Plasma Glucose–Lowering Effect of Tramadol in Streptozotocin-Induced Diabetic Rats

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The effect of tramadol on the plasma glucose level of streptozotocin (STZ)-induced diabetic rats was investigated. A dose-dependent lowering of plasma glucose was seen in the fasting STZ-induced diabetic rats 30 min after intravenous injection of tramadol. This effect of tramadol was abolished by pretreatment with naloxone or naloxonazine at doses sufficient to block opioid μ-receptors. However, response to tramadol was not changed in STZ-induced diabetic rats receiving *p*-chlorophenylalanine at a dose sufficient to deplete endogenous 5-hydroxytrptamine (5-HT). Therefore, mediation of 5-HT in this action of tramadol is ruled out. In isolated soleus muscle, tramadol enhanced the uptake of radioactive glucose in a concentration-dependent manner. The stimulatory effects of tramadol on glycogen synthesis were also seen in hepatocytes isolated from STZ-induced diabetic rats. The blockade of these actions by naloxone and naloxonazine indicated the mediation of opioid μ-receptors. The mRNA and protein levels of the subtype 4 form of glucose transporter in soleus muscle were increased after repeated treatments for 4 days with tramadol in STZ-induced diabetic rats. Moreover, similar repeated treatments with tramadol reversed the elevated mRNA and protein levels of phosphoenolpyruvate carboxykinase in the liver of STZ-induced diabetic rats. These results suggest that activation of opioid μ-receptors by tramadol can increase the utilization of glucose and/or decrease hepatic gluconeogenesis to lower plasma glucose in diabetic rats lacking insulin. *Diabetes* 50:2815–2821, 2001

Unlike the analgesic effects of opioids, their effects on glucose metabolism in diabetes have received little attention. In diabetic patients, β-endorphin stimulates insulin secretion (1). Also, β-endorphin is known to be involved in plasma glucose homeostasis (2,3). Opioid receptors in the pancreas have been investigated for this regulation of plasma glucose (4,5). However, the effect of opioids on glucose homeostasis does not depend entirely on insulin. In our previous study (6), we found that β-endorphin is also responsible for the reduction of plasma glucose during cold exposure in streptozotocin (STZ)-induced diabetic rats, which were used as a type 1 diabetes model. Actually, injection of exogenous β-endorphin lowered plasma glucose in STZ-induced diabetic rats (6). Moreover, we demonstrated that loperamide, an agonist of opioid μ-receptors, could lower plasma glucose in STZ-induced diabetic rats (7). Thus, it has been shown that activation of opioid μ-receptors may produce a plasma glucose–lowering effect in diabetic rats lacking insulin. Clinically, tramadol has widely been used as an analgesic through activation of opioid μ-receptors (8–11) and others (9). In the present study, we investigated the effect of tramadol on plasma glucose and characterized the role of opioid μ-receptors in the action of tramadol during the absence of insulin, both in vivo and in vitro. We also examined the influence of repeated treatments with tramadol on the mRNA and protein levels of the glucose transporter subtype 4 (GLUT4) form in skeletal muscle (12) and phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme of gluconeogenesis in the liver (13), in STZ-induced diabetic rats.

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2-DG, 2-[1-^{14}C]deoxy-D-glucose; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytrptamine; GLUT4, glucose transporter subtype 4; IRI, immunoreactive insulin; KRBB, Krebs-Ringer bicarbonate buffer; PCPA, *p*-chlorophenylalanine; PEPCK, phosphoenolpyruvate carboxykinase; RIA, radioimmunoassay; STZ, streptozotocin.

RESEARCH DESIGN AND METHODS

Animal models. Male Wistar rats weighing 200–250 g were obtained from the Animal Center of National Cheng Kung University Medical College. STZ-induced diabetic rats, used as a type 1 diabetes model, were prepared by administering an intravenous injection of STZ (Sigma Chemical, St. Louis, MO) (60 mg/kg) to male Wistar rats aged 8–10 weeks after the animals were fasted for 3 days. Rats with plasma glucose concentrations ≥20 mmol/l in addition to polyuria and other diabetic features were considered to have type 1 diabetes. All studies were carried out 2 weeks after the injection of STZ. All animal procedures were performed according to the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health as well as the guidelines of the Animal Welfare Act.

Experimental protocols.

Experiment 1: Effect of tramadol on plasma glucose. The rats were divided into two groups for the investigation. After fasting overnight, STZ-induced diabetic rats in group 1 received an intravenous injection of tramadol (Pairon Pharmaceuticals, Kaohsiung City, Taiwan) at the desired doses, and blood samples (0.1 ml) were collected under sodium pentobarbital anesthesia (30 mg/kg i.p.) from the tail vein for measurement of plasma glucose. In the preliminary experiments, tramadol was found to produce the maximal plasma glucose–lowering effect in STZ-induced diabetic rats 30 min after injection. Thus, the effects of tramadol on plasma glucose, insulin, and C-peptide were determined using blood samples collected at 30 min after the injection. STZ-induced diabetic rats receiving a similar injection of vehicle at the same volume were used as controls and defined as group 2. Further experiments were performed with pharmacological inhibitors, either naloxone or naloxonazine, which were obtained from Research Biochemical (Natick, MA).

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These inhibitors were intravenously injected into fasted rats 30 min before the injection of tramadol.

Experiment 2: Investigation for the role of 5-hydroxytrptamine in the action of tramadol. STZ-induced diabetic rats received an intravenous injection of *p*-chlorophenylalanine (PCPA) at 300 mg/kg once daily for 3 successive days. The control group received the same volume of vehicle in the schedule. Changes of 5-hydroxytrptamine (5-HT) were followed by the levels of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) in plasma obtained from STZ-induced diabetic rats receiving PCPA or vehicle. Determination of 5-HT or 5-HIAA was performed by electrochemical detection as previously reported (14). Then, plasma glucose–lowering activity of tramadol (50 μg/kg) in STZ-induced diabetic rats receiving PCPA was compared with that in vehicle-treated STZ-induced diabetic rats. Also, STZ-induced diabetic rats receiving oral administration of fluoxetine (Eli Lilly) at 20 mg/kg were used to estimate the alteration in plasma glucose (15).

Experiment 3: Effects of tramadol on glucose utilization. The effects of tramadol on glucose uptake were studied using the uptake of radioactive glucose analog, 2-[1-^{14}C]deoxy-D-glucose (2-DG), in isolated soleus muscle of STZ-induced diabetic rats. Hepatocytes isolated from another group of STZ-induced diabetic rats were also used to determine the effect of tramadol on [^{14}C]glucose incorporation into glycogen.

Experiment 4: Effect of tramadol on gene expression. STZ-induced diabetic rats were given injections of vehicle, tramadol (50 μg/kg), naloxone (10 μg/kg), or both naloxone and tramadol every 8 h, three times daily, into the tail vein. In the preliminary experiments, tramadol was found to significantly modify the mRNA and protein levels for GLUT4 and PEPCK in STZ-induced diabetic rats after 4 days of treatment. Thus, the effects of tramadol on gene expression of GLUT4 and PEPCK were determined using samples collected after 4 days of treatment. Normal rats received a similar treatment of vehicle and were used as controls. After the final treatment, animals were killed without fasting. Liver and soleus muscle were immediately removed, frozen in liquid nitrogen, and stored at −70°C for Northern and Western blot analysis. Blood samples were also collected from the femoral vein of these rats before they were killed.

Laboratory determinations. Blood samples (0.1 ml) were collected by a chilled syringe containing 10 IU heparin from the tail vein of the rats while they were under anesthesia with sodium pentobarbital (30 mg/kg i.p.). Concentration of plasma glucose was measured by the glucose oxidase method via an analyzer (Quik-Lab; Ames/Miles, Elkhart, IN) (16). Radioimmunoassay (RIA) was performed to measure plasma insulin or C-peptide using a commercial kit from Linco (St. Charles, MO). A plasma sample from an STZ-induced diabetic rat was added with standard insulin or C-peptide to raise the level into the detectable range of RIA. The given value was obtained by subtracting the added standard from the measured value.

Measurement of glucose uptake into soleus muscle. Soleus muscle was isolated from STZ-induced diabetic rats and divided into long longitudinal strips (35–25 mg per strip) as previously described (17). After a 30-min preincubation period, the muscle tissue was transferred to fresh incubation flasks with or without the presence of antagonist (either naloxone or naloxonazine) at appropriate concentrations for 30 min at 37°C and then incubated with tramadol at the desired concentrations at 37°C for another 30 min under continuous shaking at 40 cycles/min. The muscle tissue was subsequently incubated with 50 μl Krebs-Ringer bicarbonate buffer (KRBB) containing 2-DG (1 μCi/ml) (NEN Research, Boston, MA) for 5 min at 37°C. Reactions were terminated by quickly blotting the muscles and dissolving them in 0.5 ml of 0.5 N NaOH for 45 min before neutralization with 0.5 ml of 0.5 N HCl. After centrifugation, 800 μl of each supernatant was mixed with 1 ml aqueous counting scintillant (ASC; Amersham, Arlington Heights, IL) and the radioactivity was determined using a β-counter (Beckman LS6000, Beckman, Fullerton, CA) (17). Uptake of 2-DG, assessed after preincubation of the muscle with 20 μmol/l cytochalasin B (Sigma Chemical), was subtracted from the total muscle–associated radioactivity (18). Specific 2-DG uptake was expressed as the percentage of basal uptake that was obtained from soleus muscle incubated with KRBB only.

Measurement of glycogen synthesis in hepatocytes. Hepatocytes were prepared as previously described (19). After the 30-min preincubation period in KRBB at 37°C, 2×10^6 hepatocytes were transferred to fresh incubation flasks containing [U-^{14}C]glucose (0.25 μCi/ml) (NEN Research), with or without the presence of antagonist, at appropriate concentrations for 30 min at 37°C and then incubated with tramadol at the desired concentrations at 37°C for 1 h, which was the optimal time obtained from preliminary experiments under continuous shaking. The incorporation of [U-^{14}C]glucose into glycogen was determined by ethanol precipitation (20). Label incorporation into glycogen was expressed as the percentage of basal level that was obtained from hepatocytes incubated with KRBB only.

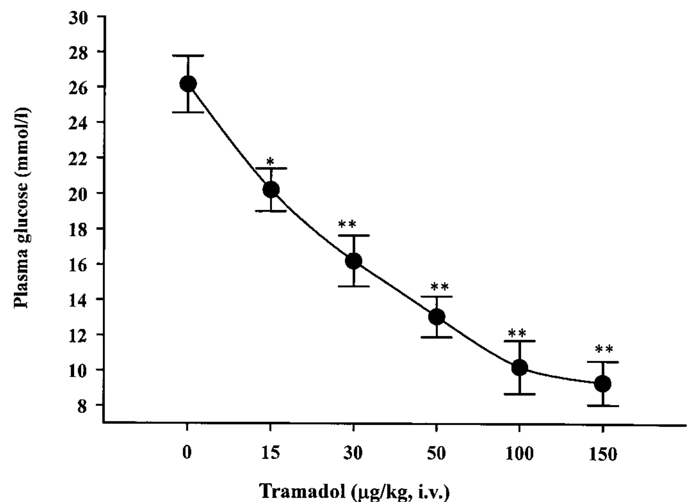

FIG. 1. Effect of tramadol on plasma glucose level in STZ-induced diabetic rats. Mean values and SE (bar) were obtained from each group of 10 animals. *P < 0.05 and **P < 0.01 vs. data from animals treated with vehicle.

Northern blotting analysis. Total RNA was extracted from liver or soleus muscle of experimental animals using the Ultraspec-II RNA extraction system (Bioteck, Houston, TX). For Northern blotting analysis, RNA (20 μg) was denatured by heating at 55°C for 15 min in a solution containing 2.2 mmol/l formaldehyde and 50% formamide (vol/vol). Aliquots of total RNA were then size-fractionated in a 1.2% agarose/formaldehyde gel. Ethidium bromide staining was used to identify the position of the 18S and 28S rRNA subunits and to confirm that equivalent amounts of undegraded RNA had been loaded. The RNA was transferred to a Hybond-N membrane (Amersham, Bucks, U.K.). GLUT4 and PEPCK mRNA levels were detected using random prime-labeled full-length cDNA under stringent hybridization conditions. Intensity of the mRNA bands on the blot was quantified by scanning densitometry (Hoefer, San Francisco, CA). The response of β-actin was used as an internal standard.

Western blot analysis. After homogenization of liver and skeletal muscle using a glass/Teflon homogenizer, the homogenates (50 μg) were separated by SDS-PAGE, and Western blot analysis was performed as previously described (17) using either anti-rat antibody to bind GLUT4 (1:1,000) (Genzyme Diagnostics, Cambridge, MA) in skeletal muscle or another anti-rat antibody (1:1,000) to bind PEPCK in liver. Blots were incubated with the appropriate peroxidase-conjugated secondary antibodies. After removal of the secondary antibody, blots were washed as described above and developed by autoradiography using the ELC-Western blotting system (Amersham, Braunschweig, Germany). Densities of the obtained immunoblots were quantified using a laser densitometer, with GLUT4 at 45 KDa and PEPCK at 69.5 KDa.

Statistical analysis. The plasma glucose–lowering activity was determined in rats that received tramadol injection under anesthesia. Data are expressed as the means ± SE for the number (n) of animals in each group, as indicated in the tables and figures. Repeated measures of analysis of variance were used to analyze the changes in plasma glucose and other parameters. Where appropriate, the Dunnett range post hoc comparisons were used to determine the source of significant differences. The concentration for 50% effect (ED_{50}) was obtained from nonlinear regression analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of tramadol on plasma glucose concentrations in STZ-induced diabetic rats. As shown in Fig. 1, a dose-dependent lowering of plasma glucose by tramadol was observed in STZ-induced diabetic rats. The maximal plasma glucose–lowering activity of tramadol in STZ-induced diabetic rats ($n = 10$) was 50.1 ± 2.2% at 50 μg/kg. This effect of tramadol was not further increased at supramaximal concentrations. Thus, 50 μg/kg tramadol was used in subsequent experiments. Plasma glucose concentrations of nonfasted STZ-induced diabetic rats were also significantly ($P < 0.01$) decreased to 15.9 ± 1.8

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TABLE 1
Effect of naloxone or naloxonazine on tramadol-induced lowering of plasma glucose in STZ-induced diabetic rats

	Plasma glucose (mmol/l)
n	10
Basal	26.1 ± 3.1
Tramadol (50 μg/kg i.v.)	
+ Vehicle	17.4 ± 2.1*
+ Naloxone (μg/kg i.v.)	
1	20.3 ± 2.2†
5	23.0 ± 2.5
10	26.6 ± 1.1
+ Naloxonazine (μg/kg i.v.)	
1	23.3 ± 1.4
5	24.8 ± 2.3
10	25.1 ± 2.9
Naloxone (10 μg/kg i.v.)	26.2 ± 2.8
Naloxonazine (10 μg/kg i.v.)	25.6 ± 3.1

Data are means ± SE. The antagonists were administered by intravenous injection 30 min before the injection of tramadol. The vehicle of distilled water containing 0.9% NaCl was used to dissolve the antagonists and was administered in the same volume. Basal level shows the value from animals receiving a similar injection of the same volume of vehicle. *$P < 0.01$ and †$P < 0.05$ vs. basal value, respectively.

mmol/l after repeated treatment with tramadol (50 μg/kg) for 4 days, as compared with vehicle-treated STZ-induced diabetic rats (26.9 ± 2.2 mmol/l). Also, the 4-day treatment of tramadol (50 μg/kg) did not influence the feeding behavior and/or body weight of STZ-induced diabetic rats. Otherwise, plasma insulin–like immunoreactivity in the STZ-induced diabetic rats was not modified by an intravenous injection of tramadol (50 μg/kg) because plasma immunoreactive insulin (IRI) in the tramadol-treated group (2.2 ± 0.6 pmol/l; $n = 10$) was not different ($P > 0.05$) from the vehicle-treated group (2.0 ± 0.6 pmol/l; $n = 10$). The value of C-peptide in plasma from the same group of STZ-induced diabetic rats was also not changed by the similar treatment of tramadol (50 μg/kg) because plasma C-peptide in the tramadol-treated group (2.5 ± 0.4 pmol/l; $n = 10$) was not markedly different ($P > 0.05$) from the vehicle-treated group (2.3 ± 0.7 pmol/l; $n = 10$). Actually, plasma IRI in STZ-induced diabetic rats was markedly ($P < 0.01$) lower than that in normal rats (223.3 ± 11.6 pmol/l; $n = 10$).

However, no change in plasma glucose level was found in normal rats receiving a similar injection of tramadol at the same dose (50 μg/kg). Tramadol, at a dose of 1–5 mg/kg, decreased the plasma glucose level in normal rats 30 min after treatment. Tramadol showed a plasma glucose–lowering activity in normal rats of ~10.5 ± 2.1%, 16.6 ± 1.8%, and 24.0 ± 1.5% after doses of 1, 3, and 5 mg/kg, respectively ($n = 6$).

In the presence of opioid μ-receptor antagonists (i.e., naloxone or naloxonazine), the plasma glucose–lowering action of tramadol in STZ-induced diabetic rats was decreased; naloxone or naloxonazine blocked the action of tramadol in a dose-dependent manner. At the highest dose, the two drugs totally abolished the action of tramadol (Table 1). However, neither naloxone nor naloxonazine alone affected the basal plasma glucose levels of STZ-induced diabetic rats (Table 1). Also, plasma IRI or C-peptide in STZ-induced diabetic rats was not modified by an intravenous injection with naloxone or naloxonazine alone at 10 μg/kg. Moreover, neither naloxone nor naloxonazine affected the plasma IRI or C-peptide in tramadol (50 μg/kg)-treated STZ-induced diabetic rats. The plasma IRI value was 2.1 ± 0.4 pmol/l ($n = 10$) in the naloxone-pretreated group and 1.9 ± 0.5 pmol/l ($n = 10$) in the naloxonazine-pretreated group of STZ-induced diabetic rats receiving tramadol (50 μg/kg). In STZ-induced diabetic rats, the plasma C-peptide value was 2.4 ± 0.5 pmol/l ($n = 10$) and 2.2 ± 0.6 pmol/l ($n = 10$) in naloxone- and naloxonazine-pretreated rats, respectively. All values were not significantly ($P > 0.05$) different from the vehicle-treated controls.

In STZ-induced diabetic rats receiving PCPA at 300 mg/kg for 3 days, concentrations of plasma 5-HT and 5-HIAA were markedly decreased, as compared with the vehicle-treated controls (Table 2). However, as shown in Table 2, the plasma glucose–lowering activity of tramadol was not changed in STZ-induced diabetic rats receiving PCPA. The plasma glucose concentration was slightly increased in STZ-induced diabetic rats receiving PCPA but was without statistical significance ($P > 0.05$). Otherwise, acute oral administration of fluoxetine at 20 mg/kg failed to modify the plasma glucose of STZ-induced diabetic rats ($n = 10$). Oral administration of fluoxetine at 20 mg/kg three times daily changed the plasma glucose of STZ-induced diabetic rats to 25.4 ± 5.1 mmol/l, which was not different from the vehicle-treated controls (26.7 ± 4.9 mmol/l).

Effect of tramadol on glucose uptake into soleus muscle. Incubation with tramadol significantly increased the glucose uptake into soleus muscle in a concentration-dependent manner from 1 nmol/l to 100 μmol/l (Fig. 2). The ED_{50} was 156 nmol/l ($n = 10$). The stimulatory effect of tramadol was not further increased even at 100 μmol/l, the supramaximal concentration (Fig. 2). In the experiments described below, 1 μmol/l tramadol was used to obtain a maximal response.

The antagonist, either naloxone or naloxonazine, was then added to the incubation medium. The basal glucose uptake was not significantly modified by naloxone and

TABLE 2
Effects of PCPA on the plasma levels of 5-HT and 5-HIAA and plasma glucose–lowering activity of tramadol in STZ-induced diabetic rats

	Vehicle-treated control	PCPA-treated group
n	9	9
Plasma concentrations		
5-HT (fmol/l)	3.2 ± 0.2	1.0 ± 0.2*
5-HIAA (fmol/l)	3.6 ± 0.3	0.9 ± 0.2*
Plasma glucose–lowering activity (%) by tramadol (50 μg/kg)	49.8 ± 4.2	45.8 ± 3.5

Data are means ± SE. Intraperitoneal injection of PCPA at 300 mg/kg was performed in STZ-induced diabetic rats for 3 successive days. Control group received the same injection of vehicle. Concentrations of 5-HT and 5-HIAA were determined using high-performance liquid chromatography–electrochemical detector from the plasma of STZ-induced diabetic rats receiving PCPA or vehicle. Plasma glucose–lowering activity (%) of tramadol (50 μg/kg) was calculated as described in RESEARCH DESIGN AND METHODS. *$P < 0.01$ compared with vehicle-treated control, respectively.

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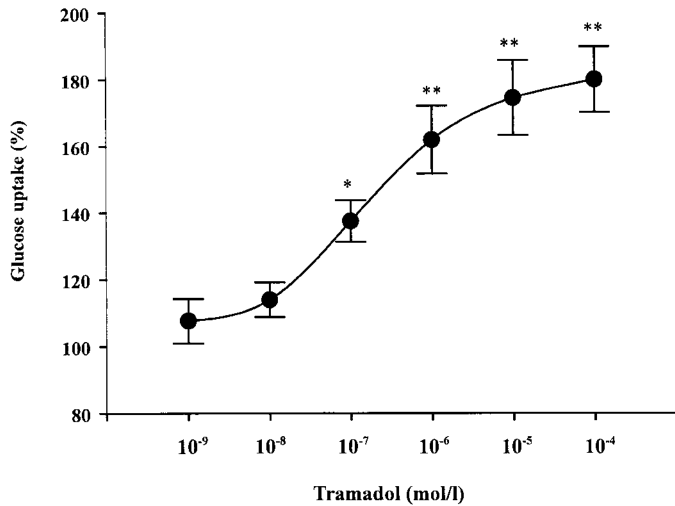

FIG. 2. Effect of tramadol on the uptake of radioactive glucose into soleus muscle isolated from STZ-induced diabetic rats. Values (means ± SE) were obtained from each group of 10 animals. Results are expressed as percentage change from control that was obtained from soleus muscle incubated with KRBB only. *P < 0.05 and **P < 0.01 vs. data from animals treated with 1 nmol/l tramadol.

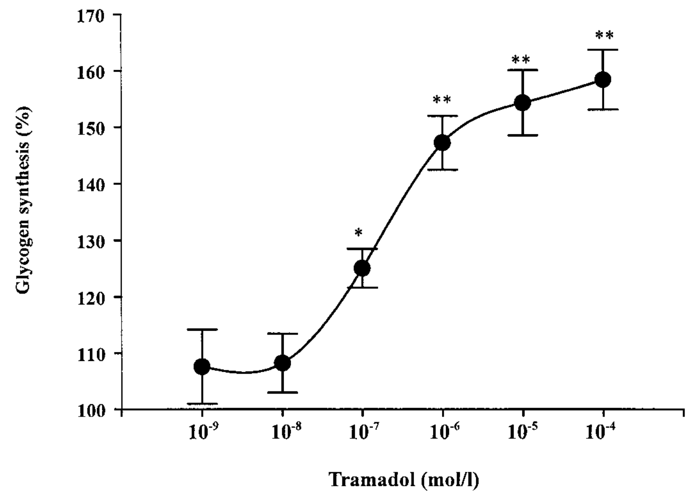

FIG. 3. Effect of tramadol on the radioactive glucose incorporation into glycogen into hepatocytes isolated from STZ-induced diabetic rats. Values (means ± SE) were obtained from each group of 10 animals. Results are expressed as percentage change from control that was obtained from hepatocytes incubated with KRBB only. *P < 0.05 and **P < 0.01 vs. data from animals treated with 1 nmol/l tramadol.

naloxonazine, but a concentration-dependent blockade of tramadol-stimulated glucose uptake was observed in soleus muscle receiving a 30-min preincubation with naloxone at concentrations ranging from 0.1 to 10 nmol/l (Table 3). Similar antagonism by naloxonazine to the action of tramadol was also obtained (Table 3).

Effect of tramadol on glycogen synthesis. In hepatocytes of STZ-induced diabetic rats, tramadol caused a marked increase of [^{14}C]glucose incorporation into glycogen in a concentration-related manner (Fig. 3). As shown in Fig. 3, a marked increase of glycogen synthesis was observed in samples treated with tramadol at concentrations of 1 nmol/l to 100 μmol/l (n = 10). Similar to the effect on glucose uptake, the level of [^{14}C]glucose incorporation into glycogen was increased by tramadol at 100 μmol/l, the supramaximal concentration, in a way not significantly ($P > 0.05$) different from the effect of tramadol at 1 μmol/l.

Data in Table 4 also show that naloxone blocked the tramadol-stimulated glycogen synthesis in hepatocytes in a concentration-dependent manner (n = 10). At 10 nmol/l, naloxone produced a marked reduction of glycogen synthesis in isolated hepatocytes to a level near to ($P > 0.05$) that of the basal level from hepatocytes incubated with vehicle only (Table 4). Also, pretreatment with naloxonazine (10 nmol/l) inhibited tramadol-stimulated glycogen synthesis to 103.4 ± 6.5% of the basal level ($P < 0.05$; n = 10). However, the basal glycogen synthesis was not directly modified by either of these inhibitors. About 95.6 ± 5.3% (n = 10) and 98.4 ± 4.1% (n = 10) of the basal glycogen synthesis in hepatocytes was maintained in rats treated with naloxone (10 nmol/l) and naloxonazine (10 nmol/l), respectively.

TABLE 3
Effect of naloxone or naloxonazine on tramadol-induced glucose uptake in soleus muscle isolated from STZ-induced diabetic rats

	Glucose uptake (%)
n	10
Tramadol (1 μmol/l)	
+ Vehicle	162.5 ± 7.4*
+ Naloxone (nmol/l)	
0.1	132.8 ± 4.9*
1	120.8 ± 4.8†
10	106.8 ± 6.1
+ Naloxonazine (nmol/l)	
0.1	135.0 ± 5.9*
1	123.3 ± 4.8*
10	107.9 ± 3.1
Naloxone (10 nmol/l)	96.6 ± 4.3
Naloxonazine (10 nmol/l)	98.1 ± 5.5

Data are means ± SE. Results are expressed as percentage changes from basal levels (100%) that were obtained from soleus muscle incubated with KRBB only. *$P < 0.01$ and †$P < 0.05$ compared with basal glucose uptake (100%), respectively.

TABLE 4
Effect of naloxone or naloxonazine on tramadol-induced glycogen synthesis in hepatocytes isolated from STZ-induced diabetic rats

	Glycogen synthesis (%)
n	10
Tramadol (1 μmol/l)	
+ Vehicle	143.8 ± 5.7*
+ Naloxone (nmol/l)	
0.1	122.7 ± 4.4*
1	116.4 ± 3.2†
10	102.8 ± 4.3
+ Naloxonazine (nmol/l)	
0.1	124.2 ± 5.2*
1	111.9 ± 6.6†
10	103.4 ± 6.5
Naloxone (10 nmol/l)	95.6 ± 5.3
Naloxonazine (10 nmol/l)	98.4 ± 4.1

Data are means ± SE. Results are expressed as percentage changes from basal levels (100%) that were obtained from hepatocytes incubated with KRBB only. *$P < 0.01$ and †$P < 0.05$ compared with basal glucose uptake (100%), respectively.

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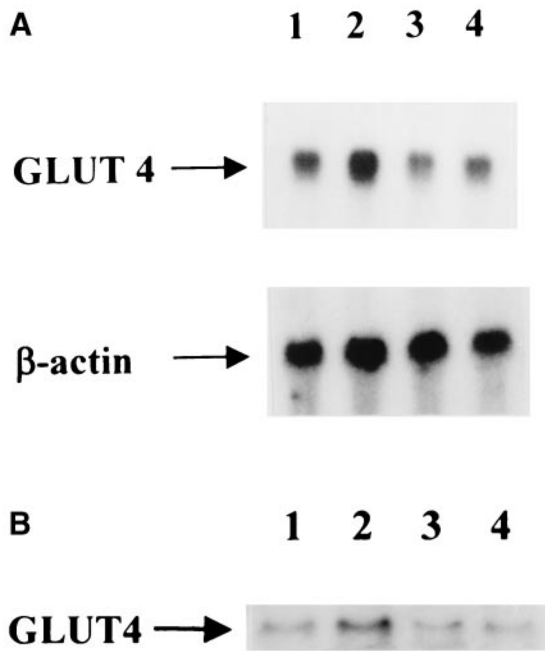

FIG. 4. ***A*****: Representative response of mRNA level for GLUT4 or β-actin in soleus muscle isolated from STZ-induced diabetic rats receiving repeated treatment with tramadol (50 μg/kg), naloxone (10 μg/kg), or both tramadol and naloxone three times daily for 4 days.** ***B*****: Identification of GLUT4 through a single band at 45 kDa using immunoblotting analysis. Lanes show vehicle-treated diabetic rats (*lane 1*), tramadol-treated diabetic rats (*lane 2*), naloxone-treated diabetic rats (*lane 3*), and tramadol plus naloxone-treated diabetic rats (*lane 4*).**

Effect of tramadol on the mRNA and protein levels of GLUT4 in soleus muscle of STZ-induced diabetic rats. Figure 4 shows the representative response of mRNA levels for GLUT4 in isolated soleus muscle from the vehicle-, tramadol-, naloxone-, or tramadol plus naloxone–treated animals, as determined by Northern blotting analysis using β-actin as the internal standard. Similar to our previous report (17), the mRNA level of GLUT4 in isolated soleus muscle from the vehicle-treated STZ-induced diabetic rats was ~52% of that from the vehicle-treated normal rats. The plasma glucose concentrations of nonfasted STZ-induced diabetic rats were decreased to 16.5 ± 3.3 mmol/l after repeated treatment with tramadol (50 μg/kg) for 4 days, as compared with vehicle-treated STZ-induced diabetic rats (26.4 ± 5.0 mmol/l) ($P < 0.01$). Repeated treatment of STZ-induced diabetic rats with tramadol (50 μg/kg) for 4 days resulted in an elevation of the GLUT4 mRNA level in soleus muscle to a level near that of vehicle-treated normal rats. Similar action of tramadol was also found on the protein levels of GLUT4 in soleus muscle from Western blotting analysis (Fig. 4). Treatment with naloxone alone (10 μg/kg) for 4 days did not affect the basal plasma glucose levels of STZ-induced diabetic rats (26.1 ± 3.8 vs. 26.3 ± 3.2 mmol/l; $P > 0.05$). Also, naloxone did not ($P > 0.05$) modify the GLUT4 mRNA and protein levels of soleus muscle isolated from diabetic rats when compared with the vehicle-treated diabetic rats (Fig. 4). The plasma glucose–lowering action of tramadol in diabetic rats was not seen after 4 days of treatment with both naloxone and tramadol. Pretreatment with naloxone (10 μg/kg) inhibited the elevation of GLUT4 mRNA level stimulated by tramadol to a level that was not

TABLE 5
Quantification of the responses of mRNA and protein levels for GLUT4 in isolated soleus muscle from STZ-induced diabetic rats

	GLUT4 (arbitrary units)	
	mRNA/β-actin	Protein
n	8	8
Vehicle	1.03 ± 0.09	1.01 ± 0.14
Tramadol (50 μg/kg)	1.62 ± 0.11*	1.52 ± 0.19*
Naloxone (10 μg/kg)	1.10 ± 0.12	1.11 ± 0.18
Tramadol (50 μg/kg) + naloxone (10 μg/kg)	1.24 ± 0.08	1.10 ± 0.20

Data are means ± SE. *$P < 0.01$ compared with vehicle-treated animals.

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different ($P > 0.05$) from the vehicle-treated diabetic rats (Fig. 4). A similar antagonism by naloxone on the elevation of the GLUT4 protein level induced by tramadol was also obtained (Fig. 4). Table 5 shows the quantification of mRNA and protein levels of GLUT4.

Effect of tramadol on the mRNA and protein levels of hepatic PEPCK in STZ-induced diabetic rats. The gene expression of PEPCK was investigated by determining the mRNA and protein levels in liver isolated from STZ-induced diabetic rats that received repeated treatments with tramadol or other regimens as described above. Similar to our previous study (17), the mRNA level of PEPCK in liver from the vehicle-treated STZ-induced diabetic rats was ~1.7-fold of that in liver of the vehicle-treated normal rats. Treatment of STZ-induced diabetic rats with tramadol three times daily for 4 days resulted in a marked reduction (62.8 ± 8.9%) of the mRNA level of PEPCK in liver, as compared with the vehicle-treated diabetic rats. However, treatment with naloxone alone in diabetic rats did not ($P > 0.05$) modify the PEPCK mRNA and protein levels in isolated liver. After pretreatment with naloxone (10 μg/kg), tramadol failed to reduce the PEPCK mRNA level in the diabetic rats; the PEPCK mRNA level from diabetic rats treated with naloxone in addition to tramadol was not different ($P > 0.05$) from the vehicle-treated diabetic rats. Similar antagonism by naloxone was seen for the reduction of PEPCK protein level induced by tramadol (Fig. 5). Table 6 shows the quantification of the mRNA and protein levels of PEPCK.

DISCUSSION

In the present study, we found that tramadol has the ability to lower the plasma glucose levels of STZ-induced diabetic

TABLE 6
Quantification of the responses of mRNA and protein levels for PEPCK in isolated liver from STZ-induced diabetic rats

	PEPCK (arbitrary units)	
	mRNA/β-actin	Protein
n	8	8
Vehicle	1.72 ± 0.09	1.60 ± 0.13
Tramadol (50 μg/kg)	1.08 ± 0.08*	1.14 ± 0.06*
Naloxone (10 μg/kg)	1.60 ± 0.07	1.53 ± 0.11
Tramadol (50 μg/kg) + naloxone (10 μg/kg)	1.65 ± 0.10	1.71 ± 0.13

Data are means ± SE. *$P < 0.01$ compared with vehicle-treated animals.

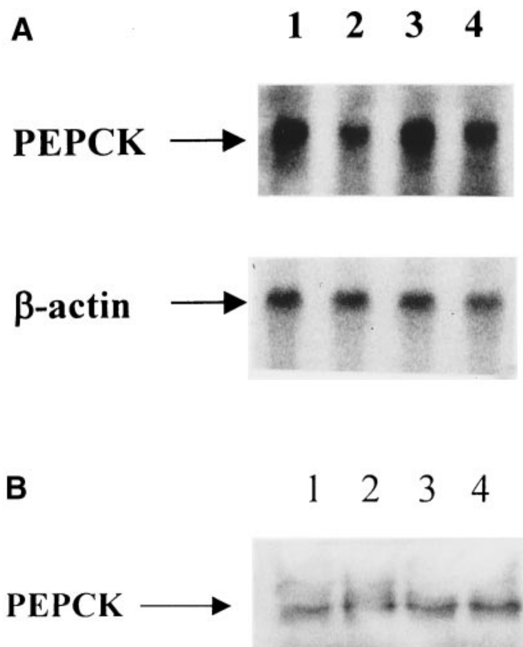

FIG. 5. ***A*: Representative response of mRNA level for PEPCK or β-actin in hepatocytes isolated from STZ-induced diabetic rats receiving repeated treatment with tramadol (50 μg/kg), naloxone (10 μg/kg), or both tramadol and naloxone three times daily for 4 days. *B*: Identification of PEPCK through a single band at 69.5 kDa using immunoblotting analysis is indicated in the lower panel. Lanes show vehicle-treated diabetic rats (*lane 1*), tramadol-treated diabetic rats (*lane 2*), naloxone-treated diabetic rats (*lane 3*), and tramadol plus naloxone-treated diabetic rats (*lane 4*).**

rats. This is consistent with our previous report that activation of opioid μ-receptors could decrease the plasma glucose concentration in STZ-induced diabetic rats (7).

Naloxone has been used in studying the relation between β-endorphin and glucose homeostasis (21). Pharmacologically, naloxone is a nonselective antagonist of opioid receptors (21), and naloxonazine is a selective antagonist of opioid μ-receptors (22). We found that naloxone and naloxonazine, at doses sufficient to block opioid μ-receptors, inhibited the plasma glucose–lowering action of tramadol in STZ-induced diabetic rats. It can thus be considered that opioid μ-receptor activation is involved in the plasma glucose–lowering action of tramadol in STZ-induced diabetic rats. Actually, either naloxone or naloxonazine given alone was ineffective in altering plasma glucose, indicating that opioid receptors should be occupied before antagonist is effective. A plasma glucose–lowering effect has also been observed with exogenous β-endorphin (6) or loperamide (7) via an activation of opioid μ-receptors.

However, tramadol has another effect in addition to the activation of opioid μ-receptor (9,23). The nonopioid effect of tramadol was documented in relation to 5-HT (9). Thus, we used PCPA to deplete the endogenous 5-HT in STZ-induced diabetic rats (24). The plasma glucose–lowering activity of tramadol was not changed in STZ-induced diabetic rats receiving PCPA (Table 2). Also, fluoxetine at a dose sufficient to block 5-HT reuptake (16) failed to modify the plasma glucose concentration in STZ-induced diabetic rats. The data that was obtained ruled out the involvement of 5-HT in the plasma glucose–lowering action of tramadol. Moreover, the effect of tramadol on plasma glucose was abolished by naloxone or naloxonazine at a dose that blocked opioid μ-receptors. There is no doubt that the lowering of plasma glucose by tramadol in STZ-induced diabetic rats is induced by an activation of opioid μ-receptors.

In STZ-induced diabetic rats, the deficiency of insulin has been documented (25). In the present study, we found that plasma insulin and C-peptide levels in STZ-induced diabetic rats were only ~1/100 of the normal rats. Therefore, the role of endogenous insulin is negligible in this STZ-induced diabetic rat model. Moreover, tramadol did not alter the plasma IRI or C-peptide levels of STZ-induced diabetic rats. Thus, the plasma glucose–lowering action of tramadol in this type 1 diabetes model was not related to the change of endogenous insulin.

In our previous study, we observed that activation of opioid μ-receptors stimulates the glucose disposal in peripheral tissues and therefore decreases plasma glucose by improving glucose utilization (7). Then, glucose uptake into skeletal muscle (26) was measured in vitro. In the present study, tramadol caused an increase in glucose uptake into soleus muscles isolated from STZ-induced diabetic rats. Blockage of this tramadol-stimulated glucose uptake by naloxonazine or naloxone was also observed. These results suggest that activation of opioid μ-receptors by tramadol can increase the utilization of glucose in peripheral tissue via an insulin-independent mechanism.

A family of glucose transporters mediates glucose transport across the cell membrane, and the GLUT4 form is predominant in skeletal muscle (12). Insulin induces a translocation of GLUT4 from microsomal membranes to plasma membranes (27). Thus, it is possible that GLUT4 is involved in the action of tramadol. We found that gene expression of GLUT4 in STZ-induced diabetic rats was increased by tramadol after repeated injection for 4 days. It has been established that long-term exposure is required for the activation of mRNA levels in cells. Because protein is generally formed with an increase in the mRNA level, it is reasonable to assume that an increase in the gene expression of GLUT4 may result in the stimulation of glucose uptake.

Mammalian cells store glycogen in liver for the production of glucose 6-phosphate in glycolysis (28). In the present study, either naloxone or naloxonazine at concentrations sufficient to block opioid μ-receptors inhibited the increase of glycogen synthesis by tramadol in STZ-induced diabetic rats. In nonfasting STZ-induced diabetic rats, tramadol was also effective in the lowering of plasma glucose after 4 days of repeated treatment. This action of tramadol was associated with a marked reduction of gene expression of PEPCK in liver. Because PEPCK catalyzes a regulatory step in gluconeogenesis, this enzyme has been widely studied in hepatic carbohydrate metabolism (13). Studies in diabetic animals have shown that augmented gluconeogenesis is a major factor in the increased plasma glucose that appears in the fasting and postabsorptive states (29). In the present study, the enhanced expression of PEPCK mRNA and protein levels in diabetic rats was suppressed by the repeated treatment with tramadol for 4 days. Gene expression of PEPCK in liver is regulated by a number of hormones (13). It may be of particular interest to determine the molecular mechanism of the effect of

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tramadol on the hepatic gene expression of PEPCK in STZ-induced diabetic rats.

Tramadol is effective in the management of pain (8), although the affinity of tramadol to opioid μ-receptors makes it less ideal than morphine and/or peptide-like agonists (30). The short half-life of peptide (31) limited its clinical development. Actually, injection of exogenous β-endorphin produced only a transient lowering of plasma glucose in STZ-induced diabetic rats (6). Otherwise, tramadol decreased the plasma glucose in normal rats at a dose of 1–5 mg/kg, a level that was higher than that required to be effective in diabetic rats. Therefore, tramadol is helpful in lowering plasma glucose in a type 1 diabetes model.

These data suggest that intravenous injection of tramadol can lower plasma glucose in STZ-induced diabetic rats by an activation of opioid μ-receptors, resulting in an increase of glucose utilization and/or a reduction of hepatic gluconeogenesis, probably via noninsulin-mediated mechanisms in peripheral tissues.

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REFERENCES

1. Curry DL, Li CH: Stimulation of insulin secretion by beta-endorphin (1-27 & 1-31). *Life Sci* 40:2053–2058, 1987
2. Feldberg W, Smyth DG: C-fragment of lipotropin—an endogenous potent analgesic peptide. *Br J Pharmacol* 60:445–453, 1977
3. Pfeiffer A, Feuerstein G, Zerbe RL, Faden AJ, Kopin IJ: Mu-receptors mediate opioid cardiovascular effects at anterior hypothalamic sites through sympatho-adrenomedullary and parasympathetic pathways. *Endocrinology* 113:929–938, 1983
4. Locatelli A, Spotti D, Caviezel F: The regulation of insulin and glucagon secretion by opiates: a study with naloxone in healthy humans. *Acta Diabetol Lat* 22:25–31, 1985
5. Khawaja XZ, Green IC, Thorpe JR, Titheradge MA: The occurrence and receptor specificity of endogenous opioid peptides with pancreas and liver of the rat: comparison with brain. *Biochem J* 267:233–240, 1990
6. Liu IM, Niu CS, Chi TC, Kuo DH, Cheng JT: Investigations of the mechanism of the reduction of plasma glucose by cold-stress in streptozotocin-induced diabetic rats. *Neurosci* 92:1137–1142, 1999
7. Liu IM, Chi TC, Chen YC, Lu FH, Cheng JT: Activation of opioid μ-receptor by loperamide to lower plasma glucose in streptozotocin-induced diabetic rats. *Neurosci Lett* 265:183–186, 1999
8. Lehmann KA: Tramadol for the management of acute pain. *Drugs* 47:19–32, 1994
9. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL: Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. *J Pharmacol Exper Ther* 260:275–285, 1992
10. Vickers MD, O'Flaherty D, Szekely SM, Read M, Yoshizumi J: Tramadol: pain relief by an opioid without depression of respiration. *Anaesthesia* 47:291–296, 1992
11. Miranda HF, Pinardi G: Antinociception, tolerance, and physical dependence comparison between morphine and tramadol. *Pharmacol Biochem Behav* 61:357–360, 1998
12. Pessin JE, Bell GI: Mammalian facilitative glucose transporter family: structure and molecular regulation. *Annu Rev Physiol* 54:911–930, 1992
13. Hanson RW, Reshef L: Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu Rev Biochem* 66:581–611, 1997
14. Cheng JT, Shen CL, Huang JJ: Decrease of catecholamine and neuropeptide Y-like immunoreactivity in the glycerol-induced acute renal failure of rats. *Res Exp Med* 190:315–322, 1990
15. Yamada J, Sugimoto Y, Kiyo I: Selective serotonin reuptake inhibitors fluoxetine and fluvoxamine induce hyperglycemia by different mechanisms. *Eur J Pharmacol* 3852:211–215, 1999
16. Chang SL, Lin JG, Chi TC, Liu I, Cheng JT: An insulin-dependent hypoglycemia induced by electroacupuncture at the Zhongwan (CV12) acupoint in diabetic rats. *Diabetologia* 42:250–255, 1999
17. Liu IM, Hsu FL, Chen CF, Cheng JT: Antihyperglycemic action of isoferulic acid in streptozotocin-induced diabetic rats. *Br J Pharmacol* 129:631–636, 2000
18. Turinsky J, Nagel GW, Elmendorf JS, Damrau-Abney A, Smith TR: Sphingomyelinase stimulates 2-deoxyglucose uptake by skeletal muscle. *Biochem J* 313:215–222, 1996
19. Fry JR, Jones CA, Wiebkin P, Bellemann P, Bridges JW: The enzymic isolation of adult rat hepatocytes in a functional and viable state. *Analyt Biochem* 71:341–350, 1976
20. Agius L, Peak M, Alberti KG: Regulation of glycogen synthesis from glucose and gluconeogenic precursors by insulin in periportal and perivenous rat hepatocytes. *Biochem J* 266:91–102, 1990
21. Martin WR: Opioid antagonists. *Pharmacol Rev* 19:463–521, 1967
22. Ling GSF, Simantov R, Clark JA, Pasternak GW: Naloxonazine actions in vivo. *Eur J Pharmacol* 129:33–38, 1986
23. Halfpenny DM, Callado LF, Hopwood SE, Bamigbade TA, Langford RM, Stamford JA: Effects of tramadol stereoisomers on norepinephrine efflux and uptake in the rat locus coeruleus measured by real time voltammetry. *Br J Anaesth* 83:909–915, 1999
24. Yamada J, Sugimoto Y, Yoshikawa T: *p*-Chloroamphetamine, a serotonin-releasing drug, elicited in rats a hyperglycemia mediated by the 5-HT_{1A} and 5-$HT_{2B/2C}$ receptors. *Eur J Pharmacol* 359:185–190, 1998
25. Wohaieb SA, Godin DV: Alterations in free radical tissue-defense mechanisms in streptozotocin-induced diabetes in rat: effects of insulin treatment. *Diabetes* 36:1014–1018, 1987
26. Ziel FH, Venkatesan N, Davidson MB: Glucose transport is rate limiting for skeletal muscle glucose metabolism in normal and STZ-induced diabetic rats. *Diabetes* 37:885–890, 1988
27. Henriksen EJ, Bourey RE, Rodnick KJ, KoranyI L, Permutt MA, Holloszy JO: Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am J Physiol* 259: E593–E598, 1990
28. Bollen M, Keppens S, Stalmans W: Specific features of glycogen metabolism in the liver. *Biochem J* 336:1–31, 1998
29. Consoli A, Nurjhan N, Capani F, Gerich J: Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes* 38:550–557, 1989
30. Chaturvedi K, Shahrestanifar M, Howells RD: mu Opioid receptor: role for the amino terminus as a determinant of ligand binding affinity. *Brain Res Mol Brain Res* 76:64–72, 2000
31. Greenwald MK, Stitzer ML, Haberny KA: Human pharmacology of the opioid neuropeptide dynorphin A(1–13). *J Pharmacol Exp Ther* 281:1154–1163, 1997

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