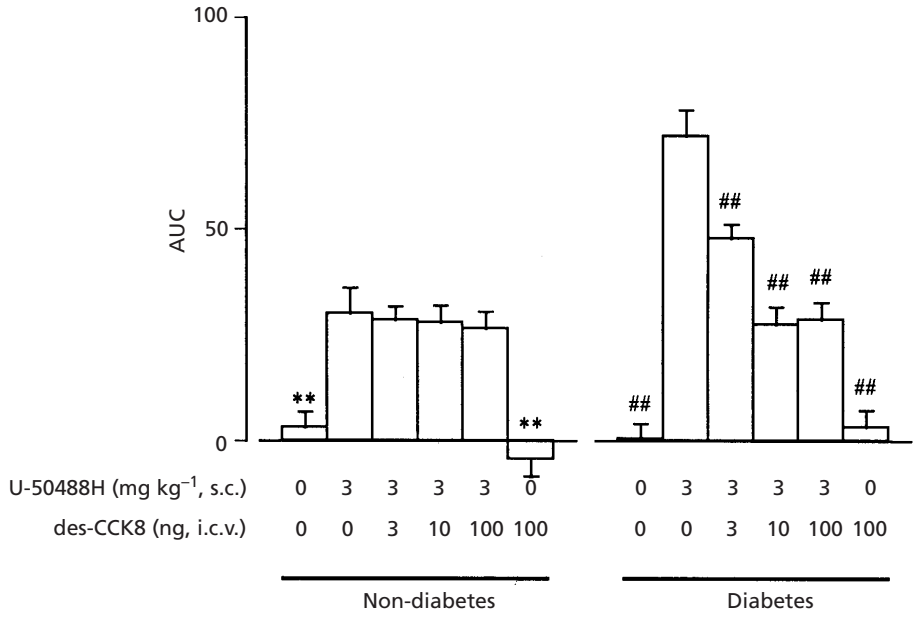

Figure 4 Effect of desulfated octapeptide of cholecystokinin (des-CCK8) on U-50488H-induced antinociception in non-diabetic and diabetic mice. The results are indicated as the area under the time–response curve (AUC). Each column and vertical bar represents the mean ± s.e.m. of results from 6–8 mice. $^{**}P < 0.01$ compared with U-50488H and saline (5 μL, i.c.v.)-treated non-diabetic mice (Dunnett's t-test). $\#\#P < 0.01$ compared with U-50488H and saline-treated diabetic mice (Dunnett's t-test).

gesia unmasked by lesions of the lateral centromedian nucleus of the thalamus or a part of the posterior hypothalamus was contradicted by intrathecal injection of antiserum of dynorphin or κ-opioid antagonist (Takeshige et al 1990). The increased antinociceptive effect of U-50488H observed in non-diabetic mice in combination with PD135158 was thought to result from inactivation of the analgesia inhibitory system mediated by CCK_B receptors. From another perspective, it was considered that the analgesia inhibitory system might be diminished in diabetic mice in which effects of PD135158 were not observed. Thus, dysfunction of the analgesia inhibitory system might induce increased antinociception via spinal κ-opioid receptors in diabetic mice. As the enhanced antinociceptive effect of U-50488H in diabetic mice diminished due to the administration of des-CCK8, we considered that the amount of CCK in the brain of diabetic mice, which is required for the stimulation of CCK_B receptors, decreased. Further investigations are necessary to elucidate the mechanism of functional enhancement of spinal κ-opioid receptors induced by diabetes mellitus.

In this study using the tail-pressure test, we also confirmed the diminution of μ_1-opioid receptor-mediated antinociception and the enhancement of δ-opioid receptor-mediated antinociception which were indicated in the tail-flick test (Kamei et al 1994a, c). We pointed out the possibility that the alteration of supraspinal μ_1-opioid receptor-mediated antinociception might be deeply involved with the hyperalgesia of diabetic animals (Kamei et al 1994a). To the contrary, since diabetic mice obviously did have a lower nociceptive threshold than non-diabetic mice, it seemed that there was only a small influence upon the functional enhancement of spinal κ-opioid receptors for the sensitivity of diabetic mice to noxious mechanical stimulation. Actually, the nociceptive threshold in diabetic mice was not changed by blockade of κ-opioid receptors with administration of nor-BNI in our study (72.5 ± 0.4 g, n = 22). This result suggested that the amount of released dynorphin in the spinal cord was insufficient for the stimulation of spinal κ-opioid receptors. Little involvement of endogenous dynorphin with nociceptive threshold may expain the continuation of hyperalgesia in diabetic mice regardless of the enhancement of spinal κ-opioid receptor-mediated antinociception.

In conclusion, the results suggest that mice with diabetes are selectively hyper-responsive to spinal κ-opioid receptor-mediated antinociception, but are normally responsive to the activation of supraspinal κ-opioid receptors. κ-Opioid agonist(s) may be useful for the treatment of painful diabetic neuropathy.

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