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Antihyperglycemic effects of M16209, a novel aldose reductase inhibitor, in normal and diabetic rats

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Abstract

The effect of a single oral administration of M16209 (1-(3-bromobenzo[*b*]furan-2-yl-sulfonyl)hydantoin), a novel aldose reductase inhibitor, on serum glucose was investigated. In normal rats, M16209 (100 mg/kg) had a weak hypoglycemic effect but markedly stimulated the disappearance of serum glucose in intravenous glucose tolerance tests. In diabetic rats, M16209 (100 mg/kg) significantly suppressed the hyperglycemia of streptozotocin-induced, mildly diabetic rats and stimulated serum glucose disappearance in neonatally streptozotocin-induced, non-insulin-dependent diabetes mellitus (NIDDM) rats in glucose tolerance tests. Additionally, M16209 augmented insulin secretion in glucose-loaded, normal and NIDDM rats and restored the reduced serum insulin in streptozotocin-induced, mildly diabetic rats. M16209, however, showed no hypoglycemic effect in severely diabetic rats. In contrast, gliclazide, a sulfonylurea, showed a much more potent hypoglycemic effect in normal rats than in mildly diabetic rats. These results suggest that M16209 suppresses hyperglycemia through augmentation of glucose-stimulated insulin secretion. The antihyperglycemic activity of M16209, combined with its potent aldose reductase inhibiting activity, is expected to be beneficial in the treatment of diabetic complications.

Keywords: M16209; Aldose reductase inhibitor; Antihyperglycemic effect; Streptozotocin-induced diabetes; Insulin secretion

1. Introduction

We previously demonstrated that M16209 (1-(3-bromobenzo[*b*]furan-2-yl-sulfonyl)hydantoin), a derivative of 1-(arylsulfonyl)hydantoin, effectively prevents cataract formation in galactose-fed rats and ameliorates delayed motor nerve conduction velocity in streptozotocin-induced diabetic rats (Kato et al., 1990, 1991b). M16209 (chemical structure is shown in Fig. 1) has been shown to act primarily by improving polyol metabolism in tissues which contain aldose reductase (Kato et al., 1990, 1991a,b).

It should be noted that part of the structure of derivatives of 1-(arylsulfonyl)hydantoin is similar to that of sulfonylureas. Thus, we designed a preliminary experiment to test the effects of these derivatives on blood glucose and found some of them to exhibit hypoglycemic activity (Miwa et al., 1984, 1988). Since a good control of blood glucose would be valuable in the prevention of diabetic complications, it seems pharmacologically beneficial if M16209, besides producing aldose reductase inhibition, also had the ability to lower blood glucose. The present study was, therefore, aimed at elucidating the acute effects of M16209 on blood glucose in (1) normal rats (serum glucose; range 6.0–7.5 mM), (2) rats with non-insulin-dependent diabetes (neonatal streptozotocin) (serum glucose; range 7.5–9.0 mM), (3) rats with mild streptozotocin-diabetes (serum glucose; range 22–26 mM) and (4) rats with

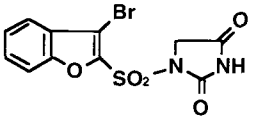

Fig. 1. Chemical structure of M16209.

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severe streptozotocin-diabetes (serum glucose; range > 27 mM).

2. Materials and methods

2.1. Assay of aldose reductase-inhibiting activity

Recombinant human aldose reductase was obtained from Wako Pure Chemical (Osaka, Japan). Assay of aldose reductase activity was performed according to the method described previously (Kato et al., 1991b). To test the effect of M16209 on the enzyme activity, the drug was dissolved in dimethylsulfoxide and added into the assay mixture to yield a solvent concentration of 0.1%.

2.2. Protocol of in vivo experiments

Male Wistar rats aged 7–8 weeks (about 200 g body weight) and pregnant Sprague-Dawley rats were obtained from Japan SLC (Hamamatsu, Japan). Diabetes was induced in rats through intravenous injection of streptozotocin (Sigma, St. Louis, MO) freshly dissolved in a 0.01-M citrate buffer, pH 4.5. Either M16209 or gliclazide (Dainippon, Osaka, Japan) was suspended in 5% gum arabic solution and administered orally to rats. Blood samples (150 μl) were obtained by retro-orbital puncture and sera (60 μl) were separated for determination of glucose and/or insulin level. Throughout the experiments, the rats were fasted 2–4 h before drug administration unless otherwise noted, so that feeding might not affect serum glucose levels. Serum glucose and 3-hydroxybutyrate were assayed using enzymatic kits from Wako Pure Chemical (Osaka, Japan) and from Sanwa Kagaku Kenkyusho (Nagoya, Japan), respectively. Serum insulin was assayed using an enzyme immunoassay kit from Sanko Junyaku (Tokyo, Japan).

Normal rats

Normal rats were divided into groups of five each so that body weight did not significantly differ between the groups. Fed rats were treated with M16209 at doses of 10, 30 or 100 mg/kg, or gliclazide at 3 or 10 mg/kg, or the vehicle. Rats which had been fasted for 40 h were treated with M16209 at doses of 30 or 100 mg/kg, gliclazide at 10 mg/kg, or the vehicle. Blood samples of fed rats were obtained 0, 1, 3 and 6 h after drug administration and those of 40-h-fasted rats were obtained at 0, 1 and 6 h, for determination of serum glucose levels. Either M16209 at 100 mg/kg or the vehicle was administered 3 h before glucose loading. For the glucose tolerance test, rats in each group were injected intravenously with a 62.5-, 125-, 250-, 500- or 1000-mg/kg bolus of glucose and blood samples were obtained 2, 5, 10 and 20 min after injection. The disappearance rate (K) value for glucose was calculated from the slope of the regression line obtained from logarithm-transformed serum glucose values between 2 and 20 min after glucose loading. The absolute area under the curve (AUC) for serum insulin was calculated through linear interpolation between 0 and 10 min after glucose loading.

Streptozotocin-induced, mildly diabetic rats

Mild diabetes was induced by intravenous injection of streptozotocin (26.5 mg/kg). Only rats with serum 3-hydroxybutyrate levels of less than 300 μM on day 2 after streptozotocin treatment and serum glucose levels of 22–26 mM on day 3 after streptozotocin treatment were used. These rats were divided into six groups of five each so that serum glucose levels did not significantly differ between the groups. The rats were treated with M16209 at 10, 30 or 100 mg/kg, gliclazide at 10 or 100 mg/kg, or the vehicle. Blood samples were obtained for determination of serum glucose levels 0, 1, 3 and 6 h after drug administration. To examine the effect of M16209 on serum insulin levels, three groups were treated with the drug at 30 or 100 mg/kg, or the vehicle, and blood samples were obtained 0, 1 and 6 h after administration. Blood samples of normal rats were also obtained for comparison of insulin levels.

NIDDM rats

NIDDM was induced according to the method of Weir et al. (1981): 1- or 2-day-old Sprague-Dawley rat pups were injected intraperitoneally with streptozotocin (90 mg/kg), freshly dissolved in a 0.01-M citrate buffer, pH 4.5. Normal controls were injected with an equivalent amount of citrate buffer. At 8 weeks of age, the diabetic rats were divided into two groups of five each so that serum glucose levels did not significantly differ between the groups. M16209 at 100 mg/kg or the vehicle was administered 3 h before glucose loading. For the glucose tolerance test, the rats were injected intravenously with a 1000-mg/kg bolus of glucose and blood samples were obtained 0, 2, 5, 10 and 20 min after injection. Normal controls also underwent the glucose tolerance test. The K value for serum glucose and AUC for serum insulin were calculated as noted above.

Streptozotocin-induced, severely diabetic rats

Severe diabetes was induced by intravenous injection of streptozotocin (40 mg/kg). Only rats with serum 3-hydroxybutyrate levels of more than 1000 μM on day 2 after streptozotocin treatment, and with serum glucose levels of more than 27 mM on day 3 after streptozotocin treatment, were used. The diabetic rats were divided into three groups of five each so that serum glucose levels did not significantly differ betwen the

groups. They were treated with M16209 at either 30 or 100 mg/kg, or the vehicle. Blood samples were obtained for determination of serum glucose levels and/or serum insulin levels 0, 1 and 6 h after drug administration. Blood samples of normal rats were also obtained for comparison of insulin levels.

2.3. Statistical analysis

After conducting a one-way analysis of variance (ANOVA), we performed multiple analyses using the Bonferroni method to compare the obtained results, and we also used the unpaired Student's t-test.

3. Results

3.1. Inhibitory effect on aldose reductase

The concentration of M16209 necessary for 50% inhibition of enzyme activity (IC_{50}) was 0.051 μM for recombinant human aldose reductase.

3.2. Effects on normal rats

Fig. 2 shows the serum glucose changes of fed, normal rats treated orally with M16209, gliclazide or the vehicle. The initial serum glucose levels of fed rats were as high as 7.1 mM. M16209 only slightly lowered serum glucose levels. The decrease at 3 h after administration of the drug at 30 and 100 mg/kg was, however, statistically significant. The decrease was less than 17%, even at a dose of 1000 mg/kg (data not shown). The reduced serum glucose level of rats treated with any of the doses of M16209 returned to the control level by 6 h after drug administration. In contrast, gliclazide at 3 and 10 mg/kg lowered the serum glucose level by 30 and 54%, respectively, 1 h after drug administration, and by 15 and 49%, respectively, 3 h after drug administration.

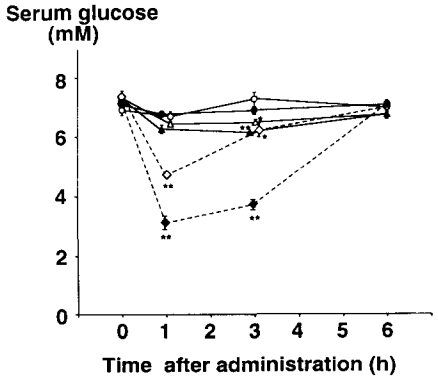

Fig. 2. Effects of oral administration of M16209 and gliclazide on serum glucose levels in normal rats. Control (○), M16209 10 mg/kg (●), M16209 30 mg/kg (△), M16209 100 mg/kg (▲), gliclazide 3 mg/kg (◇), gliclazide 10 mg/kg (◆). Values are means ± S.E. ($n = 5$). * $P < 0.05$, * * $P < 0.01$ vs. control.

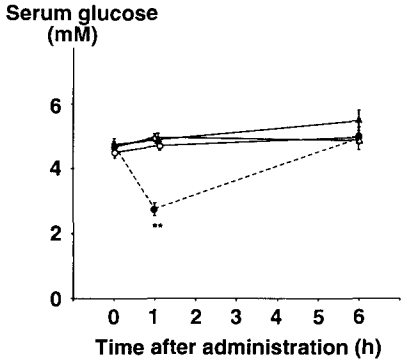

Fig. 3. Effect of oral administration of M16209 on serum glucose levels in 40-h-fasted, normal rats. Control (○), M16209 30 mg/kg (△), M16209 100 mg/kg (▲), gliclazide 10 mg/kg (●). Values are means ± S.E. ($n = 5$). * * $P < 0.01$ vs. control.

The serum glucose changes in fasted normal rats are shown in Fig. 3. The serum glucose levels of 40-h-fasted rats were reduced to 4.7 mM. M16209 at 30 and 100 mg/kg did not show any effects on serum glucose levels 1 and 6 h after administration, whereas gliclazide at 10 mg/kg lowered glucose levels by 42%, 1 h after drug administration.

Fig. 4A shows the glucose tolerance curves obtained when normal rats pretreated orally with M16209 at 100 mg/kg, or the vehicle, were given a 250-mg/kg bolus of glucose intravenously. The serum glucose level of control rats increased by about 100%, 2 min after glucose loading and then rapidly decreased. Although the preloading glucose level of M16209-treated rats was slightly, but significantly, lower than that of control rats, there was no significant difference in the 2-min postloading glucose levels between the two groups. The serum glucose levels between 5 and 20 min in the drug-treated rats were significantly lower than those in control rats. The corresponding serum insulin curves are shown in Fig. 4B. There was no apparent difference between M16209-treated rats and control groups before glucose loading. The insulin level of drug-treated rats showed a 16-fold increase 2 min after glucose loading, whereas that of control rats showed a 3.6-fold increase. The AUC for the serum insulin of M16209-treated rats, calculated 0–10 min after glucose loading, was about 5 times that of control rats.

Fig. 5A shows the relationship between glucose dose and the K value of serum glucose obtained from the results of intravenous glucose tolerance tests. The K value increased as the dose of glucose increased. Although the glucose dependency patterns of the K value in M16209-treated rats and untreated rats were comparable, the K value was significantly higher in M16209-treated rats than in control rats at every glucose dose

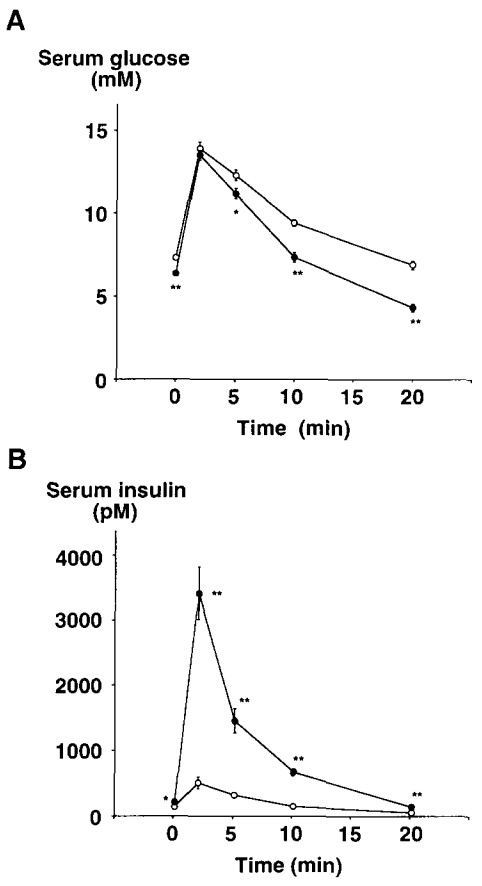

Fig. 4. Effects of oral administration of M16209 on serum glucose levels (A) and serum insulin levels (B) in normal rats injected intravenously with a 250-mg/kg bolus of glucose. Glucose was injected at time 0. M16209 was administered 3 h before glucose injection. Control (○), M16209 100 mg/kg (●). Values are means ± S.E. ($n = 5$). * $P < 0.05$, ** $P < 0.01$ vs. control.

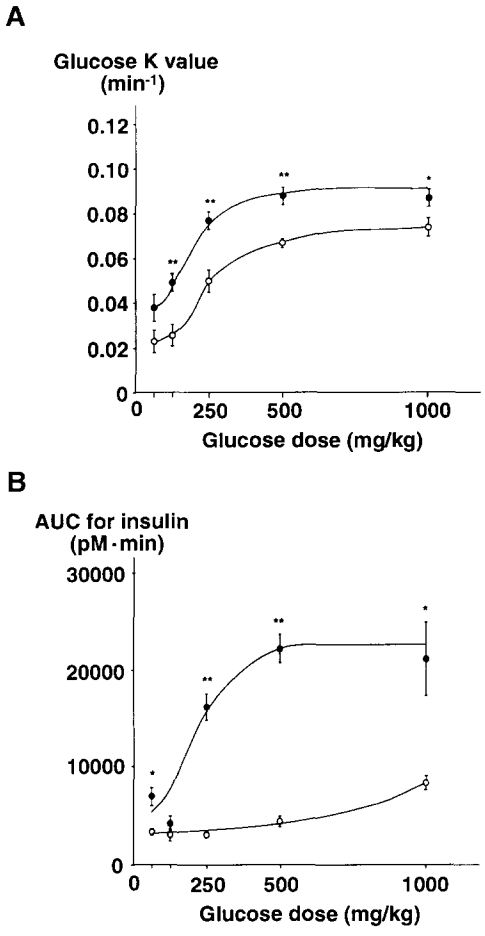

Fig. 5. Effects of oral administration of M16209 on the disappearance rate (K value) of serum glucose (A) and on the area under the curve (AUC) for serum insulin (B) in rats injected intravenously with various doses of glucose. M16209 was administered to rats 3 h before glucose injection. Control (○), M16209 100 mg/kg (●). Values are means ± S.E. ($n = 5$). * $P < 0.05$, ** $P < 0.01$ vs. control.

except 62.5 mg/kg. The corresponding relationship between glucose dose and the AUC for serum insulin is shown in Fig. 5B. The AUC for serum insulin increased as the dose of glucose increased. The AUC for serum insulin was significantly higher in M16209-treated rats than in control rats at every glucose dose except 125 mg/kg. The difference between the two groups was much more prominent at higher doses (250–1000 mg/kg) than at lower doses (62.5 and 125 mg/kg).

3.3. Effects on streptozotocin-induced, mildly diabetic rats

Fig. 6 shows the serum glucose changes of streptozotocin-induced, mildly diabetic rats treated orally with M16209, gliclazide, or the vehicle. The serum glucose levels of the diabetic rats on day 3 after streptozotocin treatment were approximately 3 times the level of fed, normal rats. M16209 did not significantly affect serum glucose level 1 h after drug administration, even at a

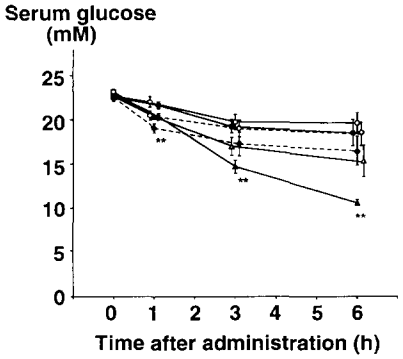

Fig. 6. Effects of oral administration of M16209 and gliclazide on serum glucose levels in STZ-induced, mildly diabetic rats. Control (○), M16209 10 mg/kg (●), M16209 30 mg/kg (△), M16209 100 mg/kg (▲), gliclazide 10 mg/kg (◇), gliclazide 100 mg/kg (◆). Values are means ± S.E. ($n = 5$). ** $P < 0.01$ vs. control.

dose of 100 mg/kg, but dose dependently suppressed the serum glucose level 3 and 6 h after drug administration. The serum glucose level of rats given M16209 at 100 mg/kg was 46% lower than that of control rats, 6 h after drug administration. However, gliclazide at 10 or 100 mg/kg only moderately (< 13%) suppressed elevated glucose levels, although the decrease in 100-mg/kg-treated rats 1 h after drug administration was statistically significant.

The serum insulin level was significantly lower in the mildly diabetic rats (108.6 ± 13.2 pM) than in normal rats (198.6 ± 32.4 pM). Fig. 7 shows the serum insulin changes of mildly diabetic rats treated with M16209 (30 or 100 mg/kg) or the vehicle. There were no significant differences in insulin levels between the groups before drug administration. M16209 at 30 and 100 mg/kg only partially restored the reduced serum insulin levels at 1 h, but had significantly restored them by the 6th h after drug administration. Gliclazide at 100 mg/kg had no significant effect at 1 and 6 h after drug administration.

3.4. Effects on NIDDM rats

Fig. 8A shows the glucose tolerance curves obtained when NIDDM rats pretreated orally with M16209 at 100 mg/kg or the vehicle, were given a 1000-mg/kg bolus of glucose intravenously. The preloading glucose level was slightly higher in NIDDM rats (9.0 ± 0.77 mM) than in normal rats (7.4 ± 0.12 mM). The serum glucose level of NIDDM rats increased by 210% 2 min after glucose loading and thereafter decreased slowly, indicating impaired glucose tolerance: K values of serum glucose in normal rats and NIDDM rats were 0.087 ± 0.004 and 0.010 ± 0.002 min^{-1}, respectively. Although M16209 did not significantly affect the preloading and 2-min postloading glucose levels, the drug accelerated the decrease in serum glucose. The corresponding serum insulin curves are shown in Fig. 8B. The insulin response to glucose was much lower in NIDDM rats than in normal rats: AUCs for serum insulin between 0 and 10 min in NIDDM rats and normal rats were 1710 ± 420 and 8405 ± 713 pM · min, respectively. The serum insulin level of M16209-treated rats increased during the first 20 min after glucose loading, whereas that of control rats did not appreciably change. There was a significant difference between the two groups 10 and 20 min after glucose loading.

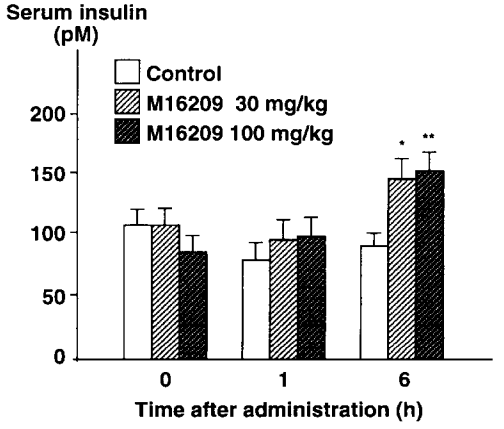

Fig. 7. Effect of oral administration of M16209 on serum insulin levels in STZ-induced, mildly diabetic rats. Values are means ± S.E. (n = 8–10). * $P < 0.05$, ** $P < 0.01$ vs. control.

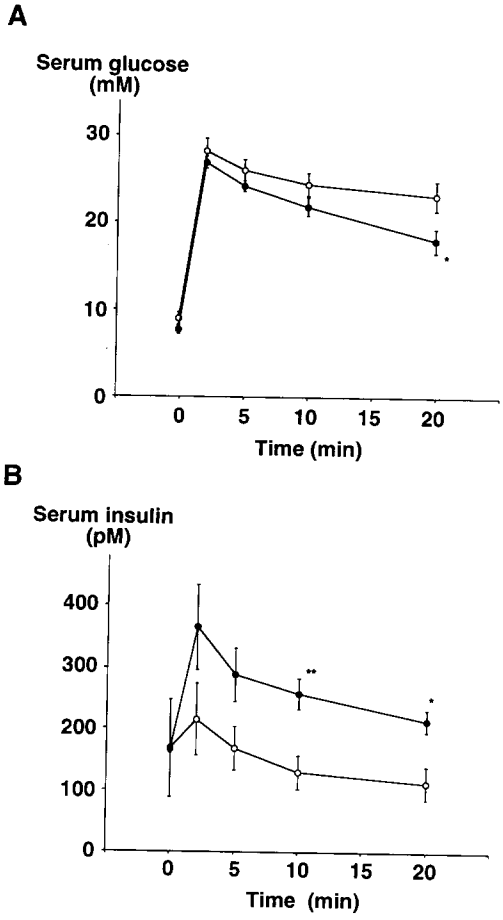

Fig. 8. Effects of oral administration of M16209 on serum glucose levels (A) and serum insulin levels (B) in NIDDM rats injected intravenously with a 1000 mg/kg bolus of glucose. Glucose was injected at time 0. M16209 was administered 3 h before glucose injection. Control (○), M16209 100 mg/kg (●). Values are means ± S.E. (n = 5). * $P < 0.05$, ** $P < 0.01$ vs. control.

3.5. Effects on streptozotocin-induced, severely diabetic rats

The serum glucose level of severely diabetic rats on day 3 after streptozotocin treatment was only slightly higher than that of mildly diabetic rats. M16209 at 30 and 100 mg/kg did not affect the serum glucose level during the first 6 h.

The serum insulin level was significantly lower in severely diabetic rats (69.6 ± 6.0 pM) than in normal rats (136.2 ± 12.0 pM). There was no significant difference in insulin level between control rats and M16209-treated rats either before or 6 h after drug administration.

4. Discussion

In normal rats, M16209 stimulated glucose disappearance in intravenous glucose tolerance tests, whereas it only slightly affected fed and fasting blood glucose levels. Concomitantly, insulin secretion stimulated by glucose loading was markedly augmented by M16209, although the preloading level was not appreciably affected. These findings indicate that the blood glucose-lowering activity of M16209 has a high selectivity for hyperglycemia and this is due to the characteristic effects of the drug on insulin secretion. In fact, the stimulatory effect of M16209 on insulin secretion appears to depend on the glucose level: an augmentation of insulin secretion was not observed when the serum glucose returned to normal levels (Fig. 4A and B) and the augmentation became more pronounced as the glucose dose increased (Fig. 5B). It is thus conceivable that M16209 is less likely to induce hypoglycemia, a well-known side-effect of sulfonylureas.

The hyperglycemia of mildly diabetic rats was greatly suppressed by M16209 at 100 mg/kg. Gliclazide was much less potent in mildly diabetic rats than in normal rats possibly due to the absence of intact β-cells in the mildly diabetic rats (Duhault et al., 1972; Jørgensen, 1977; Geisen, 1988). Restoration of reduced serum insulin levels was also observed when hyperglycemia was significantly suppressed, suggesting again that stimulation of insulin secretion is involved in the antihyperglycemic effect of M16209.

As expected from the prominent hyperketonemia in severely diabetic rats, the pancreatic insulin content of these rats was reduced to about one-tenth that of normal rats (unpublished observation). The reason M16209 failed to affect either the hyperglycemia or hypoinsulinemia of severely diabetic rats may be due, at least in part, to an inability of the compound to stimulate insulin secretion in insulin-deficient rats.

NIDDM rats whose diabetes was induced by injection of streptozotocin day 1–2 after birth, showed almost normal blood glucose levels and impaired glucose tolerance as reported previously (Bonner-Weir et al., 1981; Giroix et al., 1983). The impaired glucose tolerance of NIDDM rats was significantly improved by pretreatment with M16209. In addition, the insulin secretion in response to glucose loading was concomitantly augmented. These observations suggest that amelioration of the decreased insulin response to glucose may contribute to the improvement of impaired glucose tolerance in NIDDM rats.

Unlike sulfonylureas, M16209, even at a dose of 1000 mg/kg, showed a marginally hypoglycemic effect in normal rats. Moreover, M16209 augmented insulin secretion more pronouncedly as the serum glucose level increased. In contrast, a marked stimulation of insulin secretion with sulfonylureas was observed even under basal glucose conditions, although sulfonylureas augment the insulin-secretory effect of glucose (Basabe et al., 1976; Groop et al., 1987). We therefore concluded that the mechanism of the hypoglycemic action of M16209 is different from that of sulfonylureas.

The antihyperglycemic effect of M16209 thus appeared to be due to its distinctive action on insulin secretion. However, we found in a preliminary study using the hyperinsulinemic euglycemic clamp technique that M16209 could enhance peripheral glucose utilization in normal and insulin-resistant diabetic animals such as Zucker (fa/fa) rats and C57BL/6J (ob/ob) mice. Therefore, such extrapancreatic mechanisms may also be involved in the antihyperglycemic effect of M16209.

This study demonstrated that M16209 exhibits a blood glucose-lowering activity characterized by a selectivity for hyperglycemia. It is expected that this activity, combined with the drug's potent aldose reductase inhibiting activity, will make M16209 a useful antidiabetic agent.

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