

Gliclazide Directly Inhibits Arginine-Induced Glucagon Release

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Arginine-stimulated insulin and somatostatin release is enhanced by the sulfonylurea gliclazide. In contrast, gliclazide inhibits the glucagon response. The aim of the present study was to investigate whether this inhibition of glucagon release was mediated by a direct suppressive effect of gliclazide or was secondary to the paracrine effect of released somatostatin. To eliminate the paracrine effects of somatostatin, we first perfused isolated rat pancreata with a medium supplemented with 23% of the standard calcium content. Second, we perfused isolated rat islets with a novel and highly specific antagonist of type 2 somatostatin receptor, DC-41-33 (2 $\mu\text{mol/l}$), which fully antagonizes the suppressive somatostatin effect on rat A cells. Gliclazide (30 $\mu\text{mol/l}$) inhibited glucagon release by 54% in the perfusion experiments, whereas the somatostatin response was nearly abolished. In islet perfusions with DC-41-33, arginine-induced glucagon release was inhibited by 66%. We therefore concluded that gliclazide inhibits glucagon release by a direct action on the pancreatic A cell. *Diabetes* 51 (Suppl. 3):S381–S384, 2002

Arginine stimulates release of insulin, glucagon, and somatostatin. Sulfonylurea compounds, such as glibenclamide and gliclazide, markedly enhance the arginine effect on somatostatin release, whereas glucagon response is suppressed (1–3). In the isolated perfused rat pancreas, insulin response to arginine is not significantly altered by glibenclamide (1), whereas gliclazide induces a transient enhancement of the insulin release (3). The mechanism behind the suppression of glucagon release by sulfonylureas is not clear. They may have a direct action on A cells (2,3). Alternatively, somatostatin released from D cells by sulfonylurea compounds may decrease glucagon response by a paracrine action (1). The former hypothesis is supported by studies in which sulfonylureas inhibited glucagon release in pancreata with

abolished insulin and somatostatin responses. The insulin response was blunted by destruction of B cells with alloxan (4) or streptozotocin (STZ) (3), whereas the somatostatin release was nearly abolished by perfusing pancreata with a medium containing a low calcium concentration (4) or by treating the animals with cysteamine (3).

The action of somatostatin on target cells is mediated by five somatostatin receptor subtypes (SSTR 1–5) (5). In rodents, somatostatin inhibits glucagon and insulin release via SSTR2 and SSTR5, respectively (6,7). A novel highly specific SSTR2 antagonist, DC-41-33, has recently been synthesized (8). When applied in both perfused isolated rat islets and isolated perfused rat pancreas, this compound enhances arginine-stimulated glucagon release nearly threefold, which implies that islet somatostatin exerts a pronounced paracrine effect on A cell secretion (K.C., D.H.C., S.E., submitted manuscript).

In this study, we used DC-41-33 to investigate whether gliclazide, a representative sulfonylurea, inhibits arginine-stimulated glucagon release when somatostatin effect on A cells is completely abolished.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats, age 2–3 months, were obtained from B&K Universal (Sollentuna, Sweden). The animals were fed ad libitum with free access to water and were placed in rooms with alternate 12-h periods of light and darkness.

To destroy B cells, we treated one group of animals with a single STZ injection (65 mg/kg body wt) administered in the tail vein. STZ (Sigma, St. Louis, MO) was previously dissolved in ice-cold saline, buffered with citrate buffer (pH = 4.5). Blood glucose was measured during the 4 days after the injection to confirm that the rats developed diabetes. After that, the animals were treated with subcutaneous insulin implants (LinShin Canada, Scarborough, Canada) for 10–15 days to lower blood glucose and avoid deleterious effects of hyperglycemia on A cell function.

Perfusion of isolated rat pancreas. Each animal was anesthetized with an intraperitoneal injection of sodium thiopental (100 mg/kg body wt). The pancreata were dissected free from adjacent tissues, as previously described (9). A cannula was inserted in the abdominal aorta to enable administration of perfusion medium, which consisted of Krebs-Ringer bicarbonate buffer (KRBB; in mmol/l: 115 NaCl, 4.7 KCl, 2.6 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 20 NaHCO₃, 16 HEPES) supplemented with 3.3 mmol/l glucose and 20 g/l BSA (pH 7.4). The medium was continuously gassed with a mixture of 95% O₂:5% CO₂ and perfused through pancreata during a 20-min equilibration period. Next there was a 30- (Fig. 1) or 20-min (Fig. 2) stimulation period, with 20 mmol/l arginine used as a stimulus for hormone release. During this period, gliclazide (Servier, Paris, France) was also added to the medium where noted (10 and 30 $\mu\text{mol/l}$) (Figs. 1 and 2, respectively). Finally, the basal medium was perfused for the last 10 min of the protocol. In experiments with pancreata isolated from STZ-administered rats, only 23% of the standard amount of calcium was added to the perfusion medium. The flow rate was kept at 2.8–3.2 ml/min. Samples were collected in ice-chilled tubes containing 1,000 units aprotinin (Trasylo; Bayer, Leverkusen, Germany) at 1-min intervals and stored at –20°C for subsequent radioimmunoassay of insulin (10), glucagon (11), and somatostatin (12).

Isolation of pancreatic islets and perfusion studies. Islets were isolated by digestion with collagenase (Hoffmann-La Roche, Basel, Switzerland) and

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AUC, area under the curve; KRBB, Krebs-Ringer bicarbonate buffer; SSTR, somatostatin receptor; STZ, streptozotocin; K_{ATP}, ATP-sensitive potassium channel.

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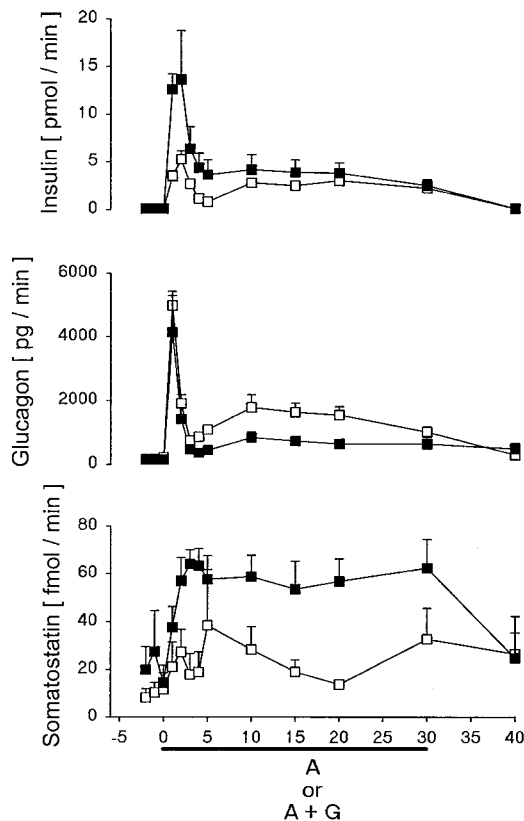


FIG. 1. Gliclazide inhibits arginine-induced glucagon release from isolated perfused rat pancreas. Hormone release was stimulated by 20 mmol/l arginine (A). Gliclazide (G) was used at the concentration of 10 μ mol/l. \square , control experiments ($n = 5$); \blacksquare , gliclazide ($n = 6$). Results are expressed as means \pm SE of n of experiments.

cultured for 20–22 h in RPMI-1640 medium supplemented with 11 mmol/l glucose and 10% (vol/vol) FCS. Before perfusion studies, the islets were preincubated for 35 min in KRBB containing 3.3 mmol/l glucose and 2 g/l BSA (Sigma, St. Louis, MO). Then 100 islets were added to each of two perfusion chambers, layered between inert polystyrene beads (Bio-Gel 200–400 mesh; Bio-Rad Laboratories, Richmond, CA). This perfusion system has been previously described (13). As a basal perfusion medium, we used the KRBB-albumin-glucose as described above, with the flow rate of 0.2 ml/min. The perfusion protocol was started by a 30-min equilibration period with basal medium, followed by a 20-min stimulation period, and finally a 10-min perfusion with the basal medium. During the stimulation period, the medium contained 20 mmol/l arginine and, where indicated, 30 μ mol/l gliclazide and/or 2 μ mol/l DC-41-33. Samples were collected at 2-min intervals, ice-chilled immediately, frozen, and kept at -20°C to be analyzed as above.

Statistics. Results are expressed as means \pm SE and were tested for significance using the unpaired Student's t test.

RESULTS

Effect of gliclazide on arginine-induced hormone release from the isolated perfused rat pancreas. In perfused pancreata of normal Wistar rats, 20 mmol/l arginine induced biphasic release of insulin, glucagon and somatostatin (Fig. 1). During the first phase (0–4 min), 10 μ mol/l gliclazide enhanced insulin response to arginine by 229% (expressed as area under the curve [AUC]; $P < 0.02$). However, gliclazide had no significant effect on the second phase of the insulin response (4–30 min). Glucagon response to arginine was not significantly affected by gliclazide during the first phase, but it was suppressed by 58% during the second phase ($P < 0.01$). Importantly, during both the first and second phases, gliclazide markedly

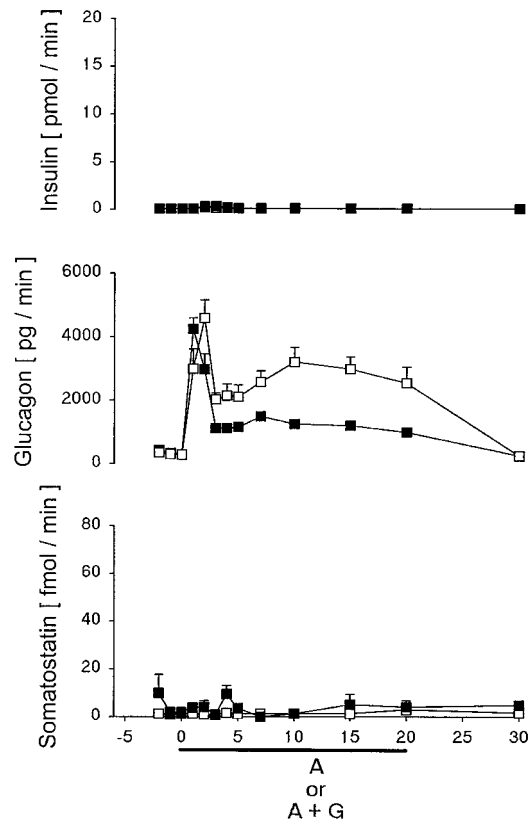


FIG. 2. Gliclazide inhibits arginine-induced glucagon release from isolated perfused pancreas of STZ-administered rats. The pancreas was perfused with buffer containing only 23% of standard calcium content. Hormone release was stimulated by 20 mmol/l arginine (A). Gliclazide (G) was used at the concentration of 30 μ mol/l. \square , control experiments ($n = 6$); \blacksquare , gliclazide ($n = 4$). Results are expressed as means \pm SE of n of experiments.

enhanced arginine-induced somatostatin release (by 299% [$P < 0.001$] and 246% [$P < 0.02$], respectively).

In pancreata isolated from STZ-treated animals and perfused with medium supplemented with 23% of standard calcium content, both insulin and somatostatin responses to 20 mmol/l arginine were blunted (Fig. 2). Although gliclazide (30 μ mol/l) did not significantly affect the release of insulin, it did enhance arginine-induced somatostatin release in some experiments, but to a level that failed to reach significance (4.6 ± 3.5 vs. 28.7 ± 33.2 fmol, expressed as AUC; $P = 0.4$, 0–20 min). Glucagon response to arginine was biphasic in this experimental model; it was suppressed by gliclazide during the second phase (4–20 min) by 62% ($P < 0.002$) and by 54% ($P < 0.002$) during the entire stimulation period (0–20 min).

Effect of gliclazide on arginine-induced hormone release from isolated perfused rat islets. In isolated perfused islets, 20 mmol/l arginine induced significant insulin and glucagon release (Fig. 3). Gliclazide (30 μ mol/l) enhanced the insulin response to arginine by 166% ($P < 0.001$) and suppressed the glucagon response by 76% ($P < 0.002$). At the concentration of 2 μ mol/l, DC-41-33 had no effect on arginine-induced insulin release, but markedly enhanced the glucagon response. Importantly, gliclazide suppressed glucagon release by 66% in the presence of DC-41-33 ($P < 0.02$).

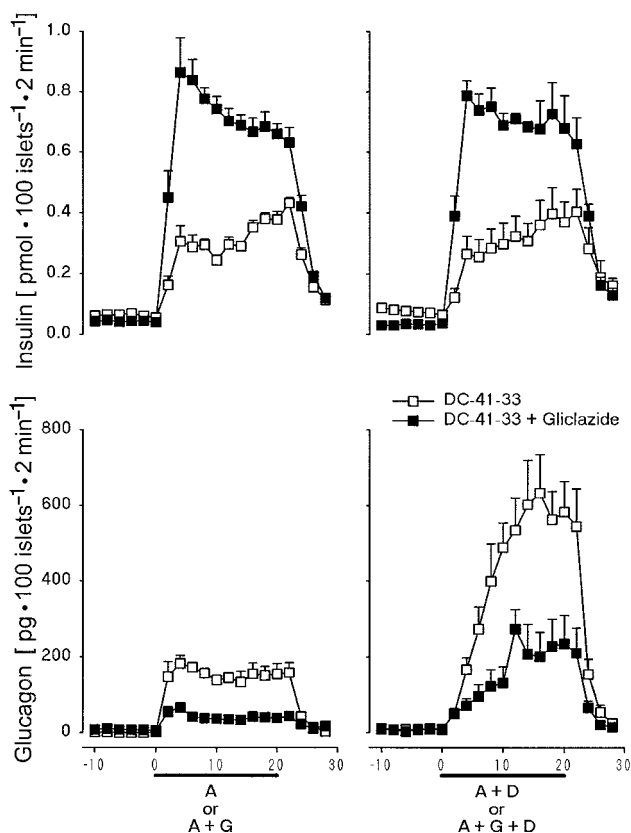


FIG. 3. Gliclazide inhibits arginine-induced glucagon release from isolated perfused rat islets. Hormone release was stimulated by 20 mmol/l arginine (A). Gliclazide (G) was used at the concentration of 30 μ mol/l and DC-41-33 (D) at the concentration of 2 μ mol/l. \square , control experiments ($n = 4$); \blacksquare , gliclazide ($n = 4$). Results are expressed as means \pm SE of n of experiments.

DISCUSSION

Somatostatin administered exogenously inhibits the release of insulin (14,15) and glucagon (16,17) from the endocrine pancreas. It has been also proposed that somatostatin released from islet D cells inhibits the secretion of insulin and glucagon by a direct paracrine action (18). However, some investigators have strongly argued against the paracrine effect of somatostatin (19–21).

The inhibitory effect of somatostatin on rodent A cells is mediated by SSTR2 (6,7). A potent and highly specific SSTR2 antagonist, DC-41-33, has been synthesized (8). Recently we used this antagonist to investigate whether paracrine interactions operate in pancreatic islets, and demonstrated that the arginine effect on glucagon release was markedly enhanced by DC-41-33 (K.C., D.H.C., S.E., submitted manuscript). Hence, islet somatostatin indeed suppresses the glucagon response by a paracrine route.

Sulfonylureas stimulate release of insulin and somatostatin (22–24). These effects are mainly mediated by closure of ATP-sensitive K^+ (K_{ATP}) channels after binding of sulfonylureas to their receptor, SUR1, which is a constitutive part of the K_{ATP} channel (25). This results in membrane depolarization that culminates in Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels and the onset of Ca^{2+} -dependent insulin (26) and, probably, somatostatin (27) secretion. In addition, it has been proposed that sulfonylureas enhance insulin release by a direct effect on exocytosis (28). In contrast, sulfonylureas have been

shown to inhibit glucagon release stimulated by arginine in both perfused isolated rat islets (2) and isolated perfused rat pancreas (1,3). Importantly, the sulfonylurea compound tolbutamide suppresses glucagon secretion in normal and type 2 diabetic subjects (29). Furthermore the sulfonylurea glibenclamide inhibits glucagon response in diabetic patients under hypoglycemic conditions (30). It has been proposed that the inhibitory action of sulfonylureas on glucagon release is mediated by a direct action, given that the effect was preserved in experiments where somatostatin release was blunted by a low extracellular calcium (4) or by cysteamine pretreatment of the animals (2,3).

Kawai et al. (19) proposed that the islet interstitium is divided into compartments, and that SSTRs are located in “somatostatin-poor compartments” and are separated from the “exocytosis-related interstitial space.” Our recent data indicate that at least a portion of somatostatin may be released directly into these SSTR-containing compartments (K.C., D.H.C., S.E., submitted manuscript). This implies that in the above-described low calcium (4) and cysteamine pretreatment experiments (3), the possibility cannot be ruled out that somatostatin attained higher concentrations in close proximity to the SSTRs than in the perfusion effluent. In the present study in pancreata isolated from STZ-treated rats and perfused with medium containing only 23% of the standard calcium concentration, gliclazide inhibited arginine-stimulated glucagon release whereas the insulin response was completely abolished (Fig. 2). A small but insignificant somatostatin response was observed. Hence, on the basis of these experiments, it is difficult to conclusively determine whether local paracrine effects of somatostatin released by gliclazide contribute, at least partially, to the inhibitory effect of the compound on glucagon secretion. To exclude this possibility, we simultaneously administered gliclazide and DC-41-33 (Fig. 3) to the isolated perfused rat islets. This SSTR2 antagonist was applied at 2 μ mol/l, a concentration that in batch-incubated rat islets abolishes the inhibitory effect of 10 nmol/l somatostatin on glucagon response to arginine (K.C., D.H.C., S.E., submitted manuscript). Because under these conditions arginine-induced glucagon release was inhibited by 66%, as compared to 76% in the absence of the antagonist, we concluded that the gliclazide effect on glucagon release is mediated mainly by a direct action of the sulfonylurea on A cells.

In situ hybridization has demonstrated the expression of the mRNA-encoding K_{ATP} channel subunits SUR1 and Kir6.2 in rat A cells (31). Moreover, using immunohistochemistry methods, SUR1 and Kir6.2 were localized in A, B, D, and PP cells (32). The sulfonylurea tolbutamide produced a concentration-dependent decrease in the whole-cell K_{ATP} conductance in isolated rat A cells and initiated action-potential firing at 20 mmol/l glucose (31). In addition, capacitance measurements in single A cells indicated that tolbutamide stimulates exocytosis evoked by depolarization or intracellular calcium infusion in these cells (33). In view of these observations, the present finding of the inhibition of arginine-induced glucagon release is unexpected and needs further explanation. We anticipated that in our studies, the A cell membrane would be depolarized in the presence of high arginine concentra-

tions (34). Therefore, we believe that the described inhibitory effect of gliclazide on glucagon release is mediated by a direct effect on exocytosis. The lack of the inhibitory effect of gliclazide on the first phase of glucagon response to arginine in the isolated perfused rat pancreas may reflect the time period necessary for this sulfonylurea compound to reach and penetrate the A cell membrane to accumulate in concentrations appropriate to inhibit exocytosis of glucagon.

In the present study, gliclazide showed marked synergism with arginine in stimulating first-phase insulin release. In contrast, in our previous study, we demonstrated only nonsignificant stimulation of arginine-induced insulin release by glibenclamide (1). This current finding is consistent with previous findings that gliclazide is more potent in stimulating the first phase of insulin release from isolated perfused pancreas (35).

In conclusion, we have provided evidence that the sulfonylurea gliclazide directly inhibits glucagon release. This may contribute to the antidiabetogenic action of the compound.

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