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Role of L-type Ca^{2+}channels in attenuated morphine antinociception in streptozotocin-diabetic rats

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Abstract

The role of L-type Ca^{2+} channels in morphine antinociception was studied in streptozotocin-induced diabetic and control rats, using [^{3}H]PN200-110 {isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycabonylpyridine-3-caboxylate} binding (0.005–1.0 nmol/l) and rat whole brain membranes, to determine if the attenuation of morphine antinociception was related to alterations in dihydropyridine-sensitive Ca^{2+} channel binding characteristics. The tail-flick antinociceptive effect of morphine (4 mg/kg, i.p.) was significantly reduced in diabetic rats in comparison to that in controls. Nimodipine (0.1–3 mg/kg, i.p.) did not produce antinociception but significantly potentiated the morphine response in control rats. Nimodipine (0.3–3 mg/kg, i.p.) reversed the attenuation of morphine antinociception in a dose-dependent manner in diabetic rats. Moreover, insulin (2 μ/kg, s.c.) reversed the attenuated morphine antinociception in streptozotocin-diabetic rats. A significant increase in the B_{max} (+41%) of [^{3}H]PN200-110 binding was observed in diabetic rat brain membranes compared to that in control rats. However, there was no change in affinity (K_d) value of [^{3}H]PN200-110. The reduced morphine response in diabetic rats, in accordance with up-regulation of dihydropyridine sites, may be due to an increased Ca^{2+} influx through L-type channels. These results suggest a functional role of L-type Ca^{2+} channels in morphine antinociception and the diabetic state may lead to alterations in their density.

Keywords: Sterptozotocin-induced diabetes; Morphine; Tail-flick antinociception; Nimodipine; [^{3}H]PN200-110; B_{max}; Saturation binding

1. Introduction

In diabetic patients, hypersensitivity to pain and attenuation of responsiveness to morphine are reported. Many studies have suggested that diabetes and hyperglycemia affect the sensitivity of laboratory animals to various pharmacological agents (Kamei and Kasuya, 1995). The antinociceptive potency of morphine in the tail-flick test was reported to be significantly decreased in streptozotocin-induced diabetic rats, mice and even in spontaneously diabetic mice when compared to that in non-diabetic control animals (Simon and Dewey, 1981). The antinociceptive effect of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO), a selective μ-opioid receptor agonist, was also found to be decreased in streptozotocin-diabetic mice (Kamei et al., 1992; Ohsawa et al., 1998). Pain tolerance has also been shown to be significantly decreased in diabetic patients and glucose-loaded normal subjects (Morley et al., 1984). On the other hand, hypoglycemia induced by insulin treatment significantly increased morphine potency in the tail-flick test in experimental animals (Simon and Dewey, 1981; Singh et al., 1983; Kamei et al., 1993, 1998; Tandon et al., 2000).

There is also considerable evidence of a close relationship between opioid antinociception and Ca^{2+} levels within the central nervous system. Agents that increase cytosolic Ca^{2+} in neurons and synaptosomes viz., ionophores, thapsigargin, have been shown to reduce μ-opioid receptor agonist-induced antinociception in normal animals (Chapman and Way, 1980; Vocci et al., 1980; Smith and Stevens, 1995; Ohsawa et al., 1998), but not to affect the already reduced antinociception in diabetic mice (Ohsawa et al., 1998). It has been postulated that Ca^{2+} alters intracellular events to antagonize the antinociceptive effects of morphine (Chapman and Way, 1980). Conversely, Ca^{2+} chelators (i.e. EGTA), Ca^{2+} channel blockers and ryanodine, which block the rise in intracellular calcium $[Ca^{2+}]_i$, have been shown to potentiate μ-opioid receptor agonist-induced antinociception

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in both control and streptozotocin-diabetic animals (Ben-Sreti et al., 1983; Hoffmeister and Tettenborn, 1986; Rani et al., 1996; Ohsawa et al., 1998). Moreover, voltage-sensitive Ca^{2+} currents through L- and N-type channels have been shown to be enhanced in dorsal root ganglion neurons of streptozotocin, Bio-Breeding/Worchester (BB/W)-diabetic rats and diabetic mice (Hall et al., 1995; Kostyuk et al., 1995; Votienko et al., 2000). Alterations in intracellular Ca^{2+} homeostasis resulting in increased $[Ca^{2+}]_i$ have been documented in several models of diabetes (Levy et al., 1994). Moreover, the complications associated with chronic diabetes mellitus such as hypertension, macrovascular and microvascular disease, cataracts, cardiomyopathy, neuropathy etc., are suggested to be related to the pronounced changes in cellular Ca^{2+} homeostasis (Votienko et al., 1999).

Thus, the literature suggests that an increase/decrease in $[Ca^{2+}]_i$ levels can antagonize/potentiate the μ-opioid receptor agonist-induced antinociception in both control and streptozotocin-diabetic animals. However, it is not clear exactly how these Ca^{2+} levels are increased in the diabetic state. One of the possible mechanisms may be up-regulation of Ca^{2+} channels, as the entry of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels is an important contributor (Thayer and Miller, 1990). An increase in binding of {isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycabonylpyridine-3-caboxylate} [^{3}H]PN200-110, a dihydropyridine type Ca^{2+} channel blocker, to cardiac muscle membranes has been shown in streptozotocin-diabetic rats, suggesting up-regulation of L-type Ca^{2+} channels as a cause for diabetic cardiomyopathies (Gotzsche et al., 1996; Nishio et al., 1990). A similar increase in the density of Ca^{2+} channels was found in the skeletal muscles of streptozotocin-diabetic rats (Lee and Dhalla, 1992). However, there are no reports regarding the status of L-type channels in the central nervous system of diabetic animals. The present study was undertaken to find the characteristics of dihydropyridine L-type Ca^{2+} channels in whole brain membranes of both streptozotocin-diabetic rats and their age-matched controls, using the radioligand, [^{3}H]PN200-110. The effect of a Ca^{2+} channel blocker, nimodipine, and insulin on morphine-induced antinociception was studied in control and diabetic rats.

2. Materials and methods

2.1. Animals

Experiments were performed on male Sprague–Dawley rats weighing 150–200 g (Central Animal Facility, NIPER, India), housed four per cage in a room with controlled ambient temperature (23±1 °C), humidity (50±10%) and 12-h light/dark cycle. The animals were allowed free access to food and water throughout the study except during the experiment. All experiments were performed between 09.00 to 17.00 h to minimize diurnal variation. The experimental protocols were duly approved by the Institutional Animal Ethical Committee (IAEC/99/004).

2.2. Drugs

Morphine was obtained from the Government Opium and Alkaloid Factory, Ghazipur, India. Streptozotocin was obtained from Sigma, St. Louis, MO, USA. Insulin (zinc insulin crystals) was gifted by Lilly laboratories, Indianapolis, USA. The Ca^{2+} channel blocker, nimodipine, was gifted by USV, Mumbai, India. Radioligand [^{3}H]PN200-110 {isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-methoxycabonylpyridine-3-caboxylate} (Isradipine) (80 Ci/mmol), a dihydropyridine type Ca^{2+} channel blocker, was obtained from Amersham Lifesciences. Morphine was dissolved in distilled water, streptozotocin in 0.1 mol/l citrate buffer and nimodipine was solubilized in 20% dimethyl sulfoxide, 20% ethanol and 60% distilled water.

2.3. Induction of diabetes

Rats were injected with a single dose of 65 mg/kg (i.p.) streptozotocin dissolved in cold 0.1 mol/l citrate buffer. The plasma samples were analyzed for glucose with a glucose oxidase peroxidase (GOD-POD) kit (Qualigens, Glaxo, Mumbai, India). Animals showing blood glucose levels higher than 250 mg/dl were considered diabetic and the 4-week-old diabetic rats were used for the study with age-matched rats as control group.

2.4. Antinociception

The modified tail-flick technique of D'Amour and Smith (1941) was employed to measure antinociception (Hicon–Medicraft tail-flick analgesiometer). Two basal (pre-drug) readings were taken at an interval of 30 min. The intensity of the thermal stimulus was adjusted to give a basal reaction latency between 4 and 6 s. The cut-off time was fixed at 20 s in order to avoid any damage to the tail. Morphine or its vehicle was administered to the diabetic group or the control group, respectively, and the reaction latencies were determined at regular intervals for a period of 180 min (0, 30, 60, 90, 120 and 180 min). Nimodipine (1 mg/kg, i.p.) or its appropriate vehicle was given 15 min before morphine treatment. The tail-flick latencies were converted to percent antinociception according to the following formula.

$$\%\text{Antinociceptive effect} = \frac{\text{Reaction time of test} - \text{Basal reaction time}}{\text{Cut off time} - \text{Basal reaction time}} \times 100$$

From the %antinociception vs. time plot, the area under the curve (AUC) was calculated using the trapezoidal method. Antinociception ($AUC_{0-180\ min}$) was expressed as the mean±S.E.M. (Nemmani et al., 2001).

2.5. Effect of nimodipine on morphine antinociception in control and diabetic rats

Morphine (2, 4 and 8 mg/kg, i.p.) antinociception was determined in 4-week-old male streptozotocin-diabetic rats and their age-matched controls. Based on the dose-dependent effect, the 4-mg/kg dose of morphine producing a submaximal response was selected for the interaction study with nimodipine. The effect of pretreatment with nimodipine (0.1, 0.3, 1 and 3 mg/kg, i.p., 15 min before) or vehicle on morphine (4 mg/kg, i.p.)-induced antinociception was determined in streptozotocin-diabetic and control rats.

2.6. Effect of insulin on morphine antinociception in control and diabetic rats

The effect of insulin (2 U/kg, s.c.) or vehicle on morphine (4 mg/kg, i.p.) antinociception was determined in male streptozotocin-diabetic and control rats (n=4). Insulin was administered 20 min before morphine (4 mg/kg) administration. The blood samples were collected from different groups at 30 min after morphine administration and analyzed for glucose levels as described above.

2.7. Saturation binding of [^{3}H]PN200-110

Saturation binding was carried out with the slightly modified method of Guarneri et al. (1996).

2.7.1. Membrane preparation

The rats were killed by decapitation, using a guillotine. The brain (minus cerebellum) was removed immediately, wrapped in aluminium foil and kept on ice. The tissue was homogenised in 25 volumes of ice-cold Tris–hydrochloride (HCl) buffer (0.05 mol/l, and pH 7.7) using a Polytron homogenizer at 20,000 rpm for 20 s. The homogenate was centrifuged (SORVALL 5B RC plus, high-speed centrifuge floor model) at 49,000×g for 20 min and the pellet was resuspended in the same buffer. The homogenate was incubated at 37 °C for 30 min in order to remove the endogenous ligands from their binding sites. The suspension was centrifuged a second time, at 49,000×g for 20 min, and the pellet obtained was resuspended in Tris–HCl buffer and used for binding studies. The protein content was determined by the method of Lowry et al. (1951).

2.7.2. Binding assay

The saturation binding of [^{3}H]PN200-110 (0.005–1.0 nmol/l) to whole brain (minus cerebellum) membranes of both diabetic and normal rats was carried out in a total volume of 0.25 ml, which contained Tris–HCl buffer (0.05 mol/l) with or without nimodipine (1 μmol/l). Binding was initiated by adding tissue protein equivalent to 250–300 μg per tube. The binding assay was carried out in triplicate at 25 °C for 60 min. Specific binding was determined as the difference in binding in the absence and presence of 1 μmol/l unlabelled nimodipine. Binding was terminated by rapidly washing with ice-cold Tris–HCl buffer and filtering the contents of the incubation tubes through Whatman GF/B filter paper under reduced pressure using a Brandel cell harvester (Biomedical Research and Development Laboratories, Gaithersberg, MD, USA). The filter discs were washed thrice with 5 ml of the same ice-cold buffer. The filter discs were then transferred to liquid scintillation vials and 5 ml of scintillation cocktail, containing 3-g PPO (2,5-diphenyloxazole) and 100 mg of POPOP (2,2′ -phenylene-bis (5-phenyloxazole) in 1000 ml of sulphur-free xylene, was dispensed using a Brandel cocktail dispenser. After a 4- to 6-h equilibration period, the radioactivity in the samples was determined using a Wallac (model 1409) liquid scintillation counter.

The density (B_{max}) and dissociation constant (K_d) of [^{3}H]PN200-110 for Ca^{2+} channels were determined from the saturation curve and Scatchard plot analysis using the LIGAND program (Munson and Rodbard, 1980).

2.8. Statistical analysis

The results are expressed as means±S.E.M. The difference in %antinociception between diabetic and normal rats over the time course of the study was determined by two-way analysis of variance (ANOVA) followed by the Newman–Keuls test with 5% level of significance (P<0.05) using the Jandel Scientific's Sigma Stat software. The B_{max} values from the binding study were analyzed in an unpaired t-test with 5% level of significance.

3. Results

3.1. Morphine antinociception in control and diabetic rats

In the present study, the blood glucose levels were found to range from 287 to 460 mg/dl with a mean value of 412.0 ± 20.5 (n = 30). The vehicle of morphine produced no change in the tail-flick latency in either diabetic or control rats. The morphine-induced antinociception in streptozotocin-diabetic rats was significantly (P < 0.05) less than that in control non-diabetic rats. Morphine (2, 4 and 8 mg/kg) produced dose-dependent antinociception in control rats with a maximal %antinociception of 14.1 ± 0.9, 32.7 ± 2.5 and 86.4 ± 4.7 ($AUC_{0-180\ min}$: 533.00 ± 21.14, 1408.94 ± 60.73 and 5014.19 ± 181.9), respectively (Fig. 1). There was a significant reduction in morphine-induced antinociception in streptozotocin-diabetic rats with a maximal %antinociception of 3.1 ± 1.6, 10.9 ± 0.9 and 30.6 ± 1.2 ($AUC_{0-180\ min}$: 138.41 ± 61.18, 407.55 ± 123.02 and 1094.10 ± 170.28) for the respective doses (Fig. 1). To determine the potentiating effect of nimodipine on morphine antinociception in normal rats, a 4-mg/kg dose of morphine producing submaximal response was selected for the interaction study with nimodipine (0.1–3 mg/kg, i.p.) in both diabetic and control rats.

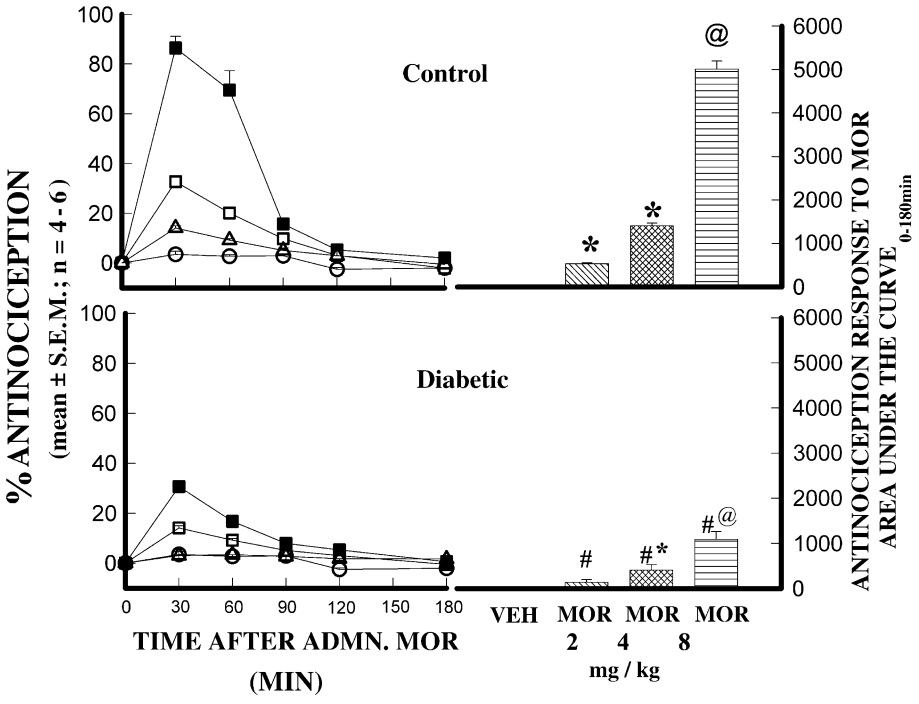

Fig. 1. Time course of action and $AUC_{0-180min}$ of morphine (MOR) tail-flick antinociception in 4-week-old male streptozotocin-induced diabetic and age-matched control Sprague–Dawley rats. Results are expressed as means±S.E.M. (n=4–6). Vehicle [○], MOR 2 mg/kg [△], MOR 4 mg/kg [□] and MOR 8 mg/kg [■]; *P<0.05 vs. Vehicle; #P<0.05 vs. corresponding control; @P<0.05 vs. MOR groups.

3.2. Effect of nimodipine on morphine antinociception in control and diabetic rats

The male rats receiving the vehicle of nimodipine did not show any antinociception in either control or diabetic groups (Table 1). Nimodipine (0.1–3 mg/kg, i.p.) per se did not produce antinociception in control or in diabetic rats (Table 1). The %antinociception at time points 30, 60 and 90 min and $AUC_{0-180min}$ of morphine in combination with nimodipine or insulin are presented in Table 1 or 2. The %antinociception up to 90 min was observed to account for 70% to 85% of the $AUC_{0-180\ min}$. The μ-opioid receptor agonist, morphine (4 mg/kg, i.p.), produced significant antinociception in control rats. In contrast, the morphine (4 mg/kg, i.p.)-induced antinociception was significantly (P<0.05) attenuated in streptozotocin-diabetic rats (Table 1). Prior administration of nimodipine (0.3, 1 and 3 mg/kg) significantly (P<0.05) potentiated the maximal %antinociception by morphine in control rats (Table 1). Nimodipine (0.1 mg/kg, i.p.) did not modify the morphine antinocicep-

Table 1
Acute effect of nimodipine (0.1–3 mg/kg, i.p.) on morphine (4 mg/kg, i.p.) antinociception at 30, 60 and 90 min after administration and the area under the curve ($AUC_{0-180\ min}$) in control and streptozotocin-diabetic rats

Treatment	%Analgesia in control rats				%Analgesia in diabetic rats			
	30 min	60 min	90 min	AUC_{0-180}	30 min	60 min	90 min	AUC_{0-180}
Vehicle	1.0 ± 0.3	0.4 ± 0.3	− 0.3 ± 0.3	17.6 ± 4.0	− 1.0 ± 0.6	− 0.2 ± 1.0	0.5 ± 0.6	37.5 ± 28.0
Nimodipine 3 mg/kg	1.6 ± 0.1	0.7 ± 0.2	− 0.7 ± 0.3	38.9 ± 5.0	1.4 ± 0.1	0.5 ± 0.1	− 0.7 ± 0.3	28.6 ± 9.0
Morphine 4 mg/kg	23.7 ± 1.1[a]	14.6 ± 1.3[a]	4.6 ± 1.0	1294.0 ± 45.0[a]	2.5 ± 0.6[b]	3.5 ± 1.3[b]	3.8 ± 0.9	341.6 ± 64[a,b]
+ nimodipine 0.1 mg/kg	20.2 ± 0.9[a]	14.6 ± 2.3[a]	9.0 ± 0.7[a]	1338.5 ± 95.0[a]	7.3 ± 1.9[a,b]	4.2 ± 2.7[b]	0.7 ± 0.8[b]	482.1 ± 113.0[a,b]
+ nimodipine 0.3 mg/kg	25.3 ± 1.0[a]	23.9 ± 4.8[c]	7.8 ± 0.7[a]	1843.6 ± 128.0[c]	10.7 ± 0.7[a,b]	9.1 ± 2.5[a,b]	6.6 ± 1.9[a]	884.8 ± 113.0[b,c]
+ nimodipine 1.0 mg/kg	51.8 ± 5.2[c]	28.3 ± 2.1[c]	10.2 ± 1.3[c]	3208.1 ± 124.0[c]	25.8 ± 1.9[b,c]	23.0 ± 1.4[c]	20.0 ± 2.4[b,c]	2899.9 ± 194.0[c]
+ nimodipine 3.0 mg/kg	51.4 ± 4.1[c]	31.3 ± 1.9[c]	16.6 ± 3.0[c]	3518.7 ± 108.0[c]	28.5 ± 1.3[b,c]	21.7 ± 1.8[b,c]	20.0 ± 0.9[c]	2926.6 ± 119.0[c]

Data given as means ± S.E.M. (n=4–6).
[a] P<0.05 vs. vehicle.
[b] P<0.05 vs. corresponding control rats.
[c] P<0.05 vs. corresponding morphine group.

Table 2
Acute effect of insulin (2 U/kg, s.c.) on morphine (4 mg/kg, i.p.) antinociception at 30, 60 and 90 min after administration, area under the curve ($AUC_{0-180\ min}$) and plasma glucose levels in control and streptozotocin-diabetic rats

Treatment group	Blood glucose (mg/dl)	%Analgesia in control rats				Blood glucose (mg/dl)	%Analgesia in diabetic rats			
		30 min	60 min	90 min	AUC_{0-180}		30 min	60 min	90 min	AUC_{0-180}
Vehicle + morphine	72.2 ± 5.0	19.1 ± 1.4	13.5 ± 2.1	12.3 ± 2.7	1534.8 ± 22.7	419.9 ± 44.7[a]	− 1.0 ± 1.1[a]	− 2.4 ± 2.3[a]	2.0 ± 4.2[a]	330.5 ± 126.1[a]
Insulin + morphine	27.3 ± 3.5[b]	27.3 ± 0.9[b]	31.6 ± 4.3[b]	19.3 ± 4.3	2630.0 ± 196.4[b]	143.7 ± 37.4[a,b]	20.7 ± 3.4[b]	25.4 ± 4.5[b]	20.9 ± 4.6[b]	2998.9 ± 303.8[b]

Data given as means ± S.E.M. (n = 4).
[a] $P < 0.05$ vs. corresponding control rats.
[b] $P < 0.05$ vs. corresponding morphine group.

tion. In addition, nimodipine (1 and 3 mg/kg, i.p.) was equally effective to potentiate morphine antinociception and an increased duration of action was observed with the 3-mg/kg dose of nimodipine (Table 1).

Moreover, in the streptozotocin-diabetic rats, nimodipine (0.3, 1 and 3 mg/kg, i.p.) dose-dependently reversed the attenuated morphine antinociception (Table 1). The pretreatment with nimodipine (1 and 3 mg/kg) not only reversed the attenuation of morphine (4 mg/kg) antinociception in diabetic rats to a level comparable to that in vehicle treated control rats but also prolonged the duration of action in diabetic rats (Table 1).

3.3. Effect of insulin on morphine antinociception in control and diabetic rats

Insulin (2 U/kg, s.c.) significantly increased the %antinociception of morphine in control rats and significantly reversed the attenuation of morphine antinociception in the diabetic rats with lowered blood glucose levels (Table 2).

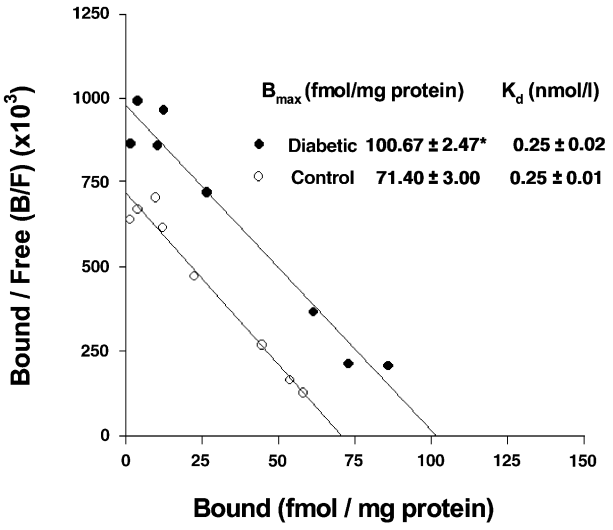

Fig. 2. Scatchard plot of saturation binding of [^{3}H]PN200-110 to rat whole brain membrane in control [○] and streptozotocin-diabetic [●] male rats. Results are expressed as means ± S.E.M. (n=3). *P<0.05 vs. Control.

Insulin not only reversed the attenuated antinociception of morphine in diabetic rats but also prolonged the duration of the effect in diabetic rats (Table 2).

3.4. Saturation binding of [^{3}H]PN200-110

The B_{max} values in control and diabetic rats (n=3) were 71.4±3.00 and 100.67±2.47 fmol/mg protein, respectively (Fig. 2). The K_d values were 0.25±0.02 and 0.25±0.01 nmol/l, respectively. There was a significant increase of B_{max} (41%) (P<0.05) of [^{3}H]PN200-110 in diabetic rat brain membranes with no change in affinity (K_d) compared to that in control rats.

4. Discussion

There was a significant attenuation of morphine-induced tail-flick antinociception in streptozotocin-diabetic rats when compared to that in control non-diabetic rats, which agrees with previous reports (Simon and Dewey, 1981; Singh et al., 1983; Tandon et al., 2000). The antinociception produced by morphine (4 mg/kg, i.p.) in control rats was potentiated by nimodipine (0.3, 1 and 3 mg/kg, i.p.) in a dose-dependent manner, which is similar to an earlier report (Hoffmeister and Tettenborn, 1986). The extent of potentiation of morphine antinociception was similar with the 1-and the 3-mg/kg dose of nimodipine with a prolonged duration of action being observed at 3-mg/kg of morphine. Nimodipine (0.3, 1 and 3 mg/kg, i.p.) also reversed the attenuated morphine antinociception in diabetic rats in a dose-dependent manner. In addition, nimodipine (1 and 3 mg/kg, i.p.) not only significantly reversed the attenuation to the control level but also increased the duration of morphine antinociception in streptozotocin-diabetic rats. This further confirms the felodipine potentiation of morphine antinociception in both control and streptozotocin-diabetic rats (Rani et al., 1996). Furthermore, insulin administration produced supersensitivity to the antinociceptive effect of morphine in control rats in addition to its hypoglycemic effect. Moreover, reversal of the hyperglycemia in streptozotocin-diabetic rats by insulin normalized the antinociceptive effect of

morphine. These findings thus indicate a possible relation of blood glucose levels to the antinociceptive responsiveness to morphine in control and streptozotocin-diabetic rats. These results are consistent with previous reports (Simon and Dewey, 1981; Singh et al., 1983; Kamei et al., 1993, 1998; Tandon et al., 2000). In the present study, a significant increase in dihydropyridine binding was observed in streptozotocin-diabetic rats, as evident from the 41% increase in the B_{max} value, compared to that in control rats. However, there was no change in K_d value of [^{3}H]PN200-110 for L-type channels in control and streptozotocin-diabetic rat brain membranes. The present study showed an up-regulation of L-type Ca^{2+} channel binding in accordance with the attenuated morphine antinociception in diabetic rats, indicating a role of Ca^{2+} in regulating analgesia in diabetes.

The mechanism(s) of the attenuation of morphine potency in the diabetic or hyperglycemic state is not clear, although some explanations have been proposed. A decrease in affinity (K_d) of [^{3}H]naloxone for the opioid receptor has been shown in brain membranes of diabetic (db/db) mice (Brase et al., 1987). A decreased release of serotonin from the bulbospinal pathways has been reported in diabetic rats (Suh et al., 1996). On the other hand, no significant change in K_d and B_{max} of [^{3}H]DAMGO for μ-opioid receptors was reported in streptozotocin-diabetic rat brain membranes (Courteix et al., 1998). In addition, the functional coupling of μ-opioid receptors and G-proteins was shown to be unaltered in streptozotocin-diabetic mice (Ohsawa et al., 2000) and genetic-diabetic NOD (non-obese diabetes) mice (Pieper et al., 2000) as indicated by an equivalent μ-opioid receptor agonist-stimulated guanosine-5′-*O*-(3-[^{3}S]thio) triphosphate ([^{35}S]GTPγS) binding in diabetic and control mice. These reports did not support down-regulation of μ-opioid receptors as a possible mechanism of decreased μ-opioid receptor agonist-antinociception in diabetes. Furthermore, the attenuation was suggested to be caused by alterations of second messengers and/or ion channels (Ohsawa et al., 2000).

Agents that reduce $[Ca^{2+}]_i$ (e.g. EGTA and ryanodine) have been shown to significantly potentiate the antinociceptive effect of DAMGO in diabetic mice (Ohsawa et al., 1998). The dihydropyridine Ca^{2+} channel blocker, felodipine, has also been shown to potentiate μ-opioid receptor agonist-antinociception in diabetic animals (Rani et al., 1996). Moreover, the agents that increase $[Ca^{2+}]_i$ (e.g. Ca^{2+} and thapsigargin) decreased the DAMGO-induced antinociception only in non-diabetic but not in diabetic mice (Ohsawa et al., 1998). The antinociceptive effect of DAMGO in diabetic mice was already less than that in non-diabetic mice (Ohsawa et al., 1998). It is likely that the attenuation of opioid-induced antinociception in the diabetic state may be due to enhanced $[Ca^{2+}]_i$ levels. Thus, the present literature suggests that an increase/decrease in $[Ca^{2+}]_i$ levels can antagonize/potentiate the μ-opioid receptor agonist-induced antinociception in both control and streptozotocin-diabetic animals. The voltage-sensitive Ca^{2+} currents through L- and N-type channels have been shown to be enhanced in dorsal root ganglion neurons of streptozotocin, Bio-Breeding/Worchester (BB/W)-diabetic rats and diabetic mice (Hall et al., 1995; Kostyuk et al., 1995; Votienko et al., 2000). These reports suggest that the diabetic state may affect $[Ca^{2+}]_i$ in neuronal tissues.

Moreover, in the present study, the hypoglycemia/normoglycemia produced by insulin in normal/streptozotocin-diabetic rats resulted in supersensitivity to/reversal of morphine antinociception, respectively. This result supports the hypothesis that hyperglycemia/hypoglycemia is responsible for decreased/increased sensitivity to the antinociceptive effect of morphine in streptozotocin-diabetic/normal rats. However, the exact mechanism involved in modulation of pain perception by glucose is not clear. The plasma levels of immunoreactive β-endorphins have been shown to be reduced significantly in anterior pituitary and hypothalamus of streptozotocin-diabetic rats (Forman et al., 1986). Insulin therapy in streptozotocin-diabetic rats has been reported to counteract the decrease in β-endorphins in the arcuate nucleus of the hypothalamus (Locatelli et al., 1986). Thus, the decreased sensitivity to morphine antinociception in diabetic rats may be due to the decreased levels of endogenous opioids. However, it has been observed that insulin-hypoglycemic rats became hypersensitive to morphine, which could be reversed by ATP-generating substrates like malate and isocitrate in spite of the fact that the rats remained hypoglycemic (Singh et al., 1983). Therefore, it appears that the metabolic effect of glucose, and not the circulating levels of glucose, is linked to cellular energetics, i.e. the ATP level and the implication of cellular ATP in glucose-induced modulation of opiate-induced antinociception is a likely possibility.

The pathophysiology and mechanism(s) of impaired Ca^{2+} homeostasis in diabetes mellitus are unclear. Insulin secretion upon increased blood glucose ultimately depends on the increased $[Ca^{2+}]_i$ levels in pancreatic β-cells through voltage-dependent Ca^{2+} channels following depolarization (Hellman, 1986; Wolf et al., 1988). A rise in $[Ca^{2+}]_i$ may be a compensatory mechanism in the hyperglycemia and impaired insulin secretion of streptozotocin-diabetic rats. It has been reported that current densities through L-type Ca^{2+} channels were increased in β-cells in diabetic cells (Kato et al., 1995). An increase in the density of Ca^{2+} channels was found with no change in their affinity for the ligands in cardiac muscle membranes (Nishio et al., 1990; Gotzsche et al., 1996) and skeletal muscles (Lee and Dhalla, 1992) of streptozotocin-diabetic rats. Insulin treatment is known to restore morphine potency in diabetic animals (Simon and Dewey, 1981; Singh et al., 1983; Kamei et al., 1993, 1998; Tandon et al., 2000). The present study showed similar results in rats. Moreover, we studied the acute effect of insulin on up-regulated L-type Ca^{2+} channels in diabetic rats. Our preliminary studies showed that the acute administration of insulin did not modify either basal [^{3}H]PN200-

110 specific binding in control rats or the increased specific binding observed in streptozotocin-diabetic rats, indicating that insulin does not alter L-type Ca^{2+} channel binding. However, the effect of chronic administration of insulin on L-type Ca^{2+} channel binding needs to be determined. In addition, insulin administration was shown to reduce contractility and $[Ca^{2+}]_i$ in vascular smooth muscle fibres, probably by stimulating the Na^+/K^+ pump and consequent membrane hyperpolarization (Kahn and Song, 1995). Thus, it may be possible that insulin is modulating Ca^{2+} channels in an indirect manner. Considerable evidence suggests that Ca^{2+}-signaling is abnormal, not only in neuronal tissues (Hall et al., 1995; Kostyuk et al., 1995; Votienko et al., 2000), but also in cardiac myocytes (Nobe et al., 1990) of diabetic animals. Reports describe diabetes as a disease of abnormal Ca^{2+} metabolism (Levy et al., 1994), inflicting diabetic cardiomyopathy to $[Ca^{2+}]_i$ overload (Heyliger et al., 1987). A diminished expression of sarcoplasmic reticulum Ca^{2+}-ATPase and ryanodine-sensitive Ca^{2+} channel mRNA has been shown in streptozotocin-diabetic rat heart (Teshima et al., 2000). It has also been shown that serum from type I diabetic patients increases L-type Ca^{2+} channel activity in cultured insulin-producing cells and GH_3 cells derived from a pituitary tumor (Juntti-Berggren et al., 1993). Similar effects were found for serum from diabetic BB/W rats, which enhanced Ca^{2+} currents in primary sensory neurons (Ristic et al., 1998). Thus, the up-regulation of L-type Ca^{2+} channels provides a possible explanation for the increased $[Ca^{2+}]_i$ currents in neuronal tissues of diabetic rats.

Thus, the present results show for the first time a significant up-regulation of L-type Ca^{2+} channels in the central nervous system of streptozotocin-diabetic rats in relation to attenuated morphine antinociception. The results also show that nimodipine significantly potentiates morphine antinociception in diabetic rats. Thus, the results indicate a functional role of L-type Ca^{2+} channels in morphine antinociception, and the diabetic state may lead to alterations in their density.

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References

Ben-Sreti, M.M., Gonzalez, J.P., Sewell, R.D.E., 1983. Effects of elevated Ca^{2+} and Ca^{2+} antagonists on 6,7-benzomorphan-induced analgesia. Eur. J. Pharmacol. 90, 385–391.

Brase, D.A., Han, Y.-H., Dewey, W.L., 1987. Effects of glucose and diabetes on binding of naloxone and dihydromorphine to opiate receptors in mouse brain. Diabetes 36, 1173–1177.

Chapman, D.B., Way, E.L., 1980. Metal ion interactions with opiates. Annu. Rev. Pharmacol. Toxicol. 20, 553–579.

Courteix, C., Bourget, P., Caussade, F., Bardin, M., Coudore, J., Fialip, J., Eschalier, A., 1998. Is the reduced efficacy of morphine in diabetic rats caused by alterations of opiate receptors or of morphine pharmacokinetics? J. Pharmacol. Exp. Ther. 285, 63–70.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Forman, L.J., Estilow, S., Lewis, M., Vasilenko, P., 1986. Streptozotocin diabetes alters immunoreactive-endorphin levels and pain perception after 8 weeks in female rats. Diabetes 35, 1309–1313.

Gotzsche, L.B., Rosenqvist, N., Gronbaek, H., Flyvbjerg, A., Gotzsche, O., 1996. Increased number of myocardial voltage-gated Ca^{2+} channels and unchanged beta-receptor number in long-term streptozotocin-diabetic rats. Eur. J. Endocrinol. 134, 107–113.

Guarneri, L., Angelico, P., Ibba, M., Poggesi, E., Taddei, C., Leonardi, A., Testa, R., 1996. Pharmacological in vitro studies of the new 1,4-dihydropyridine calcium antagonist lercanidipine. Arzneim-Forsch/Drug Res. 46, 15–24.

Hall, K., Sima, A.A.F., Wiley, J.W., 1995. Voltage-dependent calcium currents are enhanced in dorsal root ganglion neurons from the Bio Bred/Worchester diabetic rat. J. Physiol. (London) 486, 313–322.

Hellman, B., 1986. Calcium transport in the pancreatic β-cells: implications for glucose regulation of insulin release. Diabetes Metab. Rev. 2, 215–241.

Heyliger, C., Prakash, A., McNeill, J.H., 1987. Alterations in cardiac sarcolemmal Ca^{2+} pump activity during diabetes mellitus. Am. J. Physiol. 252, H540–H544.

Hoffmeister, F., Tettenborn, D., 1986. Ca^{2+} agonists and antagonists of dihydropyridine type: antinociceptive effects, interference with opiate μ-receptor agonists and neuropharmacologial actions in rodents. Psychopharmacology 90, 299–307.

Juntti-Berggren, L., Larsson, O., Rorsman, P., Ammala, C., Bokvist, K., Wahlander, K., Nicotera, P., Dypbukt, J., Orrenius, S., Hallberg, A., 1993. Increased activity of L-type Ca^{2+} channels exposed to serum from patients with type I diabetes. Science 261, 86–90.

Kahn, A.M., Song, T., 1995. Insulin inhibits dog vascular smooth muscle contraction and lowers $[Ca^{2+}]_i$ by inhibiting Ca^{2+} influx. J. Nutr. 125, 1732S–1737S.

Kamei, J., Kasuya, Y., 1995. The effects of diabetes on opioid-induced antinociception. In: Tseng, D. (Ed.), The Pharmacology of Opioid Peptides, vol. I. Harwood Academic Publishers, New York, NY, pp. 217–286.

Kamei, J., Kawashima, N., Kasuya, Y., 1992. Naloxone-induced analgesia in diabetic mice. Eur. J. Pharmacol. 210, 339–341.

Kamei, J., Kawashima, N., Kasuya, Y., 1993. Serum glucose level-dependent and independent modulation of mu-opioid agonist-mediated analgesia in diabetic mice. Life Sci. 52, 53–60.

Kamei, J., Sodeyama, M., Ohsawa, M., Kimura, M., Tanaka, S., 1998. Modulation by serum glucose levels on morphine-induced antinociceptive effect in C57BL/KsJ-db/db mice. Life Sci. 62, L1–L6.

Kato, S., Ishida, H., Tsuura, Y., Seino, Y., 1995. Altered functions of ion channels in diabetic beta cells. Obes. Res. 5, 637S–638S.

Kostyuk, E., Pronchuk, N., Shmigol, A., 1995. Calcium signal prolongation in sensory neurons of mice with experimental diabetes. NeuroReport 6, 1010–1012.

Lee, S.L., Dhalla, N.S., 1992. Ca^{2+} channels and adrenoceptors in diabetic skeletal muscle. Biochem. Biophys. Res. Commun. 184, 353–358.

Levy, J., Gavin, J., Sowers, J., 1994. Diabetes mellitus: a disease of abnormal cellular calcium metabolism. Am. J. Med. 96, 260–273.

Locatelli, V., Petralgia, F., Tirloni, N., Muller, E.E., 1986. Beta-endorphin concentration in the hypothalamus, pituitary and plasma of streptozotocin diabetic rats with and without insulin substitution. Life Sci. 38, 379–386.

Lowry, O.H., Rosebrough, N.F., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Morley, G.K., Mooradian, A.D., Levine, A.S., Morley, J.E., 1984. Mecha-

nism of pain in diabetic peripheral neuropathy: the effect of glucose on pain perception in humans. Am. J. Med. 77, 79–83.

Munson, P., Rodbard, D., 1980. Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107, 1247–1254.

Nemmani, K.V.S., Gullapalli, S., Ramarao, P., 2001. Potentiation of kappa opioid receptor agonist induced analgesia and hypothermia by fluoxetine. Pharmacol. Biochem. Behav. 69, 189–193.

Nishio, Y., Kashiwagi, A., Ogawa, T., Asahina, T., Ikebuchi, M., Kodama, M., Shigeta, T., 1990. Increase in [^{3}H]PN200-110 binding to cardiac muscle membrane in streptozotocin-induced daibetic rats. Diabetes 39, 1064–1069.

Nobe, S., Aomine, M., Arita, M., Ito, S., Takaki, R., 1990. Chronic diabetes mellitus prolongs action potential duration of rat ventricular muscles: circumstantial evidence for impaired Ca^{2+} channel. Cardiovasc. Res. 24, 381–398.

Ohsawa, M., Nagase, H., Kamei, J., 1998. Role of intracellular calcium in modification of mu and delta opioid receptor-mediated antinociception by diabetes in mice. J. Pharmacol. Exp. Ther. 286, 780–787.

Ohsawa, M., Mizoguchi, H., Narita, M., Narita, M., Kamei, J., Nagase, H., Tseng, L.F., 2000. Effects of μ-opioid receptor agonist on G-protein activation in streptozotocin-induced diabetic mice. Eur. J. Pharmacol. 401, 55–58.

Pieper, G.M., Mizoguchi, H., Ohsawa, M., Kamei, J., Nagase, H., Tseng, L.F., 2000. Decreased opioid-induced antinociception but unaltered G-protein activation in the genetic-diabetic NOD mouse. Eur. J. Pharmacol. 401, 375–379.

Rani, M.A.N., Chandran, U., Andrade, C., Venkataraman, B.V., 1996. Felodipine potentiates morphine analgesia in control and streptozotocin diabetic female rats. Ind. J. Exp. Biol. 34, 663–666.

Ristic, H., Srinivasan, S., Hall, K.E., Sima, A.A.F., Wiley, J.W., 1998. Serum from diabetic BB/W rats enhances calcium currents in primary sensory neurons. J. Neurophysiol. 80, 1236–1244.

Simon, G., Dewey, W., 1981. Narcotics and diabetes: I. The effects of streptozotocin-induced diabetes on the antinociceptive potency of morphine. J. Pharmacol. Exp. Ther. 218, 318–323.

Singh, I.S., Chatterjee, T.K., Ghosh, J.J., 1983. Modification of morphine antinociceptive response by blood glucose status: Possible involvement of cellular energetics. Eur. J. Pharmacol. 90, 437–439.

Smith, F.L., Stevens, D.L., 1995. Calcium modulation of morphine analgesia: role of calcium channels and intracellular pool calcium. J. Pharmacol. Exp. Ther. 272, 290–299.

Suh, H.W., Song, D.-K., Wie, M.-B., Jung, J.-S., Hong, H.-E., Choi, S.-R., Kim, Y.-H., 1996. The reduction of the antinociceptive effect of morphine administered intracerebroventricularly is correlated with the decrease of serotonin release from the spinal cord in streptozotocin-induced diabetic rats. Gen. Pharmacol. 27, 445–450.

Tandon, M., Srivastava, R.K., Nagpal, R.K., Khosla, P., Singh, J., 2000. Differential modulation of nociceptive responses to mu and kappa opioid receptor directed drugs by blood glucose in experimentally induced diabetes rats. Ind. J. Exp. Biol. 38, 242–248.

Teshima, Y., Takahashi, N., Saikawa, T., Hara, M., Yasunaga, S., Hidaka, S., Sakata, T., 2000. Diminished expression of sarcoplasmic reticulum Ca^{2+}-ATPase and ryanodine sensitive Ca^{2+} channel mRNA in streptozotocin-induced diabetic rat heart. J. Mol. Cell. Cardiol. 32, 655–664.

Thayer, S., Miller, R., 1990. Regulation of the intracellular free calcium concentration in single rat dorsal root ganglion neurons in vitro. J. Physiol. (London) 425, 85–115.

Vocci, F.J., Welch, S.P., Dewey, W.L., 1980. Differential effects of divalent cations, cation chelators and an ionophore on morphine and dibutyryl guanosine 3′,5′-cyclic monophosphate antinociception. J. Pharmacol. Exp. Ther. 214, 463–466.

Votienko, N.V., Kostyuk, E.P., Kruglikov, I.A., Kostyuk, P.G., 1999. Changes in calcium signaling in dorsal horn neurons in rats with streptozotocin-induced diabetes. Neuroscience 94, 887–890.

Votienko, N.V., Kruglikov, I.A., Kostyuk, E.P., Kostyuk, P.G., 2000. Effect of streptozotocin-induced diabetes on the activity of calcium channels in rat dorsal horn neurons. Neuroscience 95, 519–524.

Wolf, B.A., Colca, J.R., Turk, J., Florholmen, J., McDaniel, M.L., 1988. Regulation of Ca^{2+} homeostasis by islet endoplasmic reticulum and its role in insulin secretion. Am. J. Physiol. 254, E121–E136.