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Effect of MK-801 on the antinociceptive effect of [D-Ala^2,*N*-MePhe^4, Gly-ol^5]enkephalin in diabetic mice

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Abstract

The role of *N*-methyl-D-aspartate (NMDA) receptors in supraspinal and spinal sites on the reduction of supraspinal μ-opioid receptor-induced antinociception in diabetic mice was examined. The antinociceptive effect of i.c.v. [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO, 20 pmol) in diabetic mice was significantly less than that in non-diabetic mice. The antinociceptive effect of i.c.v. DAMGO (20 pmol) was significantly and dose dependently reduced by i.c.v. (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK-801) in both non-diabetic (0.03–0.3 nmol) and diabetic mice (0.1–3.0 nmol). While the antinociceptive effect of i.c.v. DAMGO (10 pmol) was significantly enhanced by i.c.v NMDA (0.01–0.1 nmol) in non-diabetic mice, the same doses of i.c.v. NMDA had no significant effect on the antinociceptive effect of i.c.v. DAMGO (20 pmol) in diabetic mice. In non-diabetic mice, the antinociceptive effect of DAMGO (20 pmol, i.c.v.) was dose dependently reduced by intrathecal administration of MK-801 (0.1–1.0 nmol). The antinociceptive effect of DAMGO (20 pmol, i.c.v.) was dose-dependently enhanced by MK-801 (0.1–1.0 nmol, i.t.) in diabetic mice. Furthermore, NMDA (0.1 nmol, i.t.) significantly enhanced the antinociceptive effect of DAMGO (10 pmol, i.c.v.) in non-diabetic mice. However, in non-diabetic mice, the antinociceptive effect of DAMGO (40 pmol, i.c.v.) was dose dependently reduced by NMDA (0.03–0.3 nmol, i.t.). These results suggest that NMDA receptor function in supraspinal and spinal sites appear to be modulated differently by the diabetic state, and this functional modulation may play an important role in the reduction of supraspinal μ-opioid receptor-induced antinociception in diabetic animals.

Keywords: Diabetes; Antinociception; MK-801; NMDA receptor; μ-Opioid receptor; DAMGO ([D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin)

1. Introduction

Lipa and Kavaliers (1990) demonstrated that the microinjection of morphine and *N*-methyl-D-aspartate (NMDA) either alone or in combination into the rat periaqueductal gray produced analgesia which was blocked by (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK-801), a non-competitive NMDA receptor antagonist. Furthermore, Suh et al. (1994) demonstrated that i.c.v. administration of MK-801, at doses of 0.003 to 3.0 nmol, reduced the i.c.v. morphine-induced inhibition of the tail-flick response in mice. In addition, Heinricher et al. (2001) reported that activation of brainstem NMDA receptors is required for the analgesic actions of morphine given systemically. Thus, they suggested that NMDA receptors located at supraspinal sites may be involved in the antinociceptive effect of morphine.

We previously reported that the antinociceptive effects of the i.c.v. administration of μ-opioid receptor agonists, such as morphine and [D-Ala^2,*N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO), were markedly less in diabetic mice than in non-diabetic mice (Kamei et al., 1992a,b). Furthermore, the antinociceptive effect of i.c.v. endomorphin-2, an endogenous μ-opioid receptor agonist, was also significantly less in diabetic mice than in non-diabetic mice (Kamei et al., 2000). Based on these results, we suggested that diabetic mice were selectively hypo-responsive to μ-opioid receptor-mediated antinociception. Furthermore, Di Luca et al. (1999) reported that phosphorylation of the NR2A/B subunits of NMDA receptors was reduced in the hippocampus of streptozotocin-induced diabetic rats as compared with non-diabetic rats. Thus, they suggested that such receptors may be involved in behavioral abnormalities, such as the disturbances of learn-

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ing and memory observed in streptozotocin-induced diabetic rats (Di Luca et al., 1999). Based on these results and our findings, a lack of NMDA transmission may be involved, at least in part, in the reduction of the antinociceptive effect of i.c.v. μ-opioid receptor agonist in diabetic mice.

NMDA receptors in the spinal cord play an important role in nociceptive transmission (Woolf, 1983; Cahusac et al., 1984; Dickenson and Sullivan, 1990). The excessive activation of NMDA receptors in the spinal cord contributes to neuropathic pain (e.g., Yamamoto and Yaksh, 1992). We previously reported that thermal allodynia and thermal hyperalgesia are seen in diabetic mice (Ohsawa and Kamei, 1999a,b). Furthermore, Malcangio and Tomlinson (1998) demonstrated that 6 days of treatment with MK-801 gradually reversed mechanical hyperalgesia in the diabetic rat. They suggested that the spinal cord of diabetic rats may exhibit an increased release of glutamate and activation of NMDA receptors.

Based on these results, we hypothesized that functional changes in supraspinal or spinal NMDA receptors are involved in the reduction of supraspinal μ-opioid receptor-induced antinociception in diabetic mice. To test this hypothesis, we examined the effect of i.c.v. or i.t. administration of MK-801 on the antinociceptive effects of i.c.v. DAMGO in non-diabetic and diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 25–35 g at the beginning of the experiments, were used. They had free access to food and water in an animal room that 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 non-diabetic mice were injected with the 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 with the guidelines 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 determined by recording the latency in the tail-flick test using radiant heat. The intensity of the thermal stimulus for the tail-flick test 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. The percent antinociception was calculated for each animal as %Antinociception = 100 × (post-drug latency − pre-drug latency)/(30 − pre-drug latency).

2.3. Drugs

Streptozotocin was purchased from Sigma (St. Louis, MO, USA). [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO), a selective μ-opioid receptor agonist, *N*-methyl-D-aspartate (NMDA), an NMDA receptor agonist, and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (MK-801), a non-competitive NMDA receptor antagonist, were purchased from Research Biochemical International (Natic, MA). All of the drugs were dissolved in saline. DAMGO was injected i.c.v. 10 min before the antinociceptive assay. MK-801 or NMDA was injected i.c.v. or i.t. 10 min before the administration of DAMGO. The i.c.v. injection was performed according to the method of Haley and McCormick (1957). The i.t. injection was performed according to method of Hylden and Wilcox (1980).

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/Dunn test.

3. Results

3.1. Effects of i.c.v. administration of MK-801 on antinociception induced by DAMGO administered i.c.v. in non-diabetic and diabetic mice

The effects of i.c.v. administration of MK-801 on i.c.v. DAMGO-induced antinociception in non-diabetic and diabetic mice are shown in Fig. 1. The doses of MK-801 (0.1 to 3.0 nmol) used in this study have been reported to reduce the antinociceptive effect of i.c.v. morphine in naive mice (Suh et al., 1994). The antinociceptive effect of DAMGO (20 pmol, i.c.v.) in diabetic mice was significantly less than that in non-diabetic mice. The antinociceptive effect of DAMGO (20 pmol, i.c.v.) in non-diabetic mice was dose dependently and significantly reduced by i.c.v. pretreatment with MK-801 (0.1–1.0 nmol). MK-801 (0.1 and 1.0 nmol, i.c.v.) reduced the antinociceptive effect of DAMGO in non-diabetic mice to the level observed in diabetic mice. Furthermore, the antinociceptive effect of DAMGO (20 pmol, i.c.v.) was also significantly reduced by i.c.v. pretreatment with MK-801 (0.1–3.0 nmol) in diabetic mice (Fig. 1). MK-801 (1.0 nmol for non-diabetic mice, 3.0 nmol, for diabetic mice, i.c.v.), by itself, had no significant effect on the baseline tail-flick latency in both non-diabetic (before MK-801, 3.7 ± 0.2 s, $n = 10$; after MK-801, 3.7 ± 0.3 s,

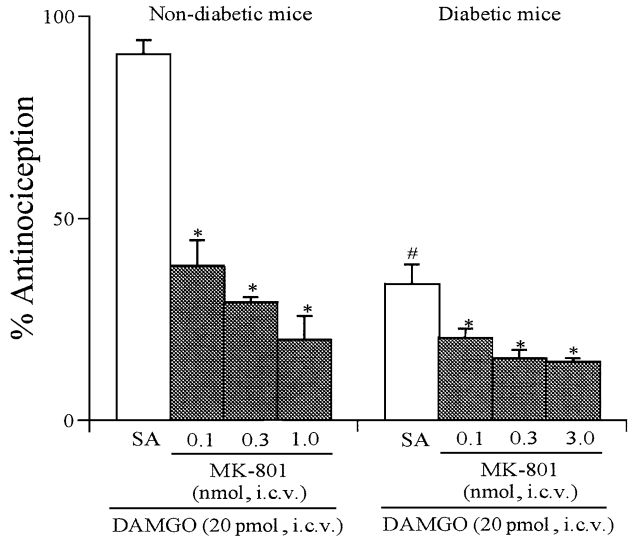

Fig. 1. Effect of i.c.v. administration of MK-801 on the antinociceptive effect of i.c.v. DAMGO in non-diabetic and diabetic mice. MK-801 was injected 10 min before the administration of DAMGO (20 pmol). The antinociceptive effect of DAMGO was measured in the tail-flick test 10 min after injection. Each column represents the mean ± S.E. for 10 mice in each group. * $P<0.05$ vs. respective saline (SA)-treated group. #$P<0.05$ vs. respective non-diabetic mice.

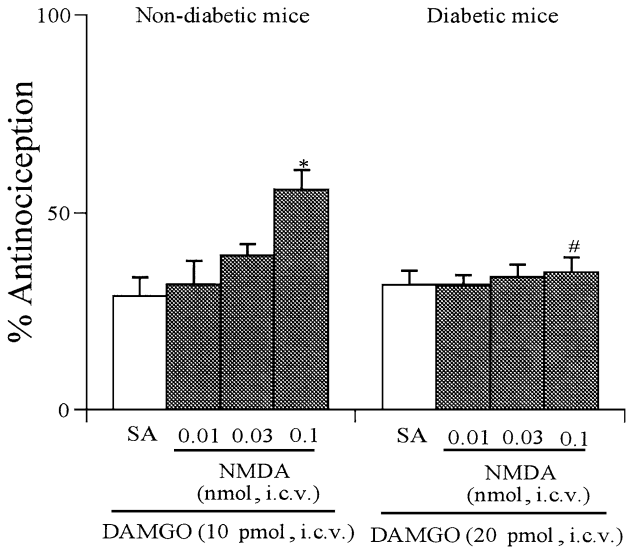

Fig. 2. Effect of i.c.v. administration of NMDA on the antinociceptive effect of i.c.v. DAMGO in non-diabetic and diabetic mice. NMDA was injected 10 min before the administration of DAMGO (non-diabetic mice, 10 pmol; diabetic mice, 20 pmol). The antinociceptive effect of DAMGO was measured in the tail-flick test 10 min after injection. Each column represents the mean ± S.E. for 10 mice in each group. * $P<0.05$ vs. respective saline (SA)-treated group. #$P<0.05$ vs. respective non-diabetic mice.

$n=10$) and diabetic mice (before MK-801, 3.6 ± 0.2 s, $n=10$; after MK-801, 3.9 ± 0.2 s, $n=10$).

3.2. Effects of i.c.v. administration of NMDA on antinociception induced by DAMGO administered i.c.v. in non-diabetic and diabetic mice

In non-diabetic mice, i.c.v. pretreatment with NMDA, at doses of 0.01 to 0.1 nmol, dose dependently enhanced DAMGO (10 pmol, i.c.v.)-induced antinociception (Fig. 2), and the effect of 0.1 nmol was significant. However, i.c.v. pretreatment with NMDA did not significantly change the antinociceptive effect of DAMGO (20 pmol, i.c.v.) in diabetic mice (Fig. 2). NMDA (0.1 nmol, i.c.v.), by itself, had no significant effect on the baseline tail-flick latency in both non-diabetic (before NMDA, 3.2 ± 0.2 s, $n=10$; after NMDA, 3.7 ± 0.3 s, $n=10$) and diabetic mice (before NMDA, 3.5 ± 0.2 s, $n=10$; after NMDA, 3.6 ± 0.3 s, $n=10$).

3.3. Effects of i.t. administration of MK-801 on antinociception induced by DAMGO administered i.c.v. in non-diabetic and diabetic mice

In non-diabetic mice, the antinociceptive effect of DAMGO (20 pmol, i.c.v.) was dose dependently and significantly reduced to the level observed in diabetic mice by i.t. pretreatment with MK-801 (0.1–1.0 nmol) (Fig. 3). The antinociceptive effect of DAMGO (20 pmol, i.c.v.) in diabetic mice was dose dependently enhanced by i.t. pretreatment with MK-801 (0.1–1.0 nmol, i.t.) (Fig. 3). Indeed, MK-801, at a dose of 1.0 nmol, i.t., significantly enhanced the antinociceptive effect of DAMGO (20 pmol, i.c.v.) in diabetic mice. Furthermore, there was no significant differ-

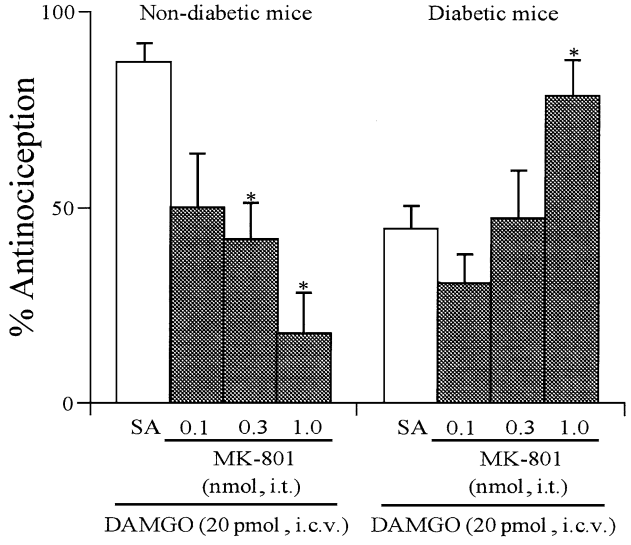

Fig. 3. Effect of i.t. administration of MK-801 on the antinociceptive effect of i.c.v. DAMGO in non-diabetic and diabetic mice. MK-801 was injected 10 min before the administration of DAMGO (20 pmol). The antinociceptive effect of DAMGO was measured in the tail-flick test 10 min after injection. Each column represents the mean ± S.E. for 10 mice in each group. * $P<0.05$ vs. respective saline (SA)-treated group.

ence in the DAMGO (20 pmol, i.c.v.)-induced antinociception between saline-treated non-diabetic mice and MK-801 (1.0 nmol, i.t.)-treated diabetic mice. MK-801(1.0 nmol, i.t.), by itself, had no significant effect on the baseline tail-flick latency in both non-diabetic (before MK-801, 3.3 ± 0.3 s, $n = 10$; after MK-801, 3.9 ± 0.3 s, $n = 10$) and diabetic mice (before MK-801, 3.1 ± 0.2 s, $n = 10$; after MK-801, 3.6 ± 0.3 s, $n = 10$).

3.4. Effects of i.t. administration of NMDA on antinociception induced by DAMGO administered i.c.v. in non-diabetic and diabetic mice

The antinociceptive effect of DAMGO (10 pmol, i.c.v.) was dose dependently enhanced by i.t. treatment with NMDA, at doses of 0.03 and 0.1 nmol, in non-diabetic mice (Fig. 4). However, a high dose of NMDA (0.3 nmol, i.t.) had no significant effect on the antinociceptive effect of DAMGO (20 pmol, i.c.v.) in non-diabetic mice (Fig. 4). In contrast, i.t. pretreatment with NMDA, at doses of 0.03 to 0.3 nmol, dose dependently and significantly reduced the antinociceptive effect of DAMGO (40 pmol, i.c.v.) in diabetic mice (Fig. 4). NMDA (0.3 nmol, i.t.), by itself, had no significant effect on the baseline tail-flick latency in both non-diabetic (before NMDA, 3.1 ± 0.2 s, $n = 10$; after NMDA, 3.4 ± 0.2 s, $n = 10$) and diabetic mice (before NMDA, 3.2 ± 0.1 s, $n = 10$; after NMDA, 3.6 ± 0.2 s, $n = 10$).

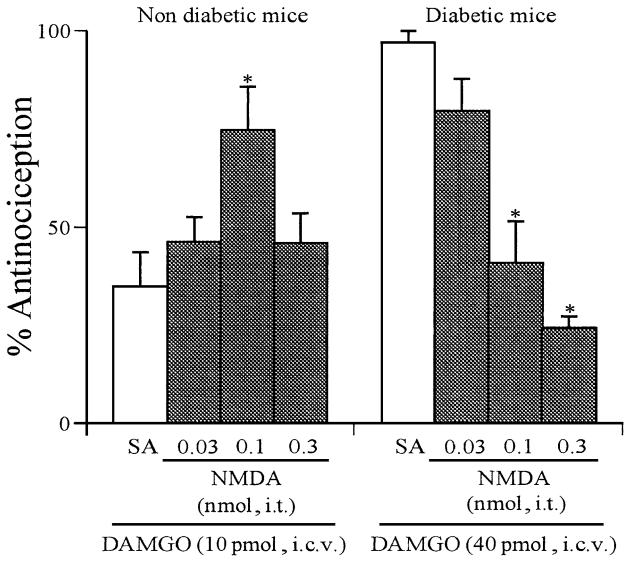

Fig. 4. Effect of i.t. administration of NMDA on the antinociceptive effect of i.c.v. DAMGO in non-diabetic and diabetic mice. NMDA was injected 10 min before the administration of DAMGO (non-diabetic mice, 10 pmol; diabetic mice, 40 pmol). The antinociceptive effect of DAMGO was measured in the tail-flick test 10 min after injection. Each column represents the mean ± S.E. for 10 mice in each group. * $P < 0.05$ vs. respective saline (SA)-treated group.

4. Discussion

In the present study, we observed that the antinociceptive effect of i.c.v. administration of DAMGO in mice was reduced by i.c.v. administration of MK-801. The i.c.v. administration of NMDA enhanced the antinociceptive effect of i.c.v. DAMGO in non-diabetic mice. These results agree with the suggestion of several authors that NMDA receptors may be actively involved in the production of antinociception when the descending analgesia system is activated by supraspinally administered μ-opioid receptor agonists (Jacquet, 1988; Lipa and Kavaliers, 1990; Suh et al., 1994; Heinricher et al., 2001).

In the present study, we also observed that i.c.v. administration of MK-801 significantly reduced the antinociceptive effect of i.c.v. DAMGO in non-diabetic mice to the level observed in diabetic mice. Although i.c.v. pretreatment with MK-801 also significantly reduced the antinociceptive effect of i.c.v. DAMGO in diabetic mice, the antagonistic potency of MK-801 in diabetic mice was less than that in non-diabetic mice. NMDA (0.1 nmol, i.c.v.), which significantly enhanced the antinociceptive effect of i.c.v. DAMGO in non-diabetic mice, had no effect on the antinociceptive effect of i.c.v. DAMGO in diabetic mice. Jacquet (1988) demonstrated that injection of the competitive NMDA receptor antagonist 2-amino-7-phosphonoheptanoic acid (AP-7) into the periaqueductal gray blocked morphine-induced antinociception. Furthermore, Heinricher et al. (1999, 2001) suggested that activation of identified nociceptive-modulating neurons, 'off-cells', in the rostral ventromedial medulla is critical to the antinociceptive effect of morphine, and this activation is at least in part mediated by NMDA receptors. Along these lines, Di Luca et al. (1999) reported that Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII)-dependent phosphorylation of NR2A and B subunits of NMDA receptors was markedly reduced in the cortex and hippocampus of streptozotocin-induced diabetic rats. An imbalance between NR2B and NR2A subunits changes the physiology of cells bearing NMDA receptors, thus influencing the kinetics of excitatory transmission in diabetic animals. Based on these findings and our results, it seems likely that dysfunction of NMDA receptors in the mediation of the inhibition of supraspinal segmental pain may play, at least in part, an important role in the reduction of supraspinal μ-opioid receptor-induced antinociception in diabetic mice.

The antinociceptive effect of i.c.v. DAMGO was dose dependently reduced by i.t. administration of MK-801 in non-diabetic mice. Furthermore, NMDA, at a dose of 0.1 nmol, i.t., significantly enhanced the antinociceptive effect of i.c.v. DAMGO in non-diabetic mice. The reasons for the enhancement of the supraspinal μ-opioid receptor-induced antinociception by NMDA remain unclear. Although the activation of spinal NMDA receptors plays an important role in nociceptive transmission (Woolf, 1983; Cahusac et al., 1984; Dickenson and Sullivan, 1990), there have been

several reports that i.t. administration of NMDA in rodents can produce antinociceptive responses (Raigorodsky and Ureca, 1987; Advokat et al., 1994; Álvarez-Vega et al., 2000). In these latter studies, the authors demonstrated that i.t. administration of NMDA inhibited the tail-flick response in rats. Since NMDA-induced antinociception is reversed by the intrathecal administration of naloxone, methysergide or phentolamine, it has been suggested that the activation of NMDA receptors may trigger the activation of endogenous antinociceptive systems (Álvarez-Vega et al., 2000). Thus, it is possible that the i.t. NMDA-induced enhancement of the antinociceptive effect of i.c.v. DAMGO in non-diabetic mice may result from the activation of endogenous antinociceptive systems, rather than from the direct activation of NMDA receptors. In contrast, in the present study, the antinociceptive effect of i.c.v. DAMGO was dose dependently enhanced by i.t. administration of MK-801 in diabetic mice. Furthermore, the antinociceptive effect of i.c.v. DAMGO in diabetic mice was dose dependently reduced by i.t. administration of NMDA. Malcangio and Tomlinson (1998) demonstrated that mechanical hyperalgesia in diabetic rats had a reduced sensitivity to the antinociceptive effect of systemic morphine. Treatment with a low dose of MK-801 for 6 days gradually reversed mechanical hyperalgesia in diabetic rats (Malcangio and Tomlinson, 1998). As with other models of neuropathic pain, diabetic peripheral neuropathy may induce chronic changes within the spinal cord that lead to an increased activity of NMDA receptors on spinal dorsal horn neurons (Davies and Lodge, 1987). Based on these results, Malcangio and Tomlinson (1998) postulated that mechanical hyperalgesia in diabetic rats is maintained by further increases in glutamate release and subsequent activation of NMDA receptors in the spinal cord. Thus, it is possible that this excessive activation of NMDA receptors in the spinal cord of diabetic mice may enhance NMDA receptor-mediated nociceptive transmission and negate NMDA receptor-mediated endogenous antinociception in the spinal cord. This possibility is supported in part by the present result that the enhancement of i.c.v. DAMGO-induced antinociception in non-diabetic mice was diminished when the dose of i.t. NMDA was increased to 0.3 nmol. Thus, it seems likely that the enhancement of i.c.v. DAMGO-induced antinociception in diabetic mice by i.t. MK-801 may be due to a reduction in NMDA receptor-mediated nociceptive transmission.

Interestingly, the present study suggested the possibility that the diabetic state might modulate supraspinal and spinal NMDA receptors differently. A separate series of investigations has suggested that supraspinal and spinal NMDA receptor subunits show differential expression (Tolle et al., 1993; Zhou et al., 2000; Sundstrom and Mo, 2001). Thus, the differential expression of supraspinal and spinal NMDA receptor subunits might be responsible for the differential modulation of the function of supraspinal and spinal NMDA receptor subunits by diabetes. However, further studies are needed before this possibility can be established with greater certainty.

In conclusion, the present results suggest that both the dysfunction of NMDA receptors in the mediation of supraspinal segmental nociceptive inhibitory mechanisms and the activation of NMDA receptor-mediated nociceptive transmission in the spinal cord may play, at least in part, an important role in the reduction of supraspinal μ-opioid receptor-induced antinociception in diabetic mice.

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