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# JTT-608 restores impaired early insulin secretion in diabetic Goto-Kakizaki rats

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**1** We investigated the pharmacological effects of a new antidiabetic agent, JTT-608, in comparison with the sulphonylurea tolbutamide, in Goto-Kakizaki (GK) rats, a genetic model of non-obese insulin-dependent diabetes mellitus (NIDDM).

**2** In isolated perfused pancreas from GK rats, JTT-608 (200 $\mu$M) enhanced 11.1 mM glucose-stimulated insulin secretion in the first and second phases, but had little effect on insulin secretion at 2.8 mM glucose. In contrast, tolbutamide (100 $\mu$M) markedly stimulated insulin secretion at 2.8 mM glucose and enhanced the second phase of insulin secretion but not the first phase at 11.1 mM glucose.

**3** *In vivo* JTT-608 also enhanced early insulin secretion only with glucose-loading. In contrast, tolbutamide enhanced insulin secretion both with and without glucose-loading.

**4** JTT-608 (10–100 mg kg$^{-1}$) improved oral glucose tolerance with enhanced insulin secretion in a meal tolerance test (MTT). In comparison with tolbutamide, JTT-608 improved glucose tolerance more efficiently in GK rats than in Wistar rats.

**5** We conclude that in diabetic GK rats JTT-608 suppressed postprandial glucose excursions with enhanced glucose-stimulated insulin secretion, especially the first phase of insulin secretion.

**Keywords:** JTT-608; antidiabetic agent; insulin secretion; perfused pancreas; GK rat

**Abbreviations:** IVGTT, intravenous glucose tolerance test; MTT, meal tolerance test; NIDDM, non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test; SUs, sulphonylurea derivatives

## Introduction

Depletion of glucose-stimulated insulin secretion, particularly loss of the first phase of insulin secretion is an important feature in the pathology of non-insulin-dependent diabetes mellitus (NIDDM) (Taylor *et al*., 1994; Porte, 1991; Polonsky *et al*., 1988). This defect is regarded as a contributing cause of postprandial hyperglycaemia (Kosaka *et al*., 1994). In the clinical management of these patients, sulphonylurea derivatives (SUs) are the most widely used hypoglycaemic agents (Groop, 1992; Gerich, 1989). However, SUs can cause severe and prolonged hypoglycaemia (Sills *et al*., 1997; Ferner *et al*., 1988; Asplund *et al*., 1983) because of their long duration of action and glucose-independent action (Zimmerman, 1997; Gerich, 1989; Jackson & Bressler, 1981).

To avoid the hypoglycaemia noted with SUs and achieve good glycaemic control in NIDDM patients, we developed JTT-608 [trans-4-(4-methylcyclohexyl)-4-oxobutyric acid] (Figure 1), which enhances the first phase of insulin secretion.

In Goto-Kakizaki (GK) rats, a genetic model of non-obese NIDDM (Goto *et al*., 1988), glucose-stimulated insulin secretion is reduced and glucose tolerance impaired. Moreover the early phase of glucose-induced insulin secretion is particularly impaired in this model, while the response of the early phase to arginine is preserved (Kimura *et al*., 1982). These reactions of insulin secretion in GK rats are similar to features of abnormal insulin secretion in NIDDM patients (Cerasi *et al*., 1972; Brunzell *et al*., 1976; Ward *et al*., 1984). It is thus considered that the GK rat offers a suitable model in which to investigate the effect of JTT-608 on insulin secretion and glucose tolerance. In the present study, we compared the effect of JTT-608 with the sulphonylurea tolbutamide.

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## Methods

### *Animals*

Male Wistar rats and GK rats were purchased from Charles River Japan (Tokyo, Japan). The animals received standard laboratory chow, CRF-1 (Oriental Yeast, Tokyo, Japan) and water *ad libitum*, and were housed in a room controlled for temperature (23 ± 3°C), humidity (55 ± 15%) and light (diurnal time; 8:00–20:00).

### *Materials*

JTT-608 was synthesized at Japan Tobacco Inc., Central Pharmaceutical Research Institute (Osaka, Japan). Tolbutamide (Wako Pure Chemical, Osaka, Japan) was used as the reference drug.

### *Perfusion of isolated pancreas of GK rats*

Rat pancreas was isolated and perfused according to the method of Grodsky & Fanska (1975) with slight modifications. In brief, after overnight fasting, the pancreas and associated spleen and duodenum were isolated under sodium pentobarbitone anaesthesia (50 mg kg$^{-1}$ i.p., Dainabot, Osaka, Japan). The isolated pancreas was perfused through the celiac artery at a flow rate of 3.5 ml min$^{-1}$ with basal Krebs Ringer bicarbonate buffer (KRB buffer in mM: NaCl 119, KCl 4.74, $CaCl_2$ 2.54, $MgCl_2$ 1.19, $KH_2PO_4$ 1.19, $NaHCO_3$ 25, pH 7.4;

equilibrated with 95% $O_2$, 5% $CO_2$) containing 2.8 mM glucose, 0.2% bovine serum albumin (fraction V, Sigma, St. Louis, MO, U.S.A.) and 4.0% dextran T-70 (Pharmacia Biotech, Uppsala, Sweden). The preparation was placed in an acryl chamber filled with the basal KRB buffer and kept at 37°C. The effluent perfusate from a portal vein cannula was collected at 1 min intervals into fraction tubes containing aprotinin (1000 u $tube^{-1}$). Collected samples were stored at −20°C and insulin concentration measured with a radioimmunoassay kit from Pharmacia Upjohn (Uppsala, Sweden) using rat insulin as a standard. After a 20 min equilibration period, basal perfusate was changed to basal KRB buffer containing a test compound and perfused for 10 min. Then the perfusate was changed to KRB buffer containing the same concentration of test compound and a high concentration of glucose (11.1 mM), and perfused for 30 min.

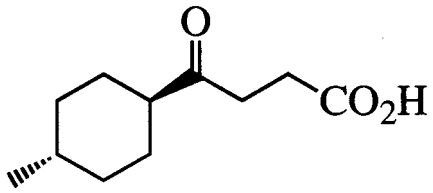


**Figure 1** Chemical structure of JTT-608.

## In vivo *experiments in GK rats*

Drugs, suspended in 0.5% methyl cellulose (MC) solution, were administered orally by a stomach tube at a volume of 5 ml $kg^{-1}$ at 10 min before glucose or meal loading. Blood samples were taken from the tail vein before and periodically after the administration of glucose or liquid meal for determination of serum glucose and serum insulin concentrations. GK rats (8 or 9-week-old, 180–270 g body weight) were fasted for 16 h before the experiments. Effect on the early insulin secretion was examined by an intravenous glucose tolerance test (IVGTT, 0.25 g glucose $kg^{-1}$) after the administration of JTT-608 or tolbutamide. Improvement of glucose tolerance and enhancement of insulin secretion with JTT-608 were examined by meal tolerance test (MTT, 20 kcal $kg^{-1}$, composition of the liquid meal; 19.2% soda-casein, 0.2% L-

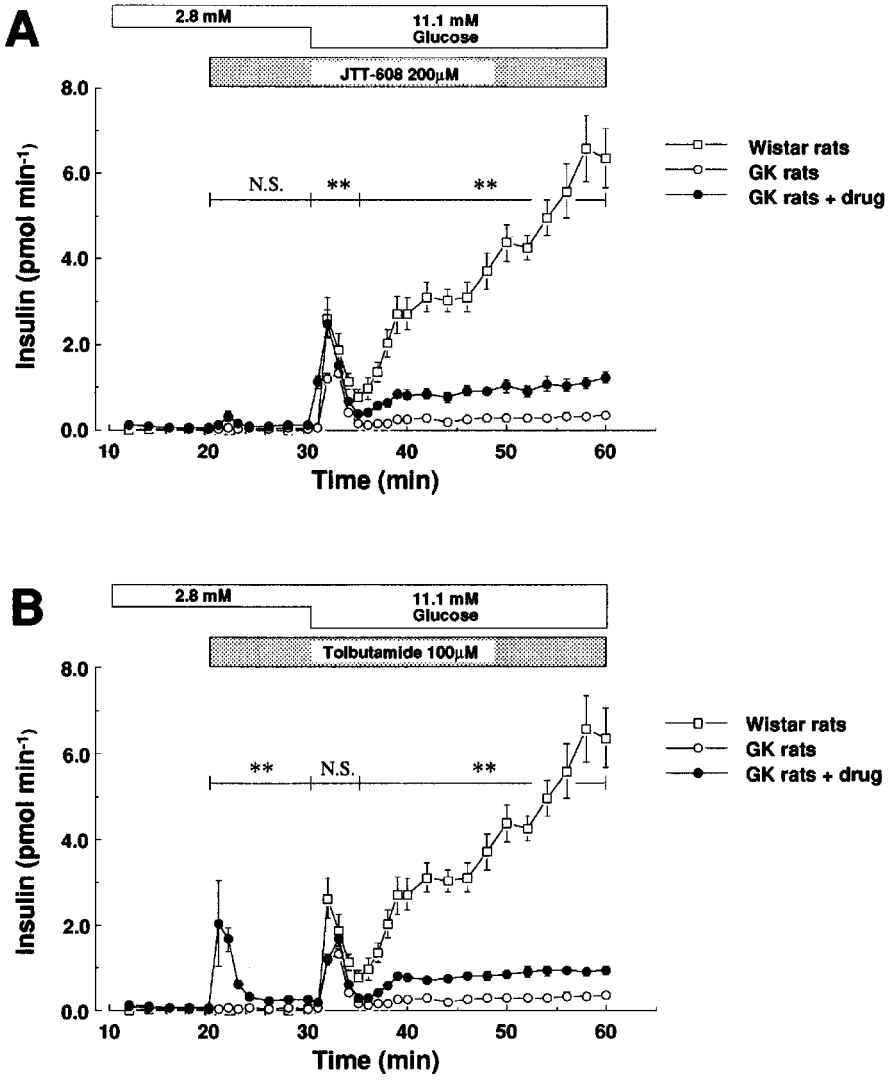


**Figure 2** Effects of JTT-608 (200 μM) (A) and tolbutamide (100 μM) (B) on insulin secretion at 2.8 mM glucose, and insulin secretion in response to 11.1 mM glucose in isolated, perfused pancreas of fasted GK rats (10-week-old) ($n=4$). Age matched Wistar rats were used as normal group ($n=5$). Data represent mean ± s.e.mean. **; AUC of GK rats + drug were significantly different from AUC of GK rats ($P<0.01$) (Tukey's compromise test). N.S.; not significant.

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, Yokohama, Japan). The insulin and glucose responses during the MTT were calculated as the incremental serum insulin concentrations integrated over a period of 30 min after the administration of glucose ($\Delta$I) and the corresponding increase in serum glucose concentrations ($\Delta$G). The insulinogenic index ($\Delta$I/$\Delta$G) represented the ratio of these two parameters. The efficacy of improvement of glucose tolerance by JTT-608 and tolbutamide was examined by an oral glucose tolerance test (OGTT, 2 g glucose $kg^{-1}$) in GK rats and Wistar rats.

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).

**Table 1** Comparison of several parameters between 8-week-old male Wistar rats and GK rats

| | *Wistar rats* (fasted) | *GK rats* (fasted) |
|---|---|---|
| Body weight (g) | 238.0±2.5 | 204.4±1.9** |
| Serum glucose (mmol $l^{-1}$) | 4.0±0.3 | 8.2±0.3** |
| Serum insulin (pmol $l^{-1}$) | 52.1±2.2 | 131.8±38.2 |
| Glucose area (mmol $l^{-1}$·h) | 12.3±1.3 | 21.2±1.3** |
| Insulinogenic index | 0.13±0.03 | −0.02±0.01** |

Glucose areas above base line from 0–3 h were calculated in glucose-loaded (2 g $kg^{-1}$ p.o.) rats. Insulinogenic index = $\Delta$ Insulin (increment from 0–30 min/$\Delta$ Glucose (increment from 0–30 min). Data represent mean±s.e.mean ($n=5$). **$P<0.01$; significantly different from the control by Student's $t$-test.

## *Statistical analysis*

All results were expressed as mean±s.e.mean. Statistical analyses of differences between mean values were performed with one-way analysis of variance (ANOVA) followed by Dunnett's two-tailed test, Tukey's compromise test or

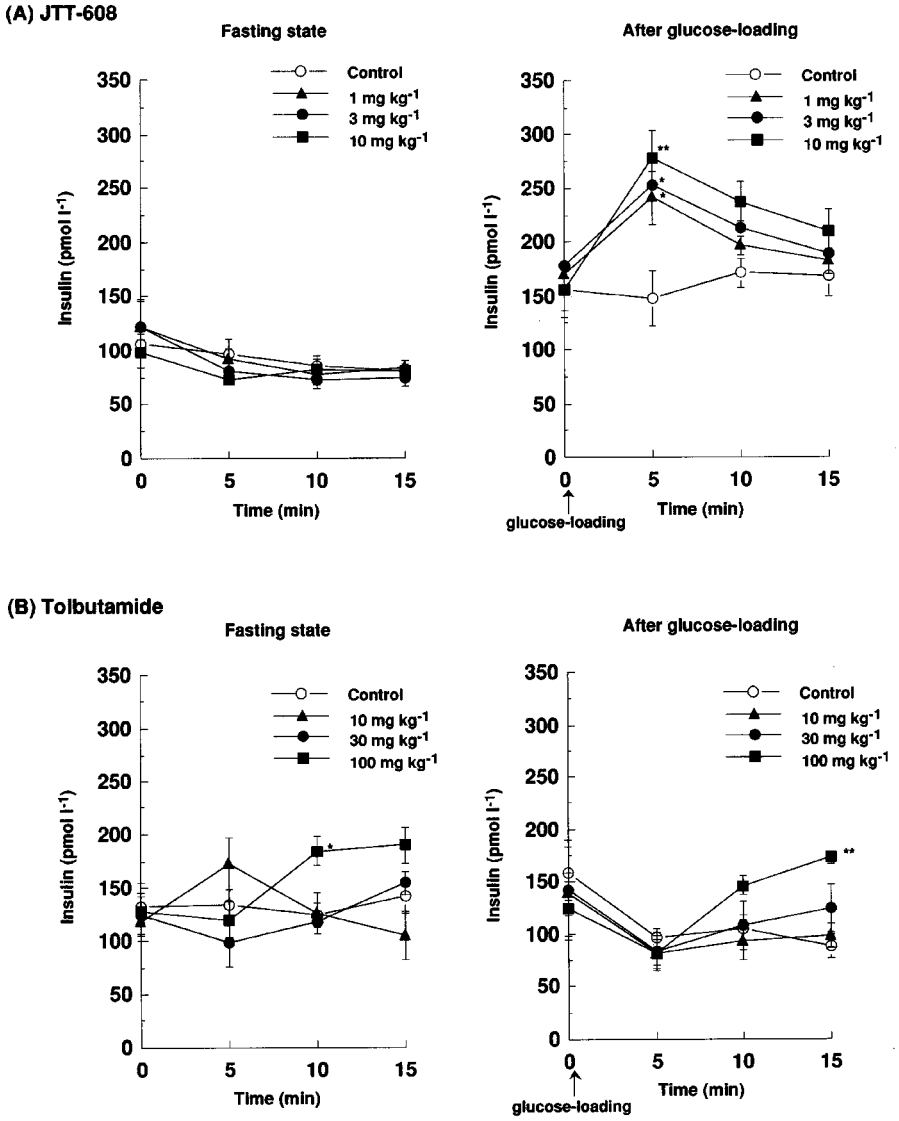


**Figure 3** Effects of JTT-608 (A) and tolbutamide (B) on serum insulin levels in fasted (8-week-old) or glucose-loaded GK rats (9-week-old). The chemicals were administered orally 10 min before glucose injection (0.25 g $kg^{-1}$ i.v.). Saline (1.25 ml $kg^{-1}$) in place of glucose was injected for the fasting state. Data represent mean±s.e.mean ($n=6$). *$P<0.05$, **$P<0.01$; significantly different from the control by Dunnett's two-tailed test.

Student's *t*-test. Differences were defined as significant at $P<0.05$.

## Results

*Effect of JTT-608 and tolbutamide on insulin secretion in isolated perfused pancreas of GK rats*

Both the first and second phases of insulin secretion at 11.1 mM glucose were highly defective in GK rats compared with age matched Wistar rats (Figure 2). JTT-608 (200 $\mu$M) markedly enhanced 11.1 mM glucose stimulated insulin secretion in the first and second phases, but minimally stimulated insulin secretion at 2.8 mM glucose. In particular, the first phase was almost restored to the levels of Wistar rats (Figure 2A). In contrast, tolbutamide (100 $\mu$M) markedly stimulated insulin secretion at 2.8 mM glucose and enhanced the second phase but not the first phase at 11.1 mM glucose (Figure 2B).

*Effect of JTT-608 and tolbutamide on the early phase of insulin secretion* in vivo

Table 1 shows the basal values, body weight, fasted serum glucose and insulin concentrations, glucose areas after glucose-loading, and insulinogenic index, in Wistar and GK rats. Body weight was lower, and the serum glucose and insulin concentrations were higher in GK rats compared to those of Wistar rats. Morever glucose tolerance and the insulinogenic index in GK rats had obviously deteriorated.

In the fasting state, JTT-608 did not stimulate insulin secretion at doses of $1-10$ mg kg$^{-1}$. After glucose-loading, however, JTT-608 enhanced insulin secretion at 5 min dose-dependently (Figure 3A). On the other hand, tolbutamide enhanced insulin secretion only at a 100 mg kg$^{-1}$, irrespective

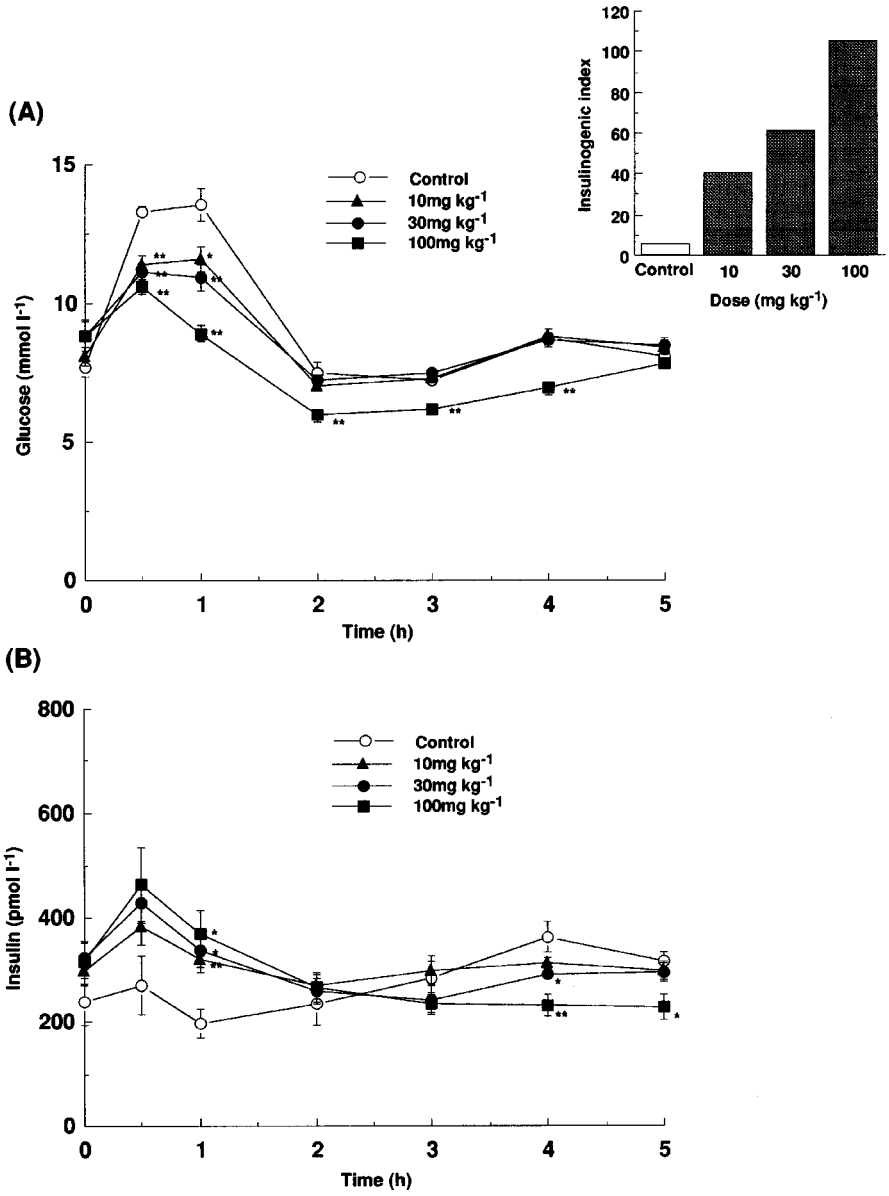


**Figure 4** Effect of JTT-608 on serum glucose (A) and serum insulin (B) levels in meal-loaded GK rats (9-week-old). JTT-608 was administered orally 10 min before meal-loading (20 kcal kg$^{-1}$). Data represent mean$\pm$s.e.mean ($n=6$). *$P<0.05$, **$P<0.01$; significantly different from the control by Dunnett's two-tailed test. The bar graph shows the insulinogenic index.

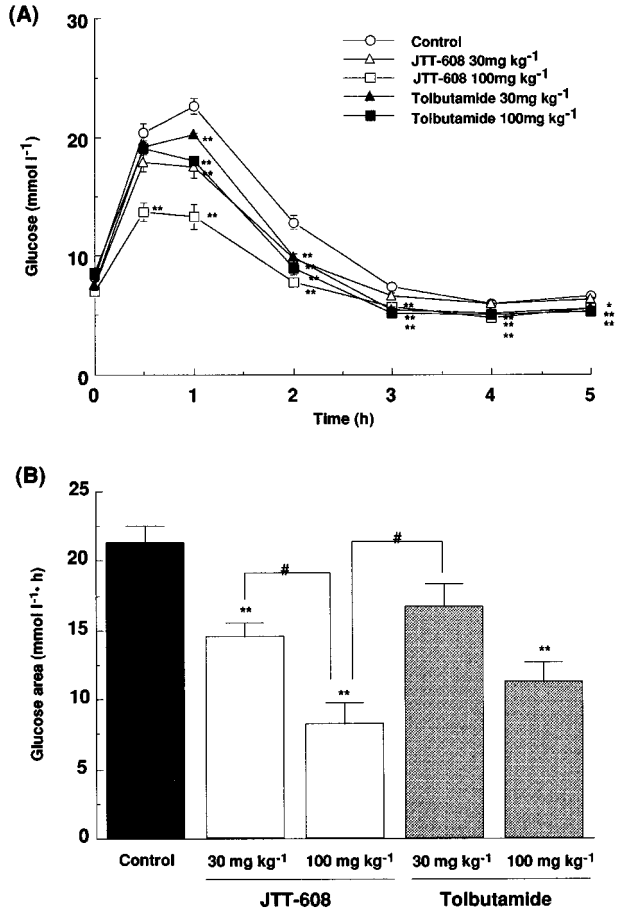


**Figure 5** (A) Effect of JTT-608 on serum glucose levels in glucose-loaded GK rats (8-week-old). The chemicals were administered orally 10 min before glucose-loading (2 g $kg^{-1}$ p.o.). Data represent mean ± s.e.mean ($n=5$). $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the control by Dunnett's two-tailed test. (B) Comparison of glucose areas above base line from 0–3 h after between JTT-608 and tolbutamide in glucose-loaded GK rats. Glucose areas were calculated on (A). Data represent mean ± s.e.mean ($n=5$). $^{**}P<0.01$; significantly different from the control by Dunnett's two-tailed test. #$P<0.05$; significantly different by Tukey's compromise test.

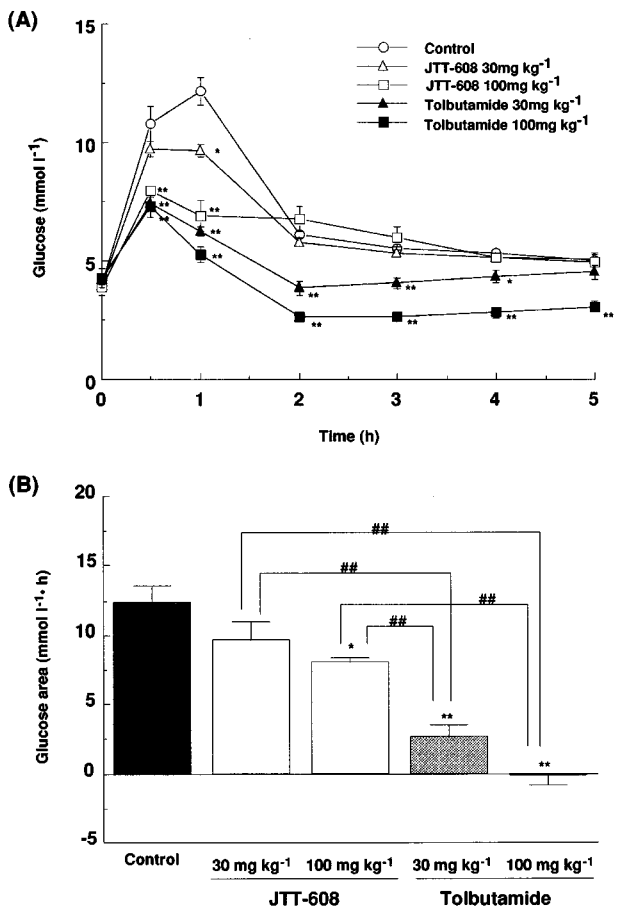


**Figure 6** (A) Effect of JTT-608 on serum glucose levels in glucose-loaded Wistar rats (8-week-old). The chemicals were administered orally 10 min before glucose-loading (2 g $kg^{-1}$ p.o.). Data represent mean ± s.e.mean ($n=5$). $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the control by Dunnett's two-tailed test. (B) Comparison of glucose areas above base line from 0–3 h after between JTT-608 and tolbutamide in glucose-loaded Wistar rats. Glucose areas were calculated on (A). Data represent mean ± s.e.mean ($n=5$). $^{*}P<0.05$, $^{**}P<0.01$; significantly different from the control by Dunnett's two-tailed test. ##$P<0.05$; significantly different by Tukey's compromise test.

of whether or not there was glucose stimulation, but did not enhance secretion at 5 min, in contrast to JTT-608 (Figure 3B).

### *Effect of JTT-608 in meal-loaded GK rats*

When JTT-608 was administered at doses of 10–100 mg $kg^{-1}$ before meal-loading, the impaired glucose tolerance was improved, with insulin secretion enhanced and insulinogenic index ameliorated dose-dependently (Figure 4). JTT-608 (100 mg $kg^{-1}$) reduced the glucose concentrations from 2–4 h and the insulin concentrations from 4–5 h, compared with control.

### *Comparison of the improvement of glucose tolerance in glucose-loaded GK and Wistar rats by JTT-608*

JTT-608 (30–100 mg $kg^{-1}$) or tolbutamide (30–100 mg $kg^{-1}$) was administered to GK rats or Wistar rats before glucose-loading. Both JTT-608 and tolbutamide inhibited the increase in blood glucose (Figures 5A and 6A). Figures 5B and 6B show the glucose areas above base line from 0–3 h after glucose-loading. In GK rats JTT-608 improved glucose tolerance from a dose of 30 mg $kg^{-1}$ (Figure 5B), but only at 100 mg $kg^{-1}$ in Wistar rats (Figure 6B). In contrast, tolbutamide improved glucose tolerance from a dose of 30 mg $kg^{-1}$ in Wistar rats (Figure 6B), but only at 100 mg $kg^{-1}$ in GK rats (Figure 5B). Tolbutamide exhibited a hypoglycaemic action at 30–100 mg $kg^{-1}$ in Wistar rats, whereas these doses produced a lesser effect in GK rats. The efficacy of tolbutamide in Wistar rats was greater than JTT-608, but in GK rats JTT-608 improved glucose tolerance more effectively than tolbutamide.

## Discussion

In the present study, we have demonstrated that JTT-608 restores the impaired early insulin secretion in GK rats. Depletion of glucose stimulated insulin secretion, particularly loss of the first phase of insulin secretion is a common feature in NIDDM patients. Insulin secretion in GK rats are similar to features of abnormal insulin secretion in NIDDM patients. In our study, both the first and second phases of glucose stimulated insulin secretion were defective in isolated perfused

pancreas of GK rats compared with those of Wistar rats. Moreover glucose tolerance and the insulinogenic index had deteriorated in GK rats. These observations are in keeping with previous reports (Portha *et al*., 1991; Östenson *et al*., 1993b). JTT-608 enhanced insulin secretion only after high glucose-stimulation *in vitro* and *in vivo*. In particular, JTT-608 restored the impaired early insulin secretion in GK rats. In contrast, tolbutamide stimulated insulin secretion at a low glucose concentration, and did not enhance early insulin secretion. Thus the characteristics of insulin secretion with JTT-608 are glucose concentration dependency and enhancement of the early phase.

A slight fall in insulin concentrations was observed after i.v. injection of glucose or saline in *in vivo* glucose-loaded study. Animal handling stress such as use of a body holder for the injection and repeated blood collection over a short time (5 min) period, is considered to cause a decrease in insulin after the injection, especially in fasting state. The stress period is too rapid here for glucocorticoids to take effect. Epinephrine released in stress inhibits insulin secretion (Niddam *et al*., 1990; Östenson *et al*., 1993a; Ling *et al*., 1998; Ogawa *et al*., 1992).

JTT-608 improved glucose tolerance with enhancement of insulin secretion in MTT. The doses of JTT-608 tested on MTT were increased 10 fold compared to those reported on IVGTT because absorption of JTT-608 was decreased by feeding. Peak plasma concentrations after oral administration of JTT-608 in non-fasted rats were about 20% in comparison with those of fasted rats. Besides, insulin concentrations of 100 mg $kg^{-1}$ decreased at 4–5 h (Figure 4B). Since JTT-608 stimulates insulin secretion glucose-concentration dependently, low levels of glucose concentrations at 2–4 h (Figure 4A) may induce a decrease of insulin secretion.

JTT-608 improved glucose tolerance more effectively in GK rats than in Wistar rats compared to tolbutamide. This may be primarily due to the enhanced first phase of insulin secretion, as shown in the IVGTT and in the perfused pancreas from GK rats. There are some reports in which glucose tolerance is evaluated by glucose area above baseline (Agardh *et al*., 1997; Tan *et al*., 1977), in order to cancel out the variation of fasted glucose levels. Therefore, an evaluation of glucose area was also made and gave the same result. The timing of drug administration 10 min before glucose-loading was established by the pharmacodynamic and pharmacokinetic effects in normal rats. Considering that plasma concentrations reached a peak at 0.25 h in rats treated orally with JTT-608 30 mg $kg^{-1}$, the timing of JTT-608 administration was considered suitable. Tolbutamide stimulated insulin secretion at 10 min after drug administration in fasted normal rats (insulin concentrations were 48.6 pmol $l^{-1}$ as pre-value, and 82.8 pmol $l^{-1}$ at 10 min). Although tolbutamide plasma concentrations reached a peak at 1–3 h in rats (Miller *et al*., 1957), it is considered to exhibit its pharmacological effect sufficiently with the present timing schedule. When the time between administration of tolbutamide and glucose-loading is longer, it caused hypoglycaemia before glucose-loading (results not shown). It is considered that the prominent difference of response to JTT-608 in GK rats compared to Wistar rats is due to deterioration of glucose-stimulated insulin secretion and especially deletion of early insulin secretion, which are characteristic of the pathology of GK rats.

It has been reported that tolbutamide stimulates insulin release in a concentration (0.3–30 $\mu$M)-dependent manner (Fujitani *et al*., 1996), and that 1 mM of tolbutamide enhances 5.5 mM glucose stimulated-insulin secretion with an amino acid mixture (Grodsky *et al*., 1977). Furthermore, sulphonylureas such as tolbutamide or glibenclamide create a first phase of their own in perfused rat pancreas (Grodsky *et al*., 1977; Östenson *et al*., 1986). However, there are also reports in which sulphonylureas either enhance the first phase of insulin secretion by glucose (Seto *et al*., 1995; van Haeften *et al*., 1991) or do not enhance it (Nakayama *et al*., 1995; Ligtenberg *et al*., 1997). The experimental conditions used, such as perfused rat pancreas and human hyperglycemic clamp, are various. It is thus considered that different results in insulin secretion of sulphonylureas depend on the technique used. In the present study, tolbutamide did not enhance the first phase of insulin secretion at 11.1 mM glucose. Early-phase insulin secretion is decreased not only in NIDDM patients but also in impaired glucose tolerance (IGT), while total insulin secretion in NIDDM patients is not different from that in normal subjects (Matsumoto *et al*., 1997). This defect of early secretion is regarded as an important cause of postprandial hyperglycaemia and it is considered that the low insulin response is an important feature of NIDDM (Kosaka *et al*., 1994). Enhancement of glucose stimulated insulin secretion and the first phase of insulin secretion with JTT-608 is thus considered to be of therapeutic value in controlling blood glucose in NIDDM patients.

In conclusion, JTT-608 is a novel antidiabetic agent which shows improvement of glucose tolerance by glucose stimulated insulin secretion and in particular an enhancement of the first phase of insulin secretion.

## References


AGARDH, C.-D., AGARDH, E., ZHANG, H. & ÖSTENSON, C.-G. (1997). Altered endothelial/pericyte ratio in Goto-Kakizaki rat retina. *J. Diab. Comp.,* **11,** 158–162.

ASPLUND, K., WIHOLM, B.E. & LITHNER, F. (1983). Glibenclamide-associated hypoglycaemia: a report on 57 cases. *Diabetologia,* **24,** 412–417.

BRUNZELL, J.D., ROBERTSON, R.P., LERNER, R.L., HAZZARD, W.R., ENSINCK, J.W., BIERMAN, E.L. & PORTE, D. (1976). Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. *J. Clin. Endocrinol. Metab.,* **42,** 222–229.

CERASI, E., LUFT, R. & EFENDIC, S. (1972). Decreased sensitivity of the pancreatic beta cells to glucose in prediabetic and diabetic subjects. *Diabetes,* **21,** 224–234.

FERNER, R.E. & NEIL, H.A.W. (1988). Sulphonylureas and hypoglycaemia. *Br. Med. J.,* **296,** 949–950.

FUJITANI, S., IKENOUE, T., AKIYOSHI, M., MAKI, T. & YADA, T. (1996). Somatostatin and insulin secretion due to common mechanisms by a new hypoglycemic agent, A-41566, in perfused rat pancreas. *Metabolism,* **45,** 184–189.

GERICH, J.E. (1989). Oral hypolgycemic agents. *N. Engl. J. Med.,* **321,** 1231–1245.

GOTO, Y., SUZUKI, K.I., SASAKI, M., ONO, T. & ABE, S. (1988). GK rat as a model of non-obese non-insulin-dependent diabetes: selective breeding over 35 generations. In *Frontiers of Diabetes Research. Lesson from Animal Diabetes II.* eds. Shafrir, E. & Reynold, A.E. pp. 301–303. London: Libbey.

GRODSKY, G.M., EPSTEIN, G.H., FANSKA, R. & KARAM, J.H. (1977). Pancreatic action of the sulfonylureas. *Fed. Proc.,* **36,** 2714–2719.

GRODSKY, G.M. & FANSKA, R.E. (1975). The in vitro perfused pancreas. *Methods Enzymol.,* **39,** 364–372.

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.

KIMURA, K., TOYOTA, T., KAKIZAKI, M., KUDO, M., TAKEBE, K. & GOTO, Y. (1982). Impaired insulin secretion in the spontaneous diabetes rats. *Tohoku J. Exp. Med.,* **137,** 453–459.

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.

LING, Z.-C., KHAN, A., DELAUNEY, F., DAVANI, B., ÖSTENSON, C.-G., GUSTAFSSON, J.-A., OKRET, S., LANDAU, B.R. & EFENDIC, S. (1998). Increased glucocorticoid sensitivity in islet beta-cells: effects on glucose 6-phosphatase, glucose cycling and insulin release. *Diabetologia,* **41,** 634–639.

MATSUMOTO, K., YAMAGUCHI, Y., MIYAKE, S., AKAZAWA, S., YANO, M., TOMINAGA, Y. & UEKI, Y. (1997). Glucose tolerance, insulin secretion, and insulin sensitivity in nonobese and obese Japanese subjects. *Diabetes Care,* **20,** 1562–1568.

MILLER, W.L., KRAKE, J.J., VANDER BROOK, M.J. & REINEKE, L.M. (1957). Studies on the absorption, mechanism of action, and excretion of tolbutamide in the rat. *Ann. N.Y. Acad. Sci. U.S.A.,* **71,** 118–124.

NAKAYAMA, K., MURAKAMI, N., OHTA, M., KATO, K., NOTSU, T., MIZOTA, M., MIKWA, I. & OKUDA, J. (1995). Effects of M16209 on insulin secretion in isolated, perfused pancreases of normal and diabetic rats. *Eur. J. Pharmacol.,* **276,** 85–91.

NIDDAM, R., ANGEL, I., BIDET, S. & LANGER, S.Z. (1990). Pharmacological characterization of alpha-2 adrenergic receptor subtype involved in the release of insulin from isolated rat pancreatic islets. *J. Pharmacol. Exp. Ther.,* **254,** 883–887.

OGAWA, A., JOHNSON, J.H. & OHNEDA, M. (1992). Roles of insulin resistance and beta-cell dysfunction in dexamethasone-induced diabetes. *J. Clin. Invest.,* **90,** 497–504.

ÖSTENSON, C.G., HJEMDAHL, P. & EFENDIC, S. (1993a). Release of catecholamines is increased but does not contribute to the impaired insulin secretion in the pancreata of diabetic rats. *Pancreas,* **8,** 34–38.

ÖSTENSON, C.G., KHAN, A., ADBEL-HALIM, S.M., GUENIFI, A., SUZUKI, K., GOTO, Y. & EFENDIC, S. (1993b). Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rats. *Diabetologia,* **36,** 3–8.

ÖSTENSON, C.G., NYLÉN, A., GUTNIAK, M. & EFENDIC, S. (1986). Sulfonylurea-induced inhibition of glucagon secretion from the perfused rat pancreas: evidence for a direct, non-paracrine effect. *Diabetologia,* **29,** 861–867.

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,** 1231–1245.

PORTE, D. (1991). $\beta$-cells in type II diabetes mellitus. *Diabetes,* **40,** 166–180.

PORTHA, B., SERRADAS, P., BAILBE, D., SUZUKI, K., GOTO, Y. & GIROIX, M.-H. (1991). $\beta$-cell insensibility to glucose in the GK rats, a spontaneous nonobese model for type II diabetes. *Diabetes,* **40,** 486–491.

SETO, Y., FUJITA, H., DAN, K., FUJITA, T. & KATO, R. (1995). Stimulating activity of A-4166 on insulin release in in situ Hamster pancreatic perfusion. *Pharmacology,* **51,** 245–253.

SILLS, M.N., OGU, C.C. & MAXA, J. (1997). Prolonged hypoglycemic crisis associated with glyburide. *Pharmacotherapy,* **17,** 1338–1340.

TAN, M.H., GRAHAM, C.A., BRADLEY, R.F., GLEASON, R.E. & SOELDNER, J.S. (1977). The effects of long-term therapy with oral hypoglycemic agents on the oral glucose tolerance test dynamics in male chemical diabetes. *Diabetes,* **26,** 561–570.

TAYLOR, S.I., ACCILI, D. & IMAI, Y. (1994). Insulin resistance or insulin deficiency. Which is the primary cause of NIDDM? *Diabetes,* **43,** 735–740.

VAN HAEFTEN, T.W., VENEMAN, T.F., GERICH, J.E. & VAN DER VEEN, E.A. (1991). Influence of gliclazide on glucose-stimulated insulin release in man. *Metabolism,* **40,** 751–755.

WARD, W.K., BEARD, J.C., HALTER, J.B., PFEIFER, M.A. & PORTE, D. (1984). Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. *Diabetes Care,* **7,** 491–502.

ZIMMERMAN, B.R. (1997). Sulfonylureas. *Endocrinol. Metab. Clin. North Am.,* **26,** 511–522.

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