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Effects of M16209 on insulin secretion in isolated, perfused pancreases of normal and diabetic rats

Kazuo Nakayama [a,*], Nobuya Murakami [a], Masahiko Ohta [a], Katsuaki Kato [a], Tatsuto Notsu [a], Masahiro Mizota [a], Ichitomo Miwa [b], Jun Okuda [b]

[a] *Department of Pharmacology, Fuji Central Research Laboratory, Mochida Pharmaceutical Co., Ltd., 1-1-1 Kamiya, Kita-ku, Tokyo 115, Japan*
[b] *Department of Clinical Biochemistry, Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya 468, Japan*

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Abstract

We investigated the stimulatory effect of M16209 (1-(3-bromobenzo[*b*]furan-2-yl-sulfonyl)hydantoin), a novel aldose reductase inhibitor, on insulin secretion using isolated, perfused pancreases of rats. In the pancreases from normal rats, M16209 (100 μM) greatly augmented glucose-stimulated insulin secretion, but showed no effect on unstimulated insulin secretion at 2.8 mM glucose. In contrast, gliclazide (10 μM), a sulfonylurea, strongly enhanced both glucose-stimulated and unstimulated insulin secretion. Sorbinil and epalrestat, potent aldose reductase inhibitors, had no stimulatory effect on insulin secretion. M16209 (100 μM) improved appreciably the decreased insulin response to 22.2 mM glucose and enhanced slightly unstimulated insulin secretion in the pancreases of rats with neonatally streptozotocin-induced, non-insulin-dependent diabetes mellitus (NIDDM). Gliclazide (10 μM), however, failed to affect the pancreases of NIDDM rats. Furthermore, M16209 showed no appreciable effect on ATP-sensitive K^+-channels in pancreatic β-cells. These results suggest that M16209, unlike sulfonylureas, selectively enhances glucose-stimulated insulin secretion in both normal and NIDDM rats through a direct action on the pancreas. The site of action remains unknown, but the inhibition of aldose reductase or the ATP-sensitive K^+ channels is unlikely to be involved.

Keywords: M16209; Aldose reductase inhibitor; Insulin secretion; Pancreas, perfused; K^+ channel, ATP-sensitive

1. Introduction

Recently, we demonstrated that M16209 (1-(3-bromobenzo[*b*]furan-2-yl-sulfonyl)hydantoin) exhibits antihyperglycemic activity as well as aldose reductase inhibitory activity (Nakayama et al., 1995). The blood glucose lowering activity of M16209 was different from that of gliclazide, a sulfonylurea: M16209 suppressed blood glucose levels more effectively in streptozotocin-induced, mildly diabetic rats, than in normal rats, whereas gliclazide produced opposite results. This indicated that a difference in mechanism of action exists between M16209 and gliclazide. In addition, our recent study indicated that M16209 lowers blood glucose by stimulating insulin secretion in vivo. Therefore, the purpose of this study was to examine the effect of M16209 on insulin secretion using isolated, perfused pancreases of rats and to compare the results with those obtained with gliclazide. In addition, we tested whether M16209 affects ATP-sensitive K^+ channels, one of the major sites of action of sulfonylureas (Malaisse and Lebrun, 1990; Arkhammar et al., 1987).

2. Materials and methods

2.1. Animals

Male Wistar rats aged 8 weeks (about 200 g body weight) and pregnant Sprague-Dawley rats were obtained from Japan SLC (Hamamatsu, Japan).

2.2. Perfusion of pancreas

The pancreas was isolated and perfused using the method of Grodsky et al. (1963). Overnight-fasted,

* Corresponding author. Tel 81-3-3913-6261, fax 81-3-3913-8393.

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normal rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and the pancreas and associated stomach, spleen and duodenum were isolated. The isolated pancreas was perfused at a flow rate of 3.6 ml/min through the abdominal aorta with Krebs Ringer bicarbonate buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, pH 7.4) containing 2.8 mM glucose, 0.2% bovine serum albumin (fraction V, Sigma, St. Louis, MO) and 4.6% dextran (M.W. 60000–90000, Wako Pure Chemical, Osaka, Japan). The buffer was equilibrated with 95% O_2, 5% CO_2 at 37°C. The effluent perfusate was collected at 4-min intervals. After a 20-min equilibration period, Krebs Ringer bicarbonate buffer containing M16209 (10–100 μM), gliclazide (10 μM, Dainippon, Osaka, Japan), sorbinil (100 μM, Pfizer pharmaceutical, New York), epalrestat (100 μM, Ono pharmaceutical, Osaka, Japan) or the vehicle (0.1% dimethylsulfoxide) was perfused for 100 min. Sorbinil and epalrestat were synthesized in our laboratory. The basal glucose concentration was 2.8 mM and the glucose concentration was raised to 5.5, 11.1 or 22.2 mM at 40 min of perfusion. The drugs were dissolved in dimethylsulfoxide and added to the perfusion buffer to yield a solvent concentration of 0.1%. Samples of perfusate were stored at −20°C and insulin was assayed using an enzyme immunoassay kit from Sanko Junyaku (Tokyo, Japan).

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 90 mg/kg streptozotocin (Sigma, St. Louis, MO), freshly dissolved in 0.01 M citrate buffer, pH 4.5. Normal controls were injected with an equivalent amount of citrate buffer. The pancreases of NIDDM rats and non-diabetic rats were isolated at 8–10 weeks of age and perfused as described above.

2.3. Measurement of ATP-sensitive K^+ channel activity

Insulin secreting cell line HIT-T15 cells (passage 67-75, Dainippon, Osaka, Japan) were cultured in

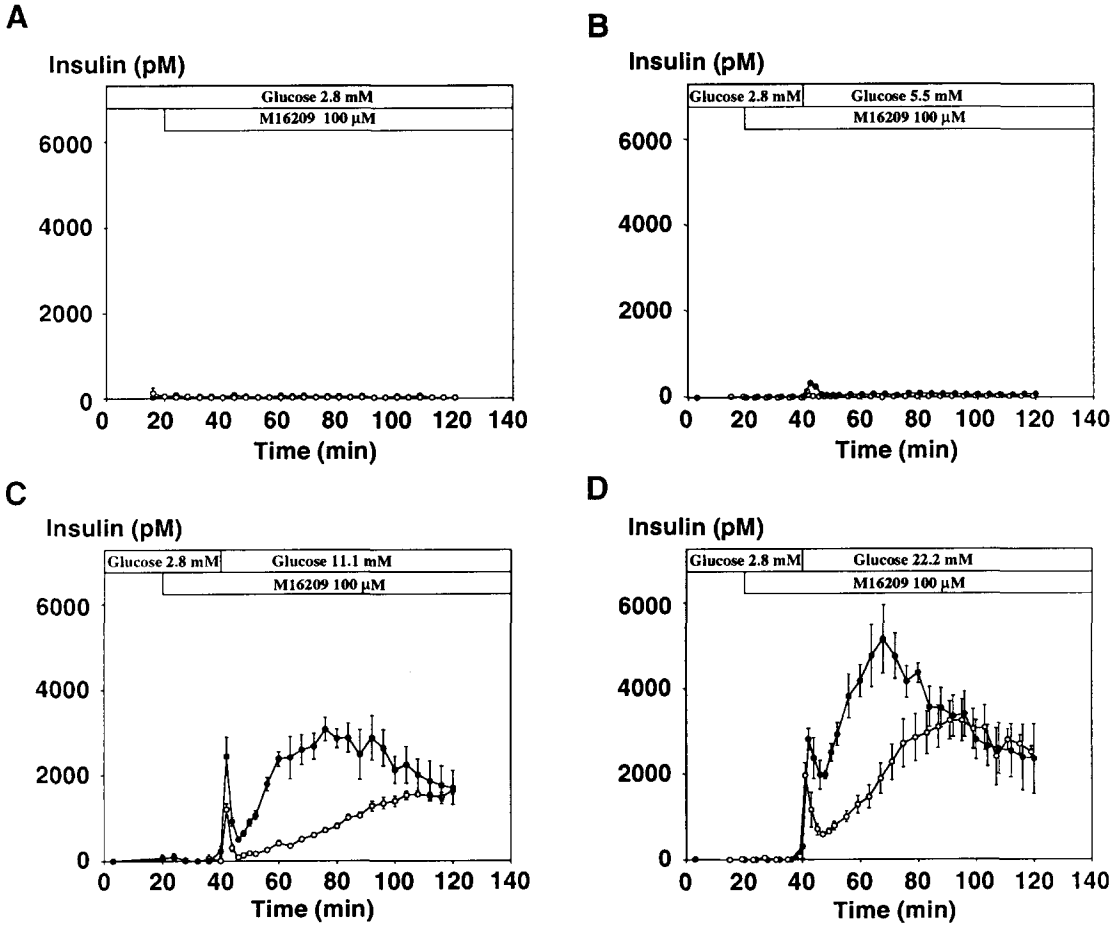

Fig. 1. Effects of M16209 on unstimulated insulin release (A) and insulin release in response to 5.5 mM glucose (B), 11.1 mM glucose (C) and 22.2 mM glucose (D) in isolated, perfused pancreases of normal rats. The basal glucose concentration was 2.8 mM and the glucose concentration was raised, starting 40 min after the perfusion started. M16209 (100 μM) was present in the perfusion buffer starting at 20 min. Control (○), M16209 100 μM (●). Values are means ±S.E. ($n = 3$).

Ham's F-12K medium containing 10% horse serum, 2.5% fetal bovine serum, 50 μg/ml streptomycin, 50 U/ml penicillin and 2.5 μg/ml amphotericin B (Dainippon, Osaka, Japan). Single channel recording techniques were essentially the same as described by Hamill et al. (1981). The cells were dispersed in a recording chamber filled with Tyrode solution (140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 5.5 mM glucose, 5.0 mM HEPES, pH 7.4). After making the giga-ohm seal, the bath solution was changed to an internal solution (150 mM KCl, 0.33 mM KH_2PO_4, 5 mM Hepes, 1 mM EGTA, pH 7.4). The ATP-sensitive K^+ channels were recorded in the inside-out configuration. The channel was identified from its current-voltage relation, which had a slope conductance of about 60 pS. ATP, applied to the bathing solution, depressed the activity of this channel. Signals from a patch clamp amplifier (EPC-7, List, Darmstadt, Germany) were recorded on a magnetic tape recorder (XR-510, TEAC, Tokyo, Japan) for later computer analysis (MBC-18SJ, SANYO, Tokyo, Japan). Experiments were performed at 30–32°C. M16209 dissolved in dimethylsulfoxide and glibenclamide (Sigma, St. Louis, MO) dissolved in ethanol were diluted with the internal solution.

2.4. Statistical analysis

The statistical difference between before and after drug treatment was evaluated using the paired Student's *t*-test

3. Results

3.1. Effect on insulin secretion in normal rats

Fig. 1 shows the effect of M16209 on unstimulated insulin secretion and glucose-stimulated insulin secretion in the isolated pancreases of normal rats. The

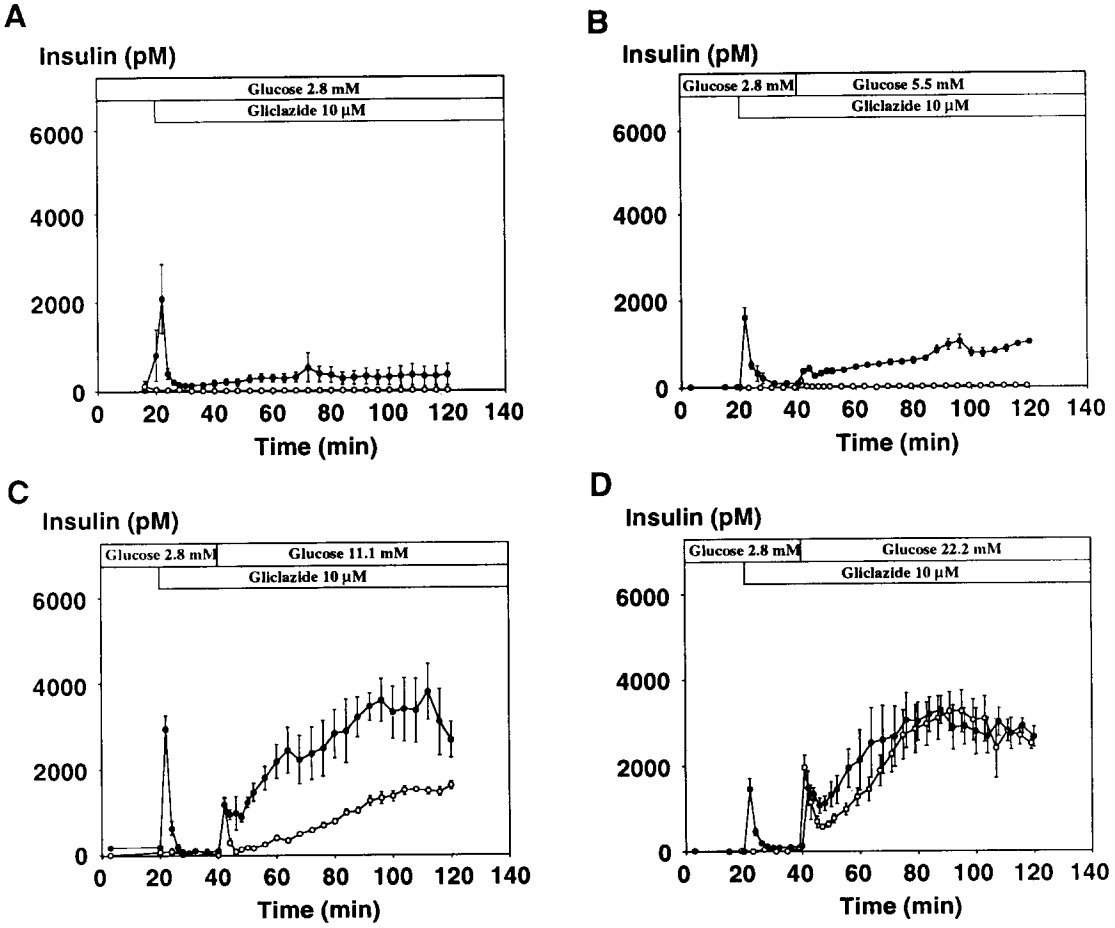

Fig. 2. Effects of gliclazide on unstimulated insulin release (A) and insulin release in response to 5.5 mM glucose (B), 11.1 mM glucose (C) and 22.2 mM glucose (D) in isolated, perfused pancreases of normal rats. The perfusion was performed as described in the legend for Fig. 1. Control (○) gliclazide 10 μM (●). Values are means ± S.E. ($n = 3$).

concentration (100 μM) of M16209 is near the maximal plasma drug concentration observed when the drug was given orally to normal rats at a dose of 10 mg/kg. M16209 markedly augmented glucose-stimulated insulin secretion (Fig. 1C and D), whereas unstimulated insulin secretion was unaffected (Fig. 1A and B). The accelerating effect was observed more pronouncedly in the second phase of insulin secretion.

Under stimulation with 11.1 mM glucose, the absolute areas under the curve for insulin between 40 and 120 min without or with M16209, at 10, 30 and 100 μM were 86.0 ± 5.8, 106.6 ± 11.7, 220.1 ± 18.4 and 204.4 ± 24.8 nM · min, respectively. The dose-dependent augmentation of insulin secretion was observed at 10–30 μM.

Insulin secretion in response to various doses of glucose in the presence of gliclazide at 10 μM is shown in Fig. 2. Gliclazide strongly enhanced unstimulated insulin secretion (Fig. 2A). The drug also augmented insulin secretion in response to 5.5 and 11.1 mM glucose in the second phase (Fig. 2B and C), whereas the insulin response to 22.2 mM glucose was almost unaffected (Fig. 2D).

Fig. 3 shows unstimulated insulin secretion in the presence and absence of either sorbinil or epalrestat, and insulin secretion in response to 11.1 mM glucose in the presence and absence of either sorbinil or epalrestat. Unstimulated insulin secretion was not affected by either drug (Fig. 3A and C), whereas the insulin response to 11.1 mM glucose was appreciably suppressed by both drugs (Fig. 3B and D).

3.2. Effect on insulin secretion in NIDDM rats

Fig. 4A and 4B show the effects of M16209 (100 μM) on unstimulated insulin secretion and insulin secretion in response to 22.2 mM glucose, respectively, in NIDDM rats. For comparison, insulin secretion in nor-

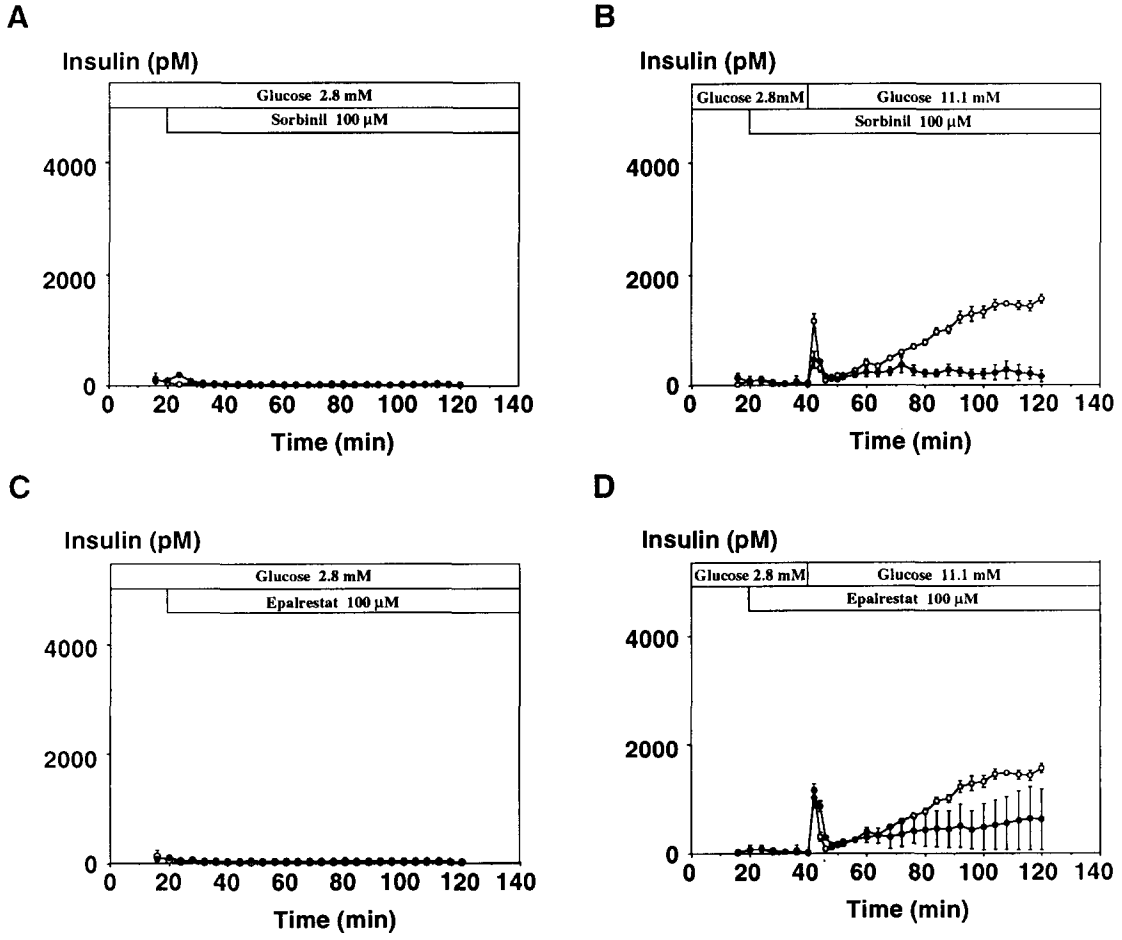

Fig. 3. Effects of sorbinil (A and B) and epalrestat (C and D) on unstimulated insulin release and insulin release in response to 11.1 mM glucose, in isolated, perfused pancreases of normal rats. The perfusion was performed as described in the legend for Fig. 1. Control (○), drug 100 μM (●). Values are means ± S.E. ($n = 3$).

mal rats without the drug is also shown in Fig. 4B. There was no difference in unstimulated insulin secretion between NIDDM and normal rats. NIDDM rats, however, exhibited a marked impairment of the insulin response to glucose compared with normal rats. The potentiating effect of M16209 on unstimulated insulin secretion was only slight, but that on insulin secretion in response to 22.2 mM glucose was appreciable: the decreased insulin response to glucose in NIDDM rats was partially, but not completely, restored in the presence of M16209. Unlike M16209, gliclazide did not affect either unstimulated or glucose-stimulated insulin secretion in NIDDM rat pancreases (Fig. 5).

3.3. Effect on ATP-sensitive K^+ channel activity

Fig. 6 shows the effect of M16209 on the open probability of ATP-sensitive K^+ channels in HIT-T15 cells. The open probability was unaffected by M16209 (30 or 100 μM), whereas it was significantly inhibited by 1 μM glibenclamide.

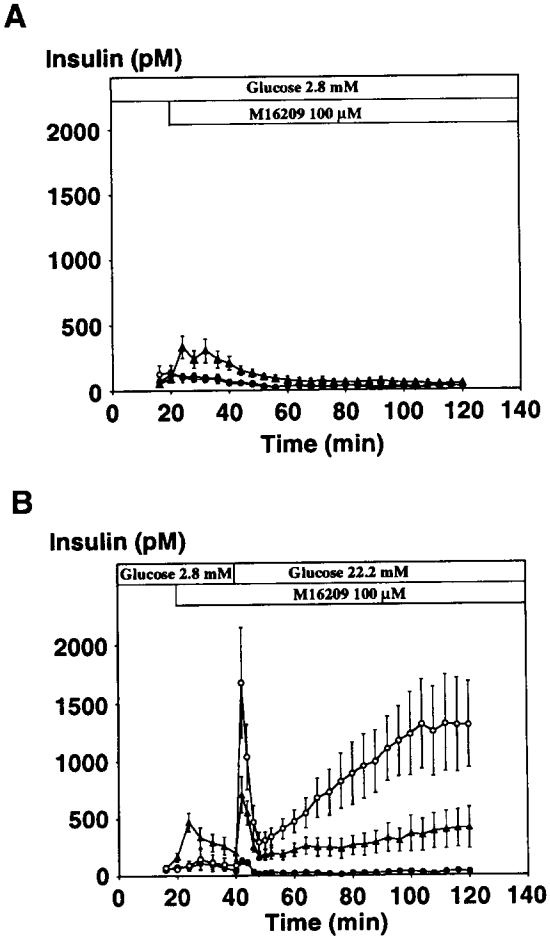

Fig. 4. Effects of M16209 on unstimulated insulin release (A) and insulin release in response to 22.2 mM glucose (B) in isolated, perfused pancreases of NIDDM rats. The perfusion was performed as described in the legend for Fig. 1. Control (●), M16209 100 μM (▲). Insulin release in normal rats not treated with the drug is also shown for comparison (○). Values are means ±S.E. ($n = 6$).

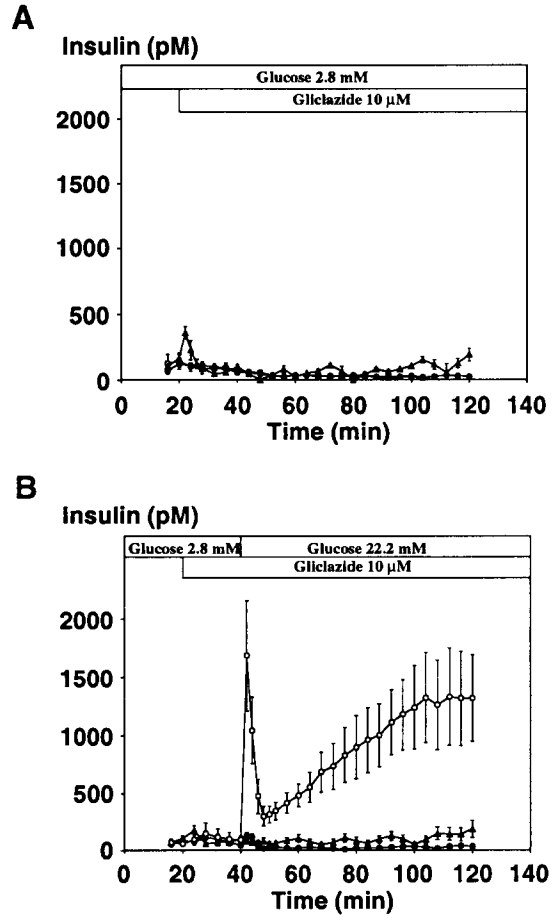

Fig. 5. Effects of gliclazide on unstimulated insulin release (A) and insulin release in response to 22.2 mM glucose (B) in isolated, perfused pancreases of NIDDM rats. The perfusion was performed as described in the legend for Fig. 1. Control (●), gliclazide 10 μM (▲). Insulin release in normal rats not treated with the drug is also shown for comparison (○). Values are means ±S.E. ($n = 3$–6).

4. Discussion

The effect of M16209 on insulin secretion in normal rats was characterized by its ability to augment glucose-stimulated insulin secretion without affecting basal insulin secretion. This is consistent with our recent findings that M16209 augmented insulin secretion in vivo in normal rats more pronouncedly as the blood glucose level increased (Nakayama et al., submitted). The concentration required to augment glucose-stimulated insulin secretion in the perfusion system can be attained in blood when M16209 at 10 mg/kg is given orally to normal rats. Thus, M16209 is likely to

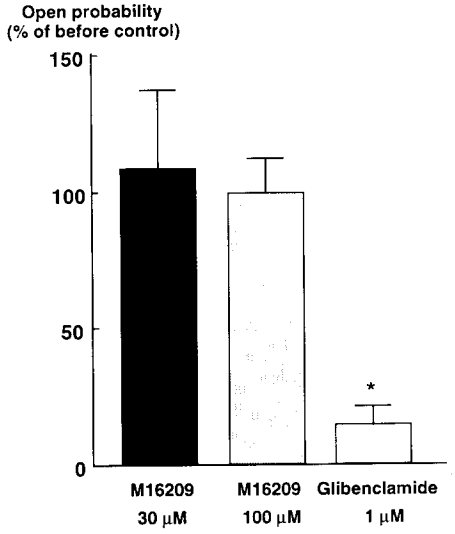

Fig. 6. Effects of M16209 and glibenclamide on the open probability of ATP-sensitive K^+ channels recorded from an inside-out patch of membrane excised from HIT-T15 cells. The experiments were performed at 30–32°C under a membrane potential of −50 mV. The drugs were added to the bathing solution. The open probability is expressed as the percentage of the corresponding before value. Values are means ±S.E. ($n = 4$–5). $^*P < 0.05$, vs. before treatment.

augment glucose-stimulated insulin secretion in vivo by acting directly on the pancreas.

The decreased insulin response to glucose observed in NIDDM rats was partially, but not completely, restored by M16209. This supported our recent findings that M16209 partially restored in vivo, glucose-induced insulin secretion in NIDDM rats (Nakayama et al., 1995). In contrast, gliclazide did not affect insulin secretion from perfused pancreases of NIDDM rats, although the drug was highly effective in pancreases of normal rats. It was previously reported that the impaired glucose tolerance of NIDDM rats was not appreciably improved by acute treatment with sulfonylureas (Marchione and Tuman, 1989) and that no augmentation of glucose-stimulated insulin secretion was observed in the isolated, perfused pancreases of NIDDM rats which had been acutely treated with sulfonylureas (Serradas et al., 1989). The results of this study support these findings.

Since aldose reductase is present also in pancreatic islets (Dufrane et al., 1984), it appeared likely that M16209 affected insulin secretion by inhibiting this enzyme. However, Malaisse et al. (1974) and Malaisse-Lagae and Malaisse (1986) reported that aldose reductase inhibitors did not affect insulin secretion in isolated rat pancreatic islets. Moreover, Giannarelli et al. (1994) recently demonstrated that tolrestat (Sestanj et al., 1984), a potent aldose reductase inhibitor, reduced glucose-induced insulin release in isolated bovine pancreatic islets. In our study using isolated rat pancreases, potent aldose reductase inhibitors, sorbinil (Peterson et al., 1979) and epalrestat (Kikkawa et al., 1983), inhibited glucose-induced insulin secretion, consistent with the latter study. The reason for this inhibition was unknown, but inhibition of aldose reductase, by which mechanism tolrestat was indicated to inhibit the glucose-induced insulin secretion (Giannarelli et al., 1994), appeared to be involved. The stimulation of glucose-induced insulin secretion by M16209, therefore, might not be caused by inhibition of aldose reductase.

Recently, the ATP-sensitive K^+ channel has been assumed to be the primary action site of sulfonylureas. In this study using HIT-T15 cells, an insulin-secreting cell line, glibenclamide, a potent sulfonylurea, strongly inhibited ATP-sensitive K^+ channels. We used glibenclamide as a positive control for inhibition of the ATP-sensitive K^+ channels in the patch clamp experiment, because the drug has been demonstrated to be a specific inhibitor of the channels (Schmid-Antomarchi et al., 1987). In addition, the HIT-T15 cells used in this study retain many differentiated functions and respond to most of the secretagogues and inhibitors of insulin secretion (Boyd et al., 1991). The presence of ATP-sensitive K^+ channels was also established in this cell line and some drug effects on the channels were tested (Hughes et al., 1992). No inhibition, however, was observed when M16209 was applied at 30 or 100 μM, which are submaximal and maximal concentrations, respectively, for stimulation of insulin secretion in the isolated, perfused pancreases of normal rats. This suggested that M16209 might not act on ATP-sensitive K^+ channels.

In future studies attempting to clarify the sites of action of M16209, it is necessary to examine its effects on intracellular free Ca^{2+} concentrations, whose rise in β-cells is a prerequisite for the induction of insulin secretion by glucose (Wollheim and Pozzan, 1984). The effect on GLUT-2, a glucose transporter, which participates in sensing glucose in pancreatic β-cells (Orci et al., 1989), also remains to be investigated.

In summary, it was demonstrated that M16209, unlike sulfonylureas, selectively augments glucose-stimulated insulin secretion in pancreases of normal and NIDDM rats. Neither the inhibition of aldose reductase nor the blocking of ATP-sensitive K^+ channels appears to be the mechanism of action of M16209.

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