ELSEVIER

European Journal of Pharmacology 367 (1999) 91–99

ejp

JTT-608 controls blood glucose by enhancement of glucose-stimulated insulin secretion in normal and diabetes mellitus rats

Takeshi Ohta *, Noboru Furukawa, Fumihiko Yonemori, Korekiyo Wakitani

Japan Tobacco, Central Pharmaceutical Research Institute, 1-1, Murasaki-cho, Takatsuki, Osaka, 569-1125, Japan

Received 23 July 1998; revised 15 December 1998; accepted 18 December 1998

Abstract

We investigated the pharmacological effects of a new anti-hyperglycemic agent, JTT-608 [*trans*-4-(4-methylcyclohexyl)-4-oxobutyric acid], in normal and neonatally streptozotocin-treated rats. In normal rats, JTT-608 improved glucose tolerance at 3–30 mg/kg, doses that did not cause a decrease in fasting blood glucose levels. In contrast, tolbutamide (10–100 mg/kg) and glibenclamide (1–3 mg/kg) caused a persistent decrease in fasting blood glucose levels, and tolbutamide only improved glucose tolerance at 10–100 mg/kg. Furthermore, JTT-608 (3–30 mg/kg) enhanced insulin secretion only with glucose stimulation, but tolbutamide (10–100 mg/kg) enhanced it both with and without glucose stimulation. In neonatally streptozotocin-treated rats, JTT-608 (10–100 mg/kg) improved glucose tolerance with enhanced insulin secretion in the oral glucose tolerance test and meal tolerance test. Additionally, JTT-608 improved glucose tolerance dose dependently, but the effect of tolbutamide reached a plateau. We conclude that JTT-608 is an enhancer of glucose-stimulated insulin secretion.

Keywords: JTT-608; Anti-hyperglycemic effect; Streptozotocin; Insulin secretion

1. Introduction

The hyperglycemia is attributed to two major defects, namely insulin resistance in insulin-sensitive tissues of non-insulin-dependent diabetes mellitus and depletion of glucose-stimulated insulin secretion from the pancreas (Perley and Kipnis, 1967; Pfeifer et al., 1981; Taylor et al., 1994). As a result of these abnormalities, patients with non-insulin-dependent diabetes mellitus usually develop elevated fasting blood glucose levels and excessive glucose fluctuations after meals (Firth et al., 1986; Kelley et al., 1994). In the clinical management of these patients, sulfonylurea derivatives, which stimulate insulin secretion directly in the pancreas, are the most widely used hypoglycemic agents (Gerich, 1989; Groop, 1992). However, sulfonylurea derivatives can cause severe and prolonged hypoglycemia (Asplund et al., 1983; Ferner and Neil, 1988) because of their long duration of action and glucose-independent action, and result in pancreatic degeneration after long-term treatment (Sodoyez et al., 1970; Dunbar and Foá, 1974; Kawai et al., 1991). For the purpose of avoiding this side effect of sulfonylurea derivatives and of achieving good control of glucose fluctuations after meals in patients with non-insulin-dependent diabetes mellitus, we developed an innovative drug, JTT-608 [*trans*-4-(4-methylcyclohexyl)-4-oxobutyric acid], which restores the defect of glucose-stimulated insulin secretion in the pancreas, presumably by stimulating insulin secretion only in the presence of high glucose concentrations.

We have already found that JTT-608 enhances insulin secretion in a mouse pancreatic β cell line, MIN (mouse insulinoma) 6 cells, in a glucose concentration-dependent manner. In isolated perfused pancreas of normal and neonatally streptozotocin-treated rats, JTT-608 enhanced insulin secretion at high glucose concentrations, but not at low concentrations. Moreover, JTT-608 did not inhibit the binding of [^{3}H]glibenclamide to membrane fractions of MIN6 cells (Furukawa et al., provisional acceptance). This lack of binding to sulfonylurea receptors suggested that JTT-608 might act by mechanisms different from those of conventional hypoglycemic drugs such as sulfonylurea derivatives.

In the present study, we investigated the pharmacological profile of JTT-608, namely its hypoglycemic action, improvement of glucose tolerance and enhancement of

* Corresponding author. Tel.: +81-726-81-9700; Fax: +81-726-81-9722

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00952-2

insulin secretion in normal rats and neonatally streptozotocin-treated rats, and compared the effects of JTT-608 with those of tolbutamide or glibenclamide.

2. Materials and methods

2.1. Chemicals

JTT-608 was synthesized at Japan Tobacco, Central Pharmaceutical Research Institute (Osaka, Japan). Tolbutamide (Wako Pure Chemical, Osaka, Japan) and glibenclamide (Research Biochemicals International, Natic, USA) were used as reference drugs.

2.2. Animals

This experiment complied with the Guidelines for Animal Experimentation of our laboratories. Male Wistar rats and pregnant Sprague–Dawley rats were purchased from Charles River Japan (Tokyo, Japan). The animals received standard laboratory chow, CRF-1 (Oriental Yeast, Tokyo,

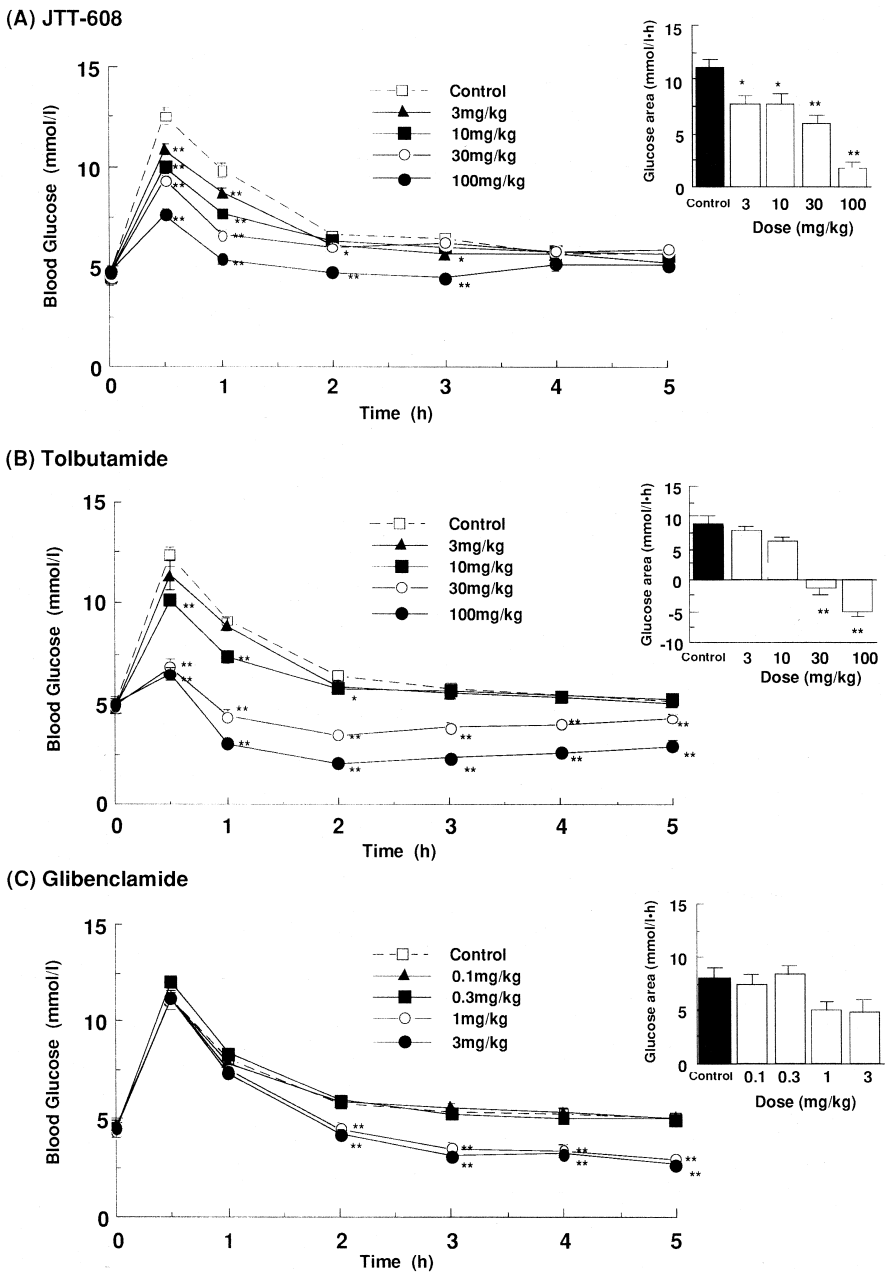

Fig. 1. The effects of JTT-608 (A), tolbutamide (B) and glibenclamide (C) on blood glucose level in glucose-loaded normal rats. The bar graphs show the area under the glucose response curve from 0 h to 3 h. The chemicals were administered orally 10 min before glucose-loading (1 g/kg i.p.). Data represent means ± S.E.M. ($n = 6$). $*\ P < 0.05$, $**\ P < 0.01$; significantly different from the control by Dunnett's two-tailed test.

Japan) and water ad libitum. They were housed in a temperature (23 ± 3°C)-, humidity (55 ± 15%)- and light (diurnal time; 0800–2000 h)-controlled room. Male Wistar rats (7-weeks-old) were used in some experiments as a normal model. Neonatally streptozotocin-treated rats were prepared according to a previously described method (Portha et al., 1974; Weir et al., 1981) with some modifications. In brief, male Sprague–Dawley pups (1.5 days old) received a single subcutaneous injection of 120 mg/kg streptozotocin (Sigma, St. Louis, MO) freshly dissolved in citrate buffer (pH = 4.3). After weaning, the rats were housed under the above conditions for 3–4 weeks. Animals that did not excrete sugars in the urine were selected 1–2 weeks before the experiments. In the selected animals, an oral glucose tolerance test (2 g glucose/kg) or a meal tolerance test (20 kcal/kg, composition of the liquid meal; 19.2% soda-casein, 0.2% L-cystine, 0.1% DL-methionine, 3.9% corn oil, 13.2% olive oil, 2.3% vitamin mixture, 4.6% salt mixture, 1.3% ethyl linoleate, 53.8% sucrose, 1.2% carrageenan; Nihon Nosan, Japan) was performed. The animals were divided into several groups according to their glucose profiles.

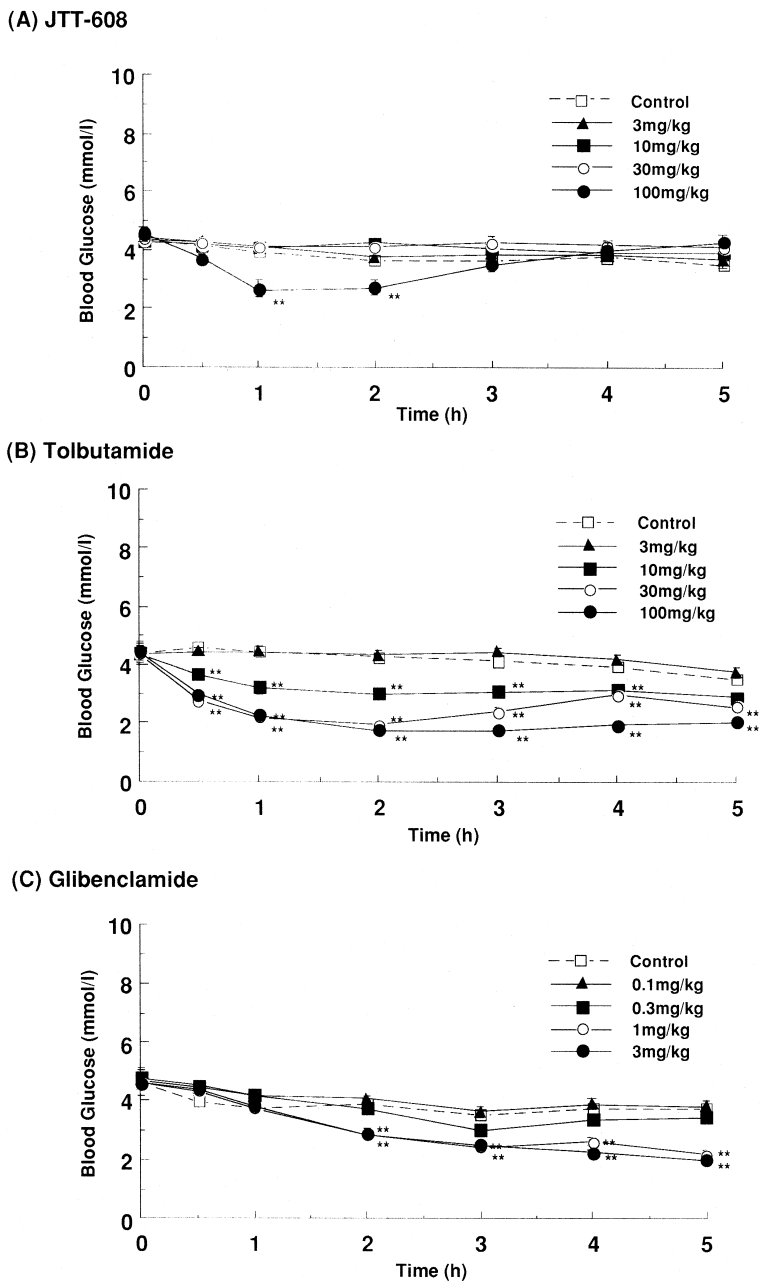

Fig. 2. The effects of JTT-608 (A), tolbutamide (B) and glibenclamide (C) on blood glucose levels in fasted normal rats. Data represent means ± S.E.M. ($n = 6$). $*\ P < 0.05$, $**\ P < 0.01$; significantly different from the control by Dunnett's two-tailed test.

2.3. Drug administration and blood sample collection

Drugs, suspended in 0.5% methyl cellulose (MC) solution, were administered orally by means of a stomach tube at a volume of 5 ml/kg. Blood samples were taken from the tail vein before and periodically after the administration of drugs, glucose or liquid meal for determination of blood glucose or insulin.

2.4. Effect of drugs in normal rats

Male Wistar rats (7-weeks-old, 170–220 g) were used after overnight fasting. The effect of drugs on blood glucose levels in glucose-loaded animals was examined by means of an intraperitoneal glucose tolerance test (1 g glucose/kg) 10 min after the oral administration of JTT-608, tolbutamide or glibenclamide. The effect of these drugs on fasting blood glucose levels was examined after their oral administration. The effect on insulin secretion of JTT-608 and tolbutamide was examined by means of an intravenous glucose tolerance test (0.25 g glucose/kg) 10 min after the oral administration of each drug.

2.5. Effect of JTT-608 in neonatally streptozotocin-treated rats

Male neonatally streptozotocin-treated rats (8 or 10-weeks-old, 180–310 g) were used after overnight fasting. The improvement of glucose tolerance and enhancement of

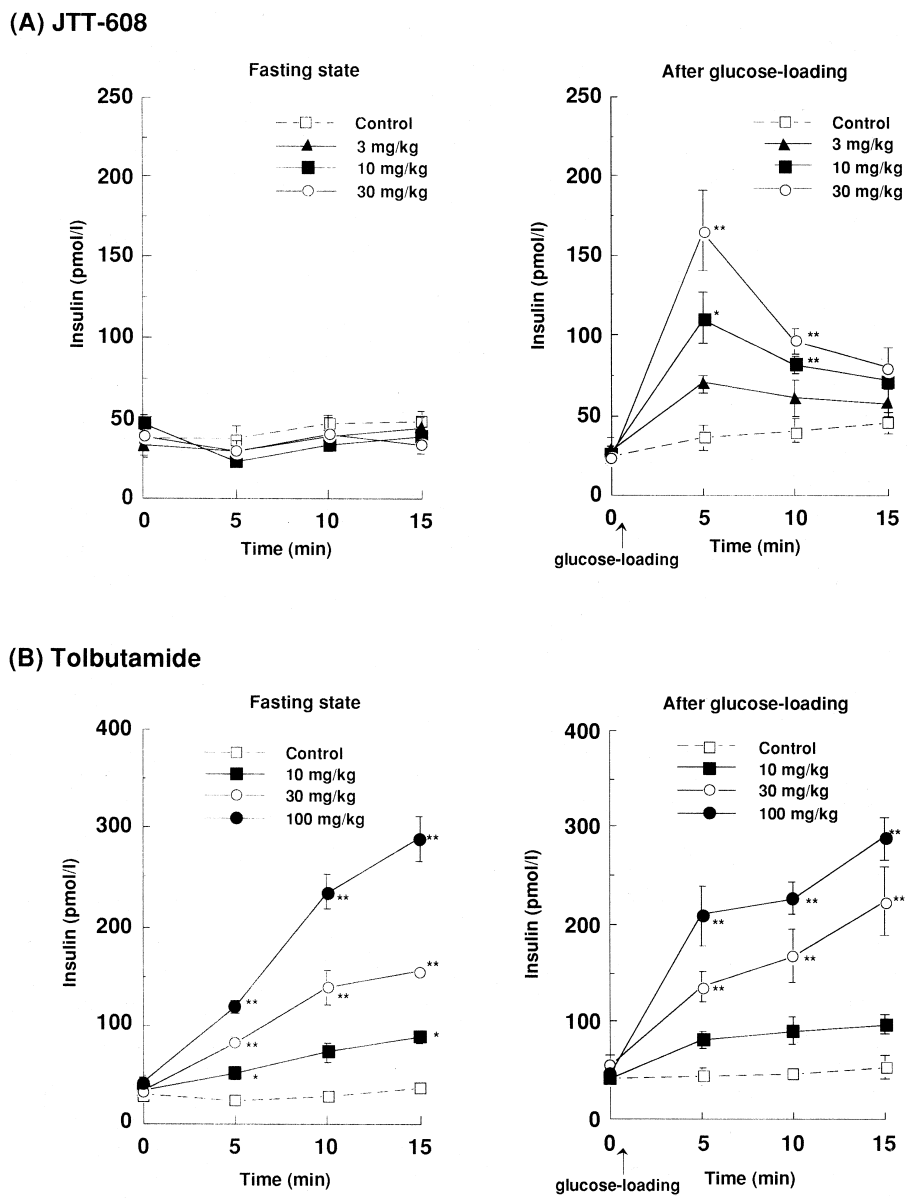

Fig. 3. The effects of JTT-608 (A) and tolbutamide (B) on blood insulin levels in fasted or glucose-loaded normal rats. The chemicals were administered orally 10 min before glucose injection (0.25 g/kg i.v.). Saline (1.25 ml/kg), in place of glucose, was injected for the fasting state. Data represent means ± S.E.M. ($n = 6$). $*P < 0.05$, $**P < 0.01$; significantly different from the control by Dunnett's two-tailed test.

insulin secretion were examined by means of an oral glucose tolerance test (2 g glucose/kg) or meal tolerance test (20 kcal/kg) 10 min after the oral administration of JTT-608. The effect on fasting blood glucose levels of oral administration of JTT-608, tolbutamide or glibenclamide was also investigated. Moreover, the efficacy of JTT-608 and tolbutamide in improving glucose tolerance was compared.

2.6. Analytical procedures

Serum glucose was measured by the hexokinase method using a commercial kit (Boehringer Mannheim, Tokyo, Japan). Serum insulin concentrations were determined by the two-antibody procedure using a radioimmunoassay kit (Shionogi, Osaka, Japan).

2.7. Statistical analysis

All results are expressed as the means ± S.E.M. Statistical analyses of differences between mean values were performed with One-way analysis of variance (ANOVA) followed by Dunnett's two-tailed test or Tukey's test. Differences were defined as significant at $P < 0.05$.

3. Results

3.1. Effect of JTT-608, tolbutamide and glibenclamide on blood glucose in glucose-loaded normal rats

The effect of JTT-608 on glycemic control in glucose-loaded rats (1 g glucose/kg i.p.) was compared with that of sulfonylurea derivatives (Fig. 1). The increase in blood glucose due to glucose-loading was inhibited dose dependently in rats receiving 3 mg/kg or higher of JTT-608. This inhibitory effect disappeared within 4 h at all doses treated (Fig. 1A). JTT-608 rapidly suppressed hyperglycemia after glucose-loading but did not cause persistent hypoglycemia.

Tolbutamide inhibited the increase in blood glucose due to glucose-loading in rats receiving 10 mg/kg or higher, but persistent hypoglycemia was seen in rats receiving 30 mg/kg or higher (Fig. 1B). In rats receiving glibenclamide, persistent hypoglycemia was only seen in rats receiving 1 mg/kg or higher, with no inhibition of the increase in blood glucose concentration due to glucose-loading being observed at any dose (Fig. 1C).

The bar graphs in Fig. 1 show the area under the curve (AUC) for glucose concentrations from 0 h to 3 h as another index of the improvement in glucose tolerance. JTT-608 decreased the glucose AUC dose dependently at 3 mg/kg or higher. Tolbutamide decreased the glucose AUC dose dependently, but glibenclamide did not cause a significant decrease. Peak glucose concentrations at 0.5 h after glucose-loading among each control group were almost equal, but there was some variation in the glucose AUC in the rats of each control group after glucose-loading.

3.2. Effect of a single oral dose of JTT-608, tolbutamide or glibenclamide on blood glucose levels in fasted normal rats

The effect of JTT-608 on blood glucose levels in fasted normal Wistar rats was compared with that of sulfonylurea derivatives (Fig. 2). JTT-608 did not decrease fasting blood glucose levels at 3–30 mg/kg, but transiently decreased them at 100 mg/kg from 1 h to 2 h (Fig. 2A). In contrast, tolbutamide and glibenclamide dose dependently caused a persistent decrease in fasting blood glucose levels (Fig. 2B, C). Tolbutamide had a hypoglycemic action at doses of 10–100 mg/kg from 0.5 h after administration until the end of the experiment. Glibenclamide exhibited a slower onset of its hypoglycemic action (2 h) and caused a

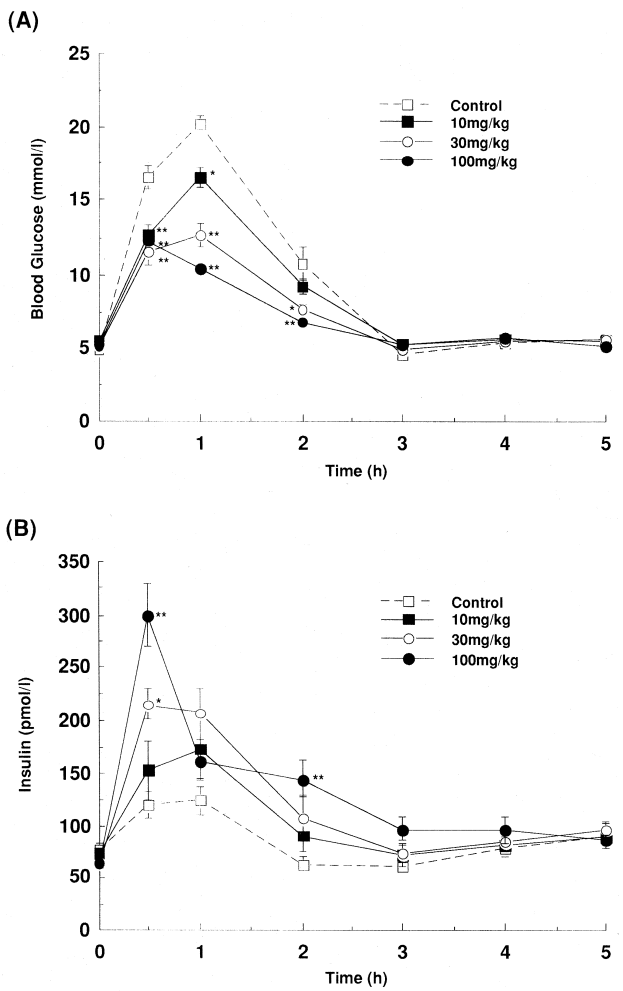

Fig. 4. The effects of JTT-608 on blood glucose (A) and insulin (B) levels in glucose-loaded neonatally streptozotocin-treated rats. JTT-608 was administered orally 10 min before glucose-loading (2 g/kg p.o.). Data represent means ± S.E.M. ($n = 6$). * $P < 0.05$, ** $P < 0.01$; significantly different from the control by Dunnett's two-tailed test.

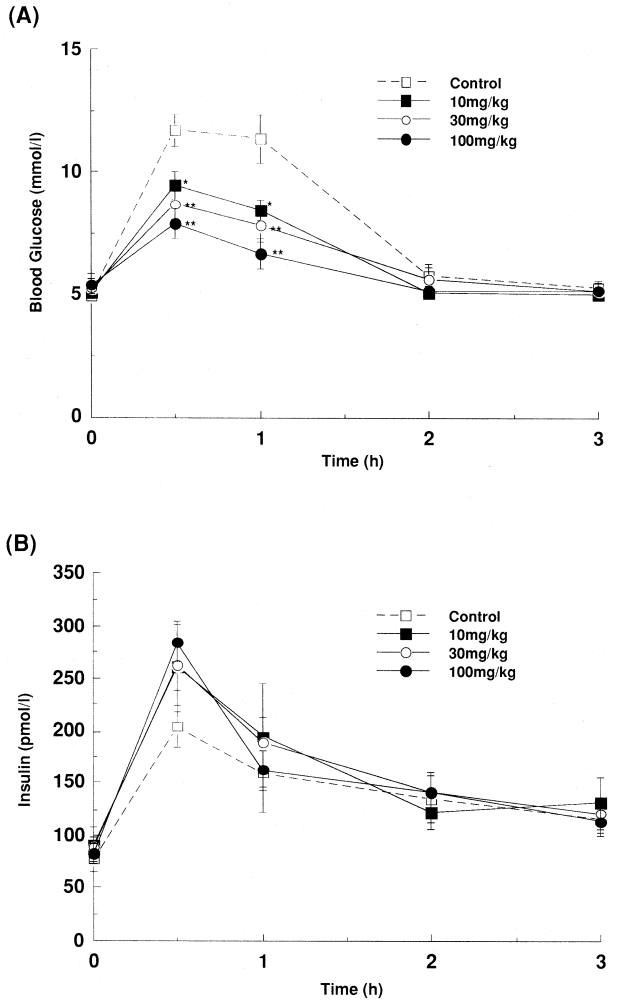

Fig. 5. The effects of JTT-608 on blood glucose (A) and insulin (B) levels in meal-loaded neonatally streptozotocin-treated rats. JTT-608 was administered orally 10 min before meal-loading (20 kcal/kg). Data represent means ± S.E.M. ($n = 6$). $*P < 0.05$, $**P < 0.01$; significantly different from the control by Dunnett's two-tailed test.

persistent decrease at 1–3 mg/kg. Thus, the effect of JTT-608 on fasting blood glucose levels was markedly different from that of tolbutamide or glibenclamide.

3.3. Effect of JTT-608 and tolbutamide on insulin secretion

In the fasting state, JTT-608 did not stimulate insulin secretion at doses of 3–30 mg/kg. After glucose-loading, however, JTT-608 enhanced insulin secretion dose dependently, especially at 5 min (Fig. 3A). Tolbutamide (10–100 mg/kg) enhanced insulin secretion dose and time dependently regardless of whether or not there was glucose stimulation (Fig. 3B).

3.4. Improvement of glucose tolerance and enhancement of insulin secretion in neonatally streptozotocin-treated rats

The efficacy of JTT-608 was investigated by means of an oral glucose tolerance test or a meal tolerance test in neonatally streptozotocin-treated rats as a model of non-insulin-dependent diabetes mellitus. When JTT-608 was administered at doses of 10–30 mg/kg 10 min before glucose-loading (2 g glucose/kg p.o.), the impaired glucose tolerance was improved dose dependently and insulin secretion was enhanced (Fig. 4). Moreover, JTT-608 improved glucose tolerance in a meal tolerance test and tended to enhance insulin secretion (Fig. 5).

3.5. Effect of a single oral dose of JTT-608, tolbutamide or glibenclamide on blood glucose levels in fasted neonatally streptozotocin-treated rats

The effect of JTT-608 on blood glucose levels in fasted neonatally streptozotocin-treated rats was compared with that of sulfonylurea derivatives (Fig. 6). JTT-608 did not decrease fasting blood glucose levels at 10–30 mg/kg, but

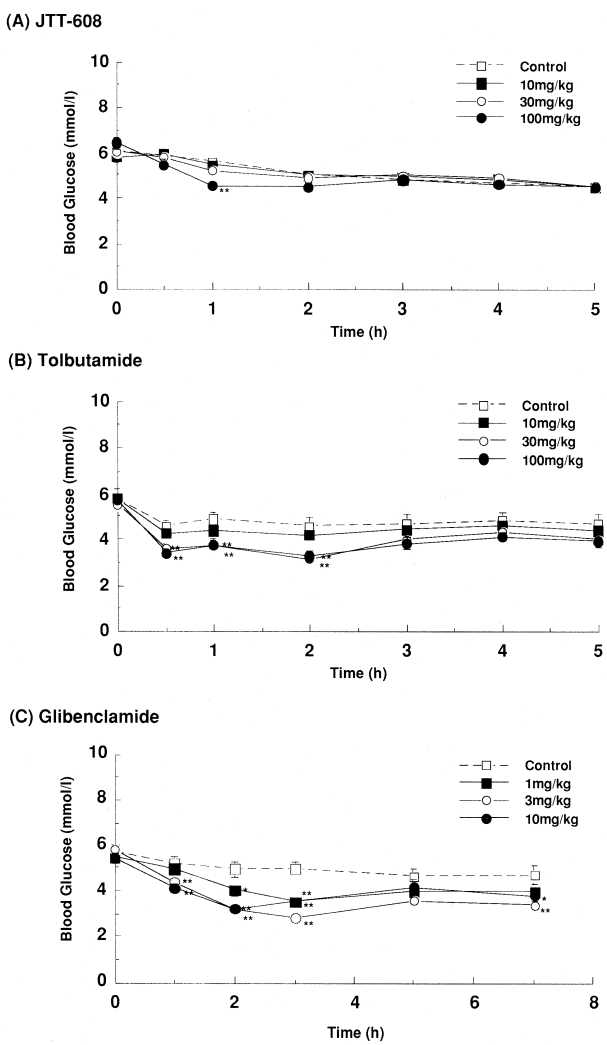

Fig. 6. The effects of JTT-608 (A), tolbutamide (B) and glibenclamide (C) on blood glucose levels in fasted neonatally streptozotocin-treated rats. Data represent means ± S.E.M. ($n = 5$). $*P < 0.05$, $**P < 0.01$; significantly different from the control by Dunnett's two-tailed test.

slightly decreased them at 100 mg/kg (Fig. 6A). In contrast, tolbutamide and glibenclamide caused a persistent decrease in fasting blood glucose levels (Fig. 6B, C). Tolbutamide had a hypoglycemic action at 10 mg/kg or higher in fasted normal rats, but not significantly at 10 mg/kg in neonatally streptozotocin-treated rats.

3.6. Effect of JTT-608 and tolbutamide on blood glucose in glucose-loaded neonatally streptozotocin-treated rats

To compare the effect on improvement of glucose tolerance, JTT-608 (30 and 100 mg/kg) or tolbutamide (30 and 100 mg/kg) was orally administered to neonatally streptozotocin-treated rats 10 min before glucose-loading (2 g glucose/kg p.o.). Fig. 7A shows the change in blood glucose level after glucose-loading. Fig. 7B shows the glucose AUC from 0 h to 3 h. Both JTT-608 and tolbutamide inhibited the increase in blood glucose level. However, the persistent hypoglycemia seen with tolbutamide (100 mg/kg) was not observed in JTT-608-treated animals (Fig. 7A). JTT-608 suppressed the glucose AUC dose dependently, but the efficacy of tolbutamide reached a plateau (Fig. 7B). It was noted that JTT-608 improved glucose tolerance more effectively than tolbutamide.

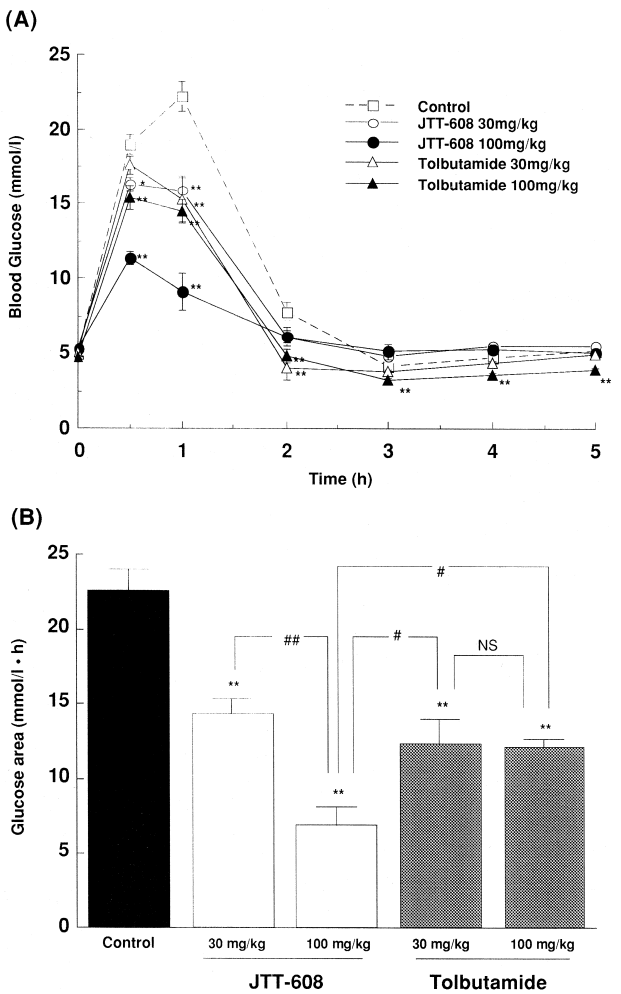

Fig. 7. (A) The effects of JTT-608 and tolbutamide on blood glucose levels in glucose-loaded neonatally streptozotocin-treated rats. (B) Comparison of the effect of JTT-608 and tolbutamide on the area under the curve (AUC) for the glucose response from 0 h to 3 h. Glucose AUCs were calculated from the data shown in (A). The chemicals were administered orally 10 min before glucose-loading (2 g/kg p.o.). Data represent means ± S.E.M. ($n = 5$). $*P < 0.05$, $**P < 0.01$; significantly different from the control by Dunnett's two-tailed test. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; significantly different by Tukey's test.

4. Discussion

In the present study, we have demonstrated that JTT-608 stimulates insulin secretion glucose concentration dependently and improves glucose tolerance in normal and neonatally streptozotocin-treated rats. Non-insulin-dependent diabetes mellitus occurs in adults rat following neonatal injection of streptozotocin. Neonatally streptozotocin-treated rats exhibit slightly lowered plasma insulin levels, slightly elevated basal plasma glucose levels, and lowered pancreatic insulin content compared with control rats. Moreover, there is no insulin response to glucose stimulation over the range of 5.5–22 mM, thus, indicating the complete loss of β-cell sensitivity to glucose. In the absence of glucose, the β-cells of neonatally streptozotocin-treated rats are hypersensitive to arginine and leucine (Portha et al., 1989). The abnormal secretion of insulin found in neonatally streptozotocin-treated rats bears a resemblance to the insulin secretory characteristics found in non-insulin-dependent diabetes mellitus patients (Giroix et al., 1983). Therefore, investigation of the effect of JTT-608 in neonatally streptozotocin-treated rats is useful in order to predict its effect in patients with non-insulin-dependent diabetes mellitus.

JTT-608 inhibited the increase in blood glucose level due to glucose-loading at doses of 3 mg/kg or higher in normal rats (Fig. 1A), or at 10 mg/kg or higher in neonatally streptozotocin-treated rats (Fig. 4A). JTT-608 did not decrease fasting blood glucose levels at doses of 30 mg/kg or lower, but transiently decreased glucose levels at 100 mg/kg in both normal and neonatally streptozotocin-treated rats (Figs. 2A and 6A). In brief, JTT-608 improved glucose tolerance dose dependently at doses of 3–30 mg/kg without causing a decrease in fasting blood glucose levels in both normal and neonatally streptozotocin-treated rats. In contrast, tolbutamide (10–100 mg/kg) and glibenclamide (1–3 mg/kg) caused a persistent decrease in fasting blood glucose levels in both normal (Fig. 2B, C) and neonatally streptozotocin-treated rats (Fig. 6B, C), and tolbutamide only inhibited the increase in blood glucose concentration due to glucose-loading at 10 mg/kg or higher in normal rats (Fig. 1B). This indicates that, irrespective of the dose, sulfonylurea derivatives cannot improve glucose tolerance without exerting a hypo-

glycemic action. The effect of JTT-608 was most pronounced when peak glucose concentrations were obtained 0 h to 0.5 h after glucose-loading. There was some variation in the glucose AUC achieved in the rats of each control group (bar graphs in Fig. 1), but peak glucose concentrations at 0.5 h after glucose-loading among each control group were almost equal. The rats were glucose-loaded 10 min after drug administration. Since in our preliminary experiments JTT-608 or tolbutamide did not decrease the blood glucose level 10 min after treatment (data not shown), the basal glucose level (0 h) at the start of the experiment probably did not change (Fig. 1). Thus, the increase in glucose level after glucose-loading is important for the effect of JTT-608. Given the observation that JTT-608 enhanced insulin secretion only in the presence of glucose (Fig. 3A), the improvement of glucose tolerance by JTT-608 without a concurrent decrease in fasting blood glucose levels was caused by the enhancement of glucose-stimulated insulin secretion. The peak plasma concentration of unchanged drug in rats treated orally with JTT-608 (30 mg/kg) was about 300 μM, which correlates with data obtained in vitro, showing an enhancement of glucose-stimulated insulin secretion at 100–300 μM of JTT-608 (Furukawa et al., provisional acceptance).

The effect of JTT-608 on glucose-stimulated insulin secretion was also examined in fasted normal rats (Fig. 3A). The observation that the glucose-loaded rats of the control group did not show an increase in insulin secretion in response to the glucose challenge can be explained by the following: (1) We used fasted rats. It is reported that glucose-stimulated insulin secretion is diminished in well-fasted rats compared with fed rats (Efendic et al., 1976). (2) The glucose load (0.25 g/kg) was small. We found that insulin secretion increased in 0.5–1 g/kg glucose-loaded rats (data not shown). (3) Basal insulin levels were low (about 30 pmol/l), and insulin levels were severely reduced by fasting. JTT-608 (3–30 mg/kg) did not enhance insulin secretion in fasted normal rats, but caused a dose-dependent increase in insulin secretion in glucose-loaded rats. Furthermore, since insulin secretion at 5 min after glucose-loading was clearly increased, it is probable that JTT-608 increases the first phase of insulin secretion in vivo. In contrast, tolbutamide enhanced insulin secretion dose dependently regardless of whether or not there was glucose stimulation and the insulin secretion was time-dependent, which was also different from the response to JTT-608 (Fig. 3B). Insulin secretion with tolbutamide was not glucose concentration-dependent and did not show specific enhancement of the first phase. It has been reported that glibenclamide increases second-phase insulin release under conditions of moderate hyperglycemia, but does not enhance the first-phase insulin release and has no additional effect on insulin release at high blood glucose levels (Ligtenberg et al., 1997). In patients with non-insulin-dependent diabetes mellitus, there is a progressive decline or loss of the first phase of insulin secretion even though the fasting plasma insulin level is normal or increased (Polonsky et al., 1988; Porte, 1991). This defect of early secretion is regarded as an important cause of postprandial hyperglycemia (Kosaka et al., 1994). Therefore, restoration of the impaired secretion of insulin may be of therapeutic value in controlling blood glucose levels in patients with non-insulin-dependent diabetes mellitus. In this regard, JTT-608 may be expected to inhibit fluctuations in postprandial glucose levels and to control blood glucose levels in patients with non-insulin-dependent diabetes mellitus. This is supported by the finding that JTT-608 improved glucose tolerance not only to an oral glucose tolerance test but also to a meal tolerance test (Figs. 4 and 5), and that JTT-608 improved glucose tolerance without causing a decrease in fasting blood glucose levels, suggesting that JTT-608 may be less likely to cause hypoglycemia than sulfonylurea derivatives.

The reason why 100 mg/kg of JTT-608 decreased fasting blood glucose levels (Figs. 2A and 6A) is not clear. This decrease may have been induced by a stimulation of insulin secretion in response to a change in sensitivity to glucose or by another action. The basal glucose level (about 5.5 mM) in neonatally streptozotocin-treated rats is not much higher than that (about 4.5 mM) of normal rats. The effect of JTT-608 was most pronounced when glucose levels were raised after glucose- or meal-loading. In particular, the enhancement of glucose-stimulated insulin secretion after glucose-loading was important for the effect of JTT-608. Moreover, the decrease in fasting blood glucose levels with JTT-608 was transient and not serious because JTT-608 is rapidly absorbed, metabolized and excreted. In rats treated orally with 30 mg/kg of JTT-608, plasma concentrations reached a peak at 0.25 h, after which they declined with a half-life of 0.43 h (data not shown). Furthermore, the enhancement of insulin secretion by JTT-608 was glucose concentration-dependent. We are currently studying the effect of JTT-608 in Goto–Kakizaki rats, a genetic model of non-obese non-insulin-dependent diabetes mellitus (Portha et al., 1991). Since the basal glucose level in Goto–Kakizaki rats is higher than that of neonatally streptozotocin-treated rats, it will be easier to see an effect of JTT-608 on the basal blood glucose level. In previous studies, tolbutamide and glibenclamide caused prolonged hypoglycemia (Jackson and Bressler, 1981; Gerich, 1989). Glibenclamide did not inhibit the increase in blood glucose level due to glucose-loading under the present experimental conditions (Fig. 1C) because the onset of its hypoglycemic action is slow. Indeed, glibenclamide is considered not to control postprandial glucose fluctuations effectively because of its slow onset but prolonged hypoglycemic action. Furthermore, several studies have shown that chronic treatment with sulfonylurea derivatives results in impairment of insulin secretion and of glucose tolerance (Dunbar and Foá, 1974; Filipponi et al., 1983; Kawai et al., 1991). The persistent hypoglycemic

action of sulfonylurea derivatives, specifically in the pancreas, may cause this impairment.

JTT-608 decreased the glucose AUC dose dependently in glucose-loaded neonatally streptozotocin-treated rats to the same extent as in normal rats (Figs. 1A and 7B). Additionally, JTT-608 (100 mg/kg) restored the dysfunction of glucose tolerance in neonatally streptozotocin-treated rats to the level of normal rats (Fig. 7A). In normal rats, peak glucose concentrations after glucose-loading (2 g/kg p.o.) were about 11–12 mM (data not shown). The effect of tolbutamide reached a plateau in the neonatally streptozotocin-treated rats, which was different from the effect in normal rats (Figs. 1B and 7B). This may be because tolbutamide does not enhance insulin secretion dose dependently at high glucose concentrations in vitro (Furukawa et al., provisional acceptance). It was also found that JTT-608 induced a glucose concentration-dependent enhancement of insulin secretion and enhanced the first phase of insulin secretion, which is not seen with sulfonylurea derivatives. The mechanism by which JTT-608 increases insulin secretion is probably different from that of sulfonylurea derivatives, which means that JTT-608 might be able to achieve better glycemic control in models of non-insulin-dependent diabetes mellitus, such as neonatally streptozotocin-treated rats.

In conclusion, JTT-608 is a novel anti-diabetic agent which improved glucose tolerance without causing a decrease in fasting blood glucose levels. This suggests that it enhances glucose-stimulated insulin secretion. JTT-608 had this effect not only in normal rats but also in rats with diabetes mellitus, which was not the case with sulfonylurea derivatives. JTT-608 might be thus expected to control postprandial hyperglycemia in patients with non-insulin-dependent diabetes mellitus without causing hypoglycemia.

References

Asplund, K., Wiholm, B.E., Lithner, F., 1983. Glibenclamide-associated hypoglycaemia: a report on 57 cases. Diabetologia 24, 412–417.

Dunbar, J.C., Foá, P.P., 1974. An inhibitory effect of tolbutamide and glibenclamide (glyburide) on the pancreatic islets of normal animals. Diabetologia 10, 27–35.

Efendic, S., Cerasi, E., Luft, R., Gladnikkoff, G., 1976. Potentiation of glucose-induced insulin release by glucose in the isolated pancreas of fed and fasted rats. Diabetes 25, 949–954.

Ferner, R.E., Neil, H.A.W., 1988. Sulphonylureas and hypoglycaemia. Br. Med. J. 296, 949–950.

Filipponi, P., Marcelli, M., Nicoletti, I., Pacifici, R., Santeusanio, F., Brunetti, P., 1983. Suppressive effect of long term sulfonylurea treatment on A, B, and D cells of normal rat pancreas. Endocrinology 113, 1972–1979.

Firth, R.G., Bell, P.M., Marsh, H.M., Hansen, I., Rizza, R.A., 1986. Postprandial hyperglycemia in patients with noninsulin-dependent diabetes mellitus. J. Clin. Invest. 77, 1525–1532.

Gerich, J.E., 1989. Oral hypoglycemic agents. N. Engl. J. Med. 321, 1231–1245.

Giroix, M.-H., Portha, B., Kergoat, M., Bailbe, D., Picon, L., 1983. Glucose insensitivity and amino-acid hypersensitivity of insulin release in rats with non-insulin-dependent diabetes. Diabetes 32, 445–451.

Groop, L.C., 1992. Sulfonylureas in NIDDM. Diabetes Care 15, 737–754.

Jackson, J.E., Bressler, R., 1981. Clinical pharmacology of sulphonylurea hypoglycaemic agents: part 1. Drugs 22, 211–245.

Kawai, K., Suzuki, S., Murayama, Y., Watanabe, Y., Yamashita, K., 1991. Comparison of B-cell function after long-term treatment with either insulin, insulin plus gliclazide or gliclazide in neonatal streptozotocin-induced non-insulin-dependent diabetic rats. Diabetes Res. Clin. Pract. 12, 163–172.

Kelley, D., Mokan, M., Veneman, T., 1994. Impaired postprandial glucose utilization in non-insulin-dependent diabetes mellitus. Metabolism 43, 1549–1557.

Kosaka, K., Kuzuya, T., Hagura, R., 1994. Insulin secretory response in Japanese type 2 (non-insulin-dependent) diabetic patients. Diabetes Res. Clin. Pract. 24, S101–S110.

Ligtenberg, J.J.M., Venker, C.E., Sluiter, W.J., Reitsma, W.D., Van Haeften, T.W., 1997. Effect of glibenclamide on insulin release at moderate and high blood glucose levels in normal man. Eur. J. Clin. Invest. 27, 685–689.

Perley, M.J., Kipnis, D.M., 1967. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J. Clin. Invest. 46, 1954–1962.

Pfeifer, M.A., Halter, J.B., Porte, D., 1981. Insulin secretion in diabetes mellitus. Am. J. Med. 70, 579–588.

Polonsky, K.S., Given, B.D., Hirsch, L.J., Tillil, H., Shapiro, E.T., Beebe, C., Frank, B.H., Galloway, J.A., Cauter, E.V., 1988. Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. N. Engl. J. Med. 318, 1231–1239.

Porte, D., 1991. β-cells in type II diabetes mellitus. Diabetes 40, 166–180.

Portha, B., Levacher, C., Picon, L., Rosselin, G., 1974. Diabetogenic effect of streptozotocin in the rat during the perinatal period. Diabetes 23, 889–895.

Portha, B., Blondel, O., Serradas, P., McEvoy, R., Giroix, M.-H., Kergoat, M., Bailbe, D., 1989. The rat models of non-insulin dependent diabetes induced by neonatal streptozotocin. Diabetes Metab. 15, 61–75.

Portha, B., Serradas, P., Bailbé, D., Suzuki, K., Goto, Y., Giroix, M.-H., 1991. β-cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. Diabetes 40, 486–491.

Sodoyez, J.C., Sodoyez-Goffaux, F., Dunbar, J.C., Foa, P.P., 1970. Reduction in the activity of the pancreatic islets induced in normal rodents by prolonged treatment with derivatives of sulfonylurea. Diabetes 19, 603–609.

Taylor, S.I., Accili, D., Imai, Y., 1994. Insulin resistance or insulin deficiency. Diabetes 43, 735–740.

Weir, J.C., Clore, E.T., Zmachinski, C.J., Bonner-weir, S., 1981. Islet secretion in a new experimental model for non-insulin-dependent diabetes. Diabetes 30, 590–595.