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THE EFFECT OF A HYPOGLYCEMIC SULFONYLUREA ON INSULIN RELEASE FROM ISOLATED RAT PANCREATIC ISLETS

(Sulfonylurea/ insulin release/ phorbol ester)

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The effects of hypoglycemic sulfonylurea gliclazide on insulin release and its interaction with tumor-promoting phorbol ester in rat pancreatic islets were examined. Lower concentrations of gliclazide stimulated insulin release in the presence of 5.6 mM glucose, but the addition of high concentrations of gliclazide resulted in a tendency to reduce insulin release. The stimulatory action of gliclazide on insulin release was abolished in the absence of Ca^{2+}. Gliclazide also failed to stimulate insulin release in the presence of 16.7 mM glucose, and 50 μM gliclazide inhibited insulin release significantly. A synergistic effect of gliclazide on insulin release was observed in the presence of phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Such a combined effect of gliclazide with TPA was inhibited when extracellular Ca^{2+} was not present. These results indicate that gliclazide stimulates insulin release to affect Ca^{2+} handling in the pancreatic B cell.

Hypoglycemic sulfonylurea gliclazide is now available to treat noninsulin-dependent diabetes mellitus (NIDDM) in Japan.

This agent shows a rather moderate action in decreasing blood sugar and is thought to be a valuable hypoglycemic drug, especially in treating eldery patients with NIDDM. However, the effects of gliclazide upon islet function are not well understood. The first aim of the present paper was to study the direct effect of gliclazide on insulin release from isolated rat pancreatic islets.

We have shown that Ca^{2+}-activated and phospholipid-dependent protein kinase (C-kinase) might be involved in insulin stimulus-secretion coupling (1). The observations that tumor-promoting phorbol ester such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA) activates C-kinase (2) and stimulates insulin release from the islets (3, 4) prompted us to study the combined effect of gliclazide and TPA on insulin release. The data reveals that a lower concentration of gliclazide markedly enhances insulin release evoked by TPA in the presence of 5.6 mM glucose.

MATERIALS AND METHODS

All experiments were performed with islets isolated from male Wistar rats (250-300 g) which had been fasted overnight. The islets were isolated by collagenase digestion (5).

Standard buffer solution was Krebs-Ringer bicarbonate (KRB) buffered solution, pH 7.4, gassed with O_2/CO_2 (95:5), with the following ionic composition (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25 and supplemented with 0.2% fatty acid-free bovine serum albumin fraction V (Sigma, St. Louis, MO) and 2.8 mM glucose.

After preincubation for 45 minutes at 37°C under O_2/CO_2 (95:5) in the buffer, five islets were transferred to an incubation vial containing 0.5 ml of the buffer with or without test substances. Incubation was then performed for 60 minutes under the same conditions as described for the preincubation. Insulin concentrations in the medium were measured by polyethylene glycol radioimmunoassay (6) using rat insulin as a standard.

A stock solution of TPA (Sigma) dissolved in ethanol at a concentration of 1 μg/10 μl was stored at -20°C. The aliquot was evaporated to dryness under a stream of N_2O and sonicated in incubation medium to obtain a final concentration of TPA (0.2 μM). Gliclazide was a gift from Dainippon Pharmaceutical Co.

(Osaka, Japan). All results are expressed as the mean±SEM together with the number of individual determinations (n). Statistical analysis was performed with the unpaired Student's t-test.

RESULTS

Gliclazide was added to the medium at concentrations ranging from 5 μM to 100 μM (Fig. 1). In the presence of 5.6 mM glucose, 25 μM gliclazide exhibited a maximal insulin secretion. Even 5 μM gliclazide augmented insulin release modestly but significantly ($p<0.01$). At higher concentrations of sulfonylurea (gliclazide: 50-100 μM), however, insulin release was markedly decreased. Sulfonylurea (gliclazide: 5-25 μM) also failed to augment insulin release in the presence of 16.7 mM glucose. At the concentration of 50 μM, insulin release was decreased significantly (386.6±38.0 vs. 217.2±18.8 μU/islet/60 min $P<0.005$).

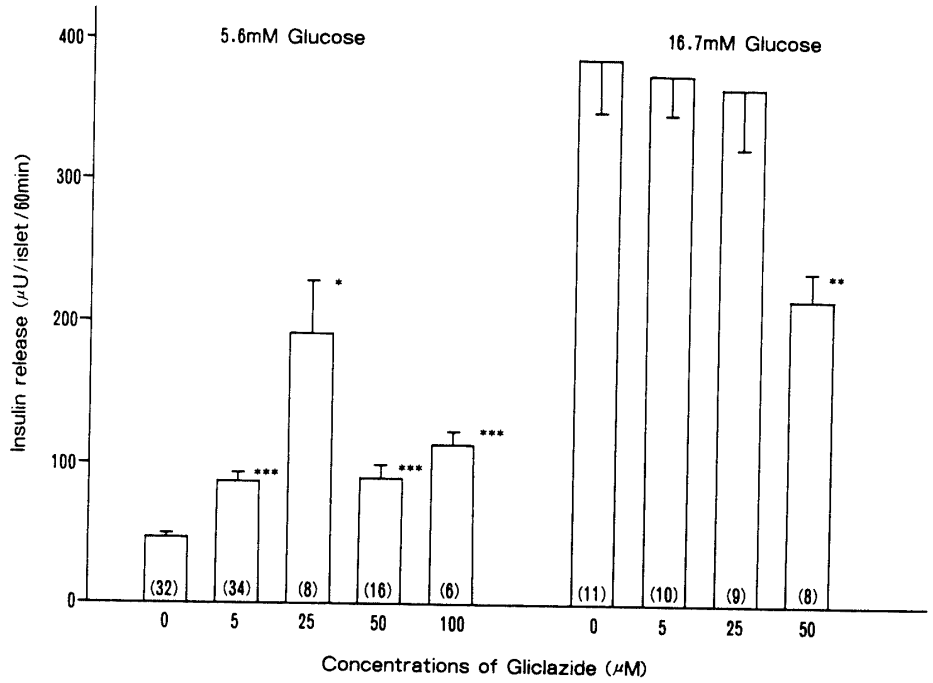

Fig. 1. Dose-dependency of gliclazide on insulin release in the presence of either 5.6 mM or 16.7 mM glucose. Each bar represents mean±SEM. * $P < 0.01$, ** $P < 0.005$, *** $P < 0.001$ versus islets incubated in the presence of glucose alone.

Table 1. Effects of gliclazide and TPA

Glucose (mM)	Gliclazide (μM)	TPA (μM)	Insulin release (μU/islet/60min)
5.6			42.5±4.9 (11)
5.6	5		85.9±7.1 (34)
5.6		0.2	99.7±7.5 (21)
5.6	5	0.2	302.7±27.5 (16)
5.6	100	0.2	312.8±34.2 (6)

Mean values (±SEM) are shown with the number of individual observations.

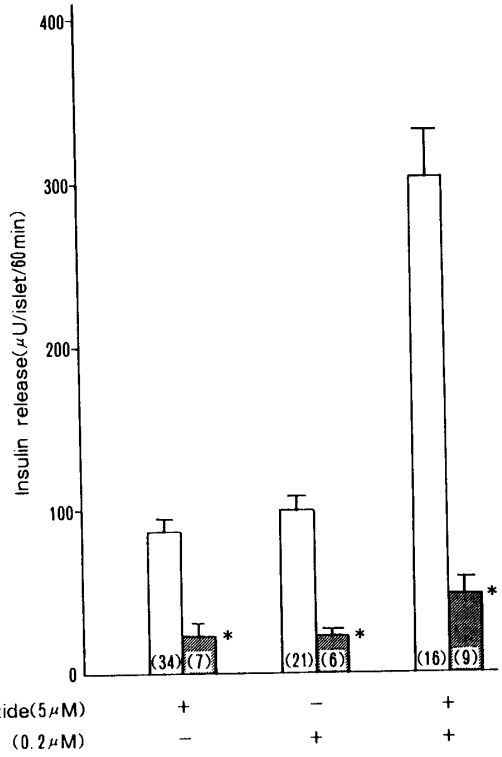

Fig. 2. Effects of extracellular Ca^{2+} on insulin release in various conditions. Glucose concentration in the medium was 5.6 mM. Each bar represents mean±SEM. Open columns indicate that the islets were incubated in the presence of $CaCl_2$. Shadded columns indicate that the islets were incubated in the absence of $CaCl_2$. * $P < 0.001$ versus islets incubated in the presence of $CaCl_2$.

As shown in Table 1, 0.2 μM TPA caused a modest but significant increase in insulin release compared with control ($P < 0.005$). The combination of gliclazide and TPA showed a synergistic effect on and dramatic increase in insulin release. When the concentration of gliclazide was increased to 100 μM, the magnitude of insulin release was similar to that observed with 5 μM gliclazide. When $CaCl_2$ was omitted from the medium, gliclazide and TPA did not stimulate insulin release (Fig. 2). The simultaneous addition of gliclazide and TPA enhanced insulin release compared with TPA alone in the absence of Ca^{2+} (22.5±3.0 vs. 45.8±6.6 μU/islet/60min, $P<0.025$), but the synergistic effect was not observed.

DISCUSSION

The present study clearly indicates that gliclazide is a potent stimulator of insulin release. Even 5 μM gliclazide stimulated insulin release significantly from the isolated islets. This finding seems very important, because the concentration of 5 μM always used in this study, covers the range in treated patients (7). The potency of gliclazide to induce insulin release is similar to tolbutamide and weaker than glibenclamide, as reported previously (8). Gliclazide did not stimulate insulin release in a dose-dependent fashion in the presence of 5.6 mM glucose. Maximal secretory activity was observed at the concentration of 25 μM, while at concentrations above 50 μM, insulin release was inhibited, but still significant compared with control. This inhibitory action of gliclazide seems a common phenomenon in sulfonylureas as previously suggested (8) and in accord with an experiment using tolbutamide. Henquin has demonstrated that tolbutamide secondarily inactivate Ca^{2+} channels and causes a decrease in Ca^{2+} influx (9). However, the mechanism of this inhibitory effect of sulfonylureas remains unclear. Gliclazide failed to augment insulin release and even had an inhibitory effect at concentrations of 50 μM in the presence of 16.7 mM glucose. This finding is also in accord with previous reports, indicating that at increasing glucose levels, only glibenclamide potentiated insulin release, and all other sulfonylureas tested produced less marked rates at high glucose levels (8). However, these phenomena still remains unclear.

TPA stimulated insulin release in the presence of 5.6 mM glucose. A synergistic effect of gliclazide and TPA on insulin release was also observed and the present result confirmed previous observation (10). It is of interest that gliclazide enhanced insulin release evoked by TPA even in the absence of extracellular Ca^{2+}, although the effect of gliclazide on insulin release was inhibited as shown in Fig. 2. A possible explanation for these results can be considered. Since sulfonylureas interact with plasma membrane (11) and with phospholipid bilayers in an artificial system (12), the combined effect might take place close to the B cell plasma membranes. Gliclazide also may act as a Ca^{2+} ionophore and directly interfere with the modality of Ca^{2+} inwards transport in the B cell (13), resulting in an activation of C-kinase in the presence of TPA. Indeed, under suitable exprerimental conditions, TPA facilitated the process of ionophore-mediated calcium exchange diffusion (14, 15). Gliclazide also enhances cyclic AMP-mediated insulin release (16). Thus it is suggested that gliclazide amplify the common process of insulin release and that an alteration of Ca^{2+} ion in the B cell have an important role of gliclazide-induced insulin release and synergistic effect with TPA. The present work reveals that an alteration of Ca^{2+} ion in the B cell might be involved in gliclazide-induced insulin release.

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