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GLYBURIDE ENHANCES INSULIN GENE EXPRESSION AND GLUCOSE-INDUCED INSULIN RELEASE IN ISOLATED RAT ISLETS

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Long-term glyburide therapy has been reported to improve glucose-induced insulin secretion in patients with NIDDM. We examined the effects of glyburide on the synthesis and release of insulin and insulin gene expression in isolated islets in vitro. Incubation with glyburide (500 ng/ml) significantly increased insulin release without affecting the insulin content. The PPI mRNA level was not increased after incubation for 1 h but was increased after incubation for 20 h. Incubation with 5 ng/ml of glyburide for 1 h or 20 h had no effect on the content or release of insulin, but incubation with 5 ng/ml of glyburide for 20 h significantly increased the PPI mRNA level and enhanced insulin release induced by 11 mM glucose. These results suggest that a high concentration of glyburide stimulates insulin release directly, while a low concentration of glyburide increases the PPI mRNA level and may thereby enhance glucose-induced insulin release.

Sulfonylureas are widely used in the therapy of NIDDM. Acute treatment of sulfonylureas is known to evoke insulin release from pancreatic B cells (1, 2) and this insulinotropic action appears to be responsible for the hypoglycemic effect of short-term therapy with sulfonylureas (3). Recent in vivo studies have shown that long-term sulfonylurea therapy improves glycemic control by increasing the responsiveness of pancreatic B cells to glucose in patients with NIDDM (4) and neonatal streptozotocin-treated rats (5). These studies suggested that sulfonylureas exert their effects not only by direct stimulation of insulin release from pancreatic B cells, but also by enhancing glucose-induced insulin release.

Glyburide, a second generation sulfonylurea, has been shown to have a more potent and prolonged insulinotropic action than other sulfonylureas (6, 7). In vitro

Abbreviations: NIDDM, non-insulin dependent diabetes mellitus; PPI, preproinsulin; GGB, Gey & Gey buffer; SSC, sodium citrate solution.

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studies showed that glyburide at a concentration of over 250 ng/ml stimulated insulin release from rat pancreatic islets (8). This concentration corresponds to the peak concentration in the serum at 1 or 2 hours after administration of 2.5 mg of glyburide per os (9). However, the concentration of glyburide falls 5 to 10 ng/ml at 12 to 24 hours after the administration of glyburide per os in man (10). At this lower concentration, glyburide has no direct insulinotropic action in vitro (8). Recent in vivo studies, however, have suggested that sulfonylureas enhance glucose-induced insulin secretion at this concentration (4, 5). To investigate the action of glyburide in vitro, we examined the effects of incubating isolated islets with glyburide on their synthesis and release of insulin and their expression of the insulin gene.

Materials and methods

Isolation and culture of islets

Male Wistar rats (150-200g) were anesthetized by intraperitoneal injection of 60 mg/kg of sodium pentobarbiturate. The pancreas was digested with collagenase. The isolated islets were preincubated for 40 h in RPMI 1640 medium containing 5.5 mM glucose and 10 % fetal calf serum, washed with GGB containing 5.5 mM glucose, and incubated in the same buffer for 1 h. The basal medium was GGB containing 5.5 mM glucose, 100 U/ml of penicillin, 100 μg/ml of streptomycin, 1 mg/ml of bovine serum albumin, 200 KIU/ml of aprotinin, and 0.1 mM phenyl-methyl-sulfonyl-fluoride. Islets were incubated in the basal medium with or without 5 to 500 ng/ml of glyburide in free-floating conditions for 1 or 20 h in an atmosphere of 5% CO_2 in humidified air at 37 °C.

To demonstrate that glyburide enhances glucose-induced insulin release from the islets, the isolated islets were incubated for 1 or 20 h with or without 5 ng/ml of glyburide in solution containing 5.5 mM glucose in the above conditions, and then washed twice with GGB and incubated with GGB containing 11 mM glucose for 1 h. Glucose-induced insulin release was expressed as the stimulation index, calculated as insulin release per hour relative to that in the preincubation period. Glyburide was kindly provided by Yamanouchi Pharmaceutical Co., Tokyo, Japan.

Measurements of release and islet content of insulin

The amount of insulin released from islets after incubation with or without glyburide was calculated from the insulin concentration of the incubation medium. The insulin concentration was measured by radioimmunoassay with purified rat insulin as a standard (Novo, Copenhagen, Denmark).

The insulin content of the islets was measured by radioimmunoassay after its extraction by the acid-ethanol method. The islets were sonicated in 300 μl of acid-ethanol solution (75 % ethanol, 1.5 % HCl; v/v) at 10 watts for 1 min. The mixture was stood overnight and then centrifuged and the supernatant was lyophilized. The residue was then dissolved in 1 ml of distilled water for determination of its insulin content.

Measurement of preproinsulin(PPI) mRNA level

After incubation for 1 or 20 h, the islets were collected by centrifugation, washed twice with GGB, and sonicated in 75 μl of lithium chloride mixture plus 75 μl of phenol solution for 15 s. The lithium chloride mixture consisted of 0.1 M lithium chloride, 0.2 M Tris (pH 8.0), 25 mM EDTA and 1 % SDS and the phenol

solution consisted of a mixture of phenol, chloroform and isoamylalcohol (50:48:2, v/v). The mixture was centrifugated and the aqueous phase was removed. The organic phase and interface were re-extracted with 50 µl of lithium chloride mixture and 30 µl of phenol solution (11). Total nucleic acids were precipitated with ethanol at -20 °C with transfer RNA as a carrier. The amount of PPI mRNA was determined by slot blot-hybridization. The nucleic acids extracted from 50 islets were denatured in 28 % formaldehyde and 7.5 x SSC; 1 x SSC = 0.15 M NaCl, 0.015 M Na citrate) at 65 °C for 15 min and dot-blotted to a nylon membrane (Hybond N, Amersham, Arlington Heights, IL). The filter was prehybridized in hybridization buffer containing 1 M NaCl, 1 % SDS, 0.4 M Tris (pH 7.0), 0.1 M EDTA, 0.1 mg/ml of salmon sperm DNA and 10 x modified Denhardt's solution (50 x modified Denhardt's solution: 1 % bovine serum albumin, 1 % Ficoll and 1 % polyvinylpyrrolidone) at 65 °C for 1 h and then hybridized with [^{32}P] dCTP-labeled rat PPI (I) cDNA probe (12) at 65 °C for 15 h in the same buffer. Then the filter was washed with 0.1 x SSC, 0.1 % SDS at 60 °C and exposed to Kodak XAR film with an intensifying screen at -80 °C for 12 h. After removal of the insulin probe, the filters were rehybridized with [^{32}P] dCTP-labeled β-actin DNA probe. The amount of hybridized mRNA was measured in a dual-wave-length flying spot analyzer CS-9000 (Shimazu, Kyoto, Japan).

Statistical analysis

Data are expressed as means±SEMs. Statistical significance was analyzed by the unpaired Student's t-test.

Results

Effect of glyburide on insulin release

Figure 1 shows the effect of glyburide on insulin release from isolated islets. On incubation for 1 h, 500 ng/ml of glyburide significantly increased insulin release

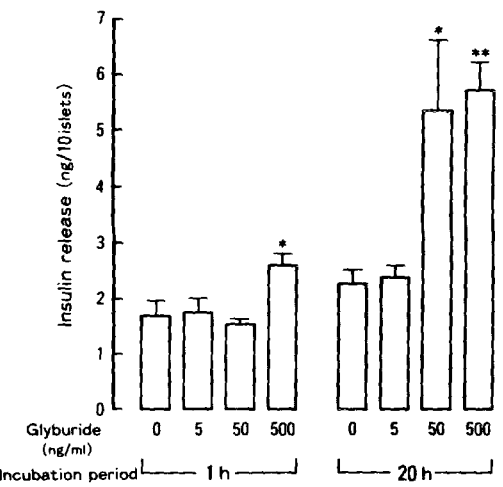

Fig.1. Effect of glyburide on insulin release from isolated rat islets. Batches of 10 isolated islets were incubated for 1 or 20 h in control buffer (5.5 mM glucose) with or without 5 to 500 ng/ml of glyburide. The concentration of insulin was measured by radioimmunoassay by the double antibody method with rat insulin as a standard. Columns and bars represent means+SEMs for 10-20 experiments. * $p<0.05$, ** $p<0.01$ vs. control value.

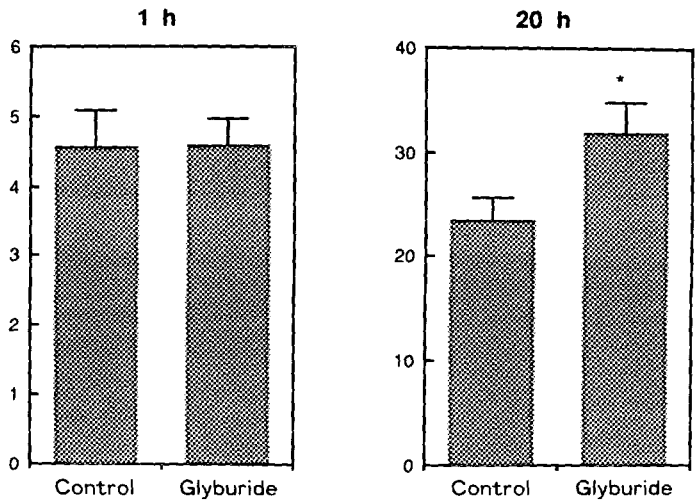

Fig. 2. Effect of pretreatment with 5 ng/ml of glyburide on glucose-induced insulin release (11 mM glucose). Glucose-induced insulin release was expressed as the stimulation index calculated as the ratio of the release of insulin per hour to that in the pretreatment period. Columns and bars represent means+SEMs for 15-20 experiments. * $p<0.05$ vs. control value.

from the control level of 1.7±0.3 to 2.6±0.2 ng/10 islets ($p<0.01$), but 5 and 50 ng/ml of glyburide did not affect insulin release (1.8±0.3 and 1.6±0.2 ng/10 islets, respectively). On incubation for 20 h, 50 and 500 ng/ml of glyburide significantly increased insulin release from the control level of 2.3±0.2 to 5.4±1.3 ($p<0.05$) and 5.7±1.0 ng/10 islets ($p<0.01$), respectively, but 5 ng/ml of glyburide did not affect insulin release from the islets (2.4±0.2 ng/10 islets).

Figure 2 shows the effect of pretreatment with 5 ng/ml of glyburide on insulin release induced by 11 mM of glucose. The isolated islets were incubated for 1 or 20 h with or without 5 ng/ml of glyburide in solution containing 5.5 mM glucose, and then washed twice with GGB and incubated with GGB containing 11 mM glucose for 1 h. Glucose-induced insulin release was expressed as the stimulation index, calculated as insulin release per hour relative to that in the preincubation period. Pretreatment with 5 ng/ml of glyburide for 1 h did not affect glucose-induced insulin release (stimulation index, 4.6±0.4 vs. control value of 4.6±0.5). In contrast, pretreatment with 5 ng/ml of glyburide for 20 h significantly enhanced glucose-induced insulin release (stimulation index, 32±3.0 vs. control value of 23±2.2; $p<0.05$)

Effect of glyburide on insulin content

Figure 3 shows the effect of glyburide on the insulin content of isolated rat islets. Incubation with 5, 50 or 500 ng/ml of glyburide did not affect the insulin content of the islets either after 1 h (74±14, 93±16 and 87±7.1 ng/20 islets, respectively, vs. control value of 101±29 ng/20 islets) or after 20 h (104±26, 109±9.4 and 127±32 ng/20 islets, respectively, vs. control value of 101±32 ng/20 islets).

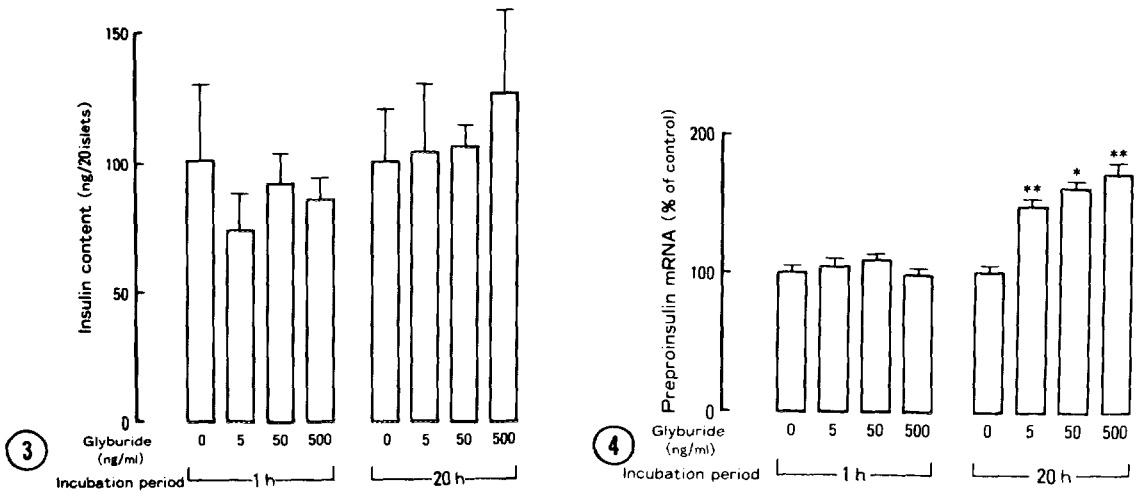

Fig. 3. Effect of glyburide on the insulin content of isolated rat islets. Experimental conditions are similar to Fig 1. Columns and bars represent means+SEMs for 5 experiments.

Fig. 4. Effect of glyburide on the PPI mRNA level in isolated rat islets. Experimental conditions are similar to Fig 1. Columns and bars represent means+SEMs for 6-7 experiments. * $p<0.05$, ** $p<0.01$ vs. control value.

Effect of glyburide on PPI mRNA level

Figure 4 shows the effect of glyburide on the PPI mRNA level in isolated rat islets. Incubation with 5, 50 and 500 ng/ml for 1 h did not affect the PPI mRNA level in islets (104±5.6 %, 109±3.5 % and 98±4.2 % of the control, respectively). In contrast, incubation with 5, 50 and 500 ng/ml of glyburide for 20 h significantly increased the PPI mRNA level in the islets in a dose-dependent manner [148±4.4 % ($p<0.01$), 161±5.3 % ($p<0.05$) and 171±7.6 % ($p<0.01$) of the control, respectively].

Discussion

Short-term treatment with sulfonylurea has been shown to increase insulin secretion in vitro and in vivo (1-3). However, prolonged treatment of the patients with NIDDM with this drug improved the glucose tolerance without any increase in the plasma insulin level (13). These observations suggest that an extrapancreatic action of sulfonylureas may contribute to improvement of glycemic control during long-term sulfonylurea therapy.

Glyburide, a second generation sulfonylurea, is reported to have a direct insulinotropic action in vitro as well as in vivo (1, 2). In our experimental conditions, incubation with 500 ng/ml of glyburide for 1 h increased insulin release. After oral administration of 2.5 mg glyburide, the concentration of glyburide is reported to reach a maximum of 250 to 500 ng/ml in 1 or 2 hours (9) and to decrease to a steady state concentration of 5 to 10 ng/ml (10) after 24 hours, although

interindividual variation was found (14). These results suggest that the acute effect of glyburide, observed in several hours after its administration, is exerted by its direct stimulation of insulin release from the pancreatic B cells.

In contrast, incubation with glyburide at a concentration of 5 ng/ml for 1 or 20 h did not stimulate insulin release from the islets. However, we found that incubation of isolated rat islets with glyburide at 5 ng/ml enhanced their glucose-induced insulin release without having a direct insulinotropic effect. These findings together with in vivo observations (4, 5) suggest that glyburide even at its steady state concentration enhances glucose-induced insulin release, and that this action may be responsible for the hypoglycemic effect of long-term treatment with this agent.

We found that incubation with glyburide for 20 h caused a dose-dependent increase in the insulin mRNA level in isolated islets. The insulin mRNA level of pancreatic islets is reported to be increased by many physiological regulators of insulin biosynthesis and release, such as glucose (15, 16), arginine (17) and leucine (15). We demonstrated that glyburide also increased the insulin mRNA level. Thus the increase in the insulin mRNA level may contribute to the insulinotropic action of sulfonylureas as well as those of glucose and amino acids. The increase in the insulin mRNA level may be due to positive control of insulin gene expression or increased half-life of mRNA or both. We are currently investigating these mechanisms and the intracellular pathway mediating glyburide-induced increase in the insulin mRNA level.

In our study, incubation with 5 ng/ml of glyburide, corresponding to the steady state concentration on its prolonged administration to patients, increased the PPI mRNA level after 20 h. This treatment did not stimulate insulin release from the islets directly, but enhanced glucose-induced insulin release from the islets. Thus glyburide may enhance glucose-induced insulin release by increasing the PPI mRNA level although the possibility that translational control may also be involved cannot be excluded. In patients with NIDDM during therapy with glyburide, the pancreatic islets are exposed to the steady state concentration of glyburide. Therefore, it is reasonable to speculate that glyburide increases the insulin mRNA level in the islets, enhances the postprandial insulin response and may contribute to improve glycemic control in patients with NIDDM.

In our experiments, even 20 h incubation with glyburide did not affect the insulin content of the isolated islets, but it increased the release of insulin from the islets and the PPI mRNA level. Curry showed that the insulin content of the pancreas was constant over long periods of high glucose stimulation (18). Schatz at al. demonstrated that glyburide increased the fractional release of newly synthesized insulin, and concluded that it enhanced release of fresh granules, but not of stored granules from beta cells (19). These observations may explain our finding that glyburide stimulated insulin release without affecting the insulin content of the islets.

In this work, we demonstrated that a high concentration of glyburide stimulated insulin release directly, whereas a low concentration, corresponding to the steady state level in patients during long-term treatment with this agent, did not increase insulin release, but increased the PPI mRNA level in the islets and enhanced the glucose-induced insulin release from the islets. These in vitro observations suggest that glyburide improves glycemic control in patients with NIDDM not only by direct stimulation of insulin release but also by enhancement of glucose-induced insulin release.

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