Effect of glucose/sulfonylurea interaction on release of insulin, glucagon, and somatostatin from isolated perfused rat pancreas

(glibenclamide)

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ABSTRACT The effect of a sulfonylurea, glibenclamide, on the release of insulin, glucagon, and somatostatin was studied in the isolated perfused rat pancreas. At glucose concentrations of 1.1 mM or less, the drug stimulated somatostatin release, whereas glucagon release, after 2–3 min of increase, was markedly inhibited. Insulin release was moderately stimulated, and maximal release occurred relatively late. A moderate glucose load (6.7 mM) inhibited glibenclamide-induced release of somatostatin, whereas the two in combination exerted an additive action on insulin release. Greater glucose loads, which by themselves would stimulate somatostatin release, only marginally suppressed glibenclamide-induced somatostatin release. The insulinogenic effect of these glucose levels was not modified by glibenclamide. Glibenclamide may thus stimulate both the alpha and beta as well as delta cells of the pancreas, depending on glucose concentration. We suggest a paracrine (local) interaction of somatostatin with the alpha and beta cells, which has an important role in the kinetics of insulin and glucagon release induced by sulfonylureas.

Sulfonylurea compounds stimulate the release of insulin and somatostatin from the pancreas (1–3). Their effect on insulin release is dependent upon the prevailing glucose concentration (4, 5). There are contradictory reports regarding the effect of sulfonylureas on glucagon release: they have been considered to either stimulate (6) or inhibit this function (2, 7, 8).

The present study demonstrates that the effect of one such sulfonylurea, glibenclamide, on somatostatin and glucagon release is glucose dependent as well and suggests a paracrine action of somatostatin on the alpha and beta cells of the islets.

MATERIAL AND METHODS

The pancreas was removed from fasting (18 hr) Sprague–Dawley rats (Cimex, Stockholm) and attached by the coeliac artery to an open-circuit perfusion system. The glands were perfused with a Krebs–Ringer bicarbonate solution (5) containing 2% bovine serum albumin, to which the various concentrations of glucose were added. After an initial equilibration period (20–40 min) with basal glucose concentration, glibenclamide (Daonil; final concentration, 1 µg/ml) or glucose (to give 6.7–33.3 mM) was added. The perfusion system was operated at a flow rate of 2.5 ml/min.

Insulin was determined by a double-antibody radioimmunoassay (9), using an insulin reagent kit (Radiochemical Centre, Amersham). Glucagon was assayed by the charcoal separation technique, using an antibody specific for pancreatic glucagon (10). Somatostatin was measured with a radioimmunoassay. Tyr-somatostatin was labeled with 125I by the lactoperoxidase method and separated on CM-cellulose column. The antibodies produced in our laboratory were used at a final dilution of 1: 56,000. Crossreactivity of the antibody was less than 0.01% with insulin, glucagon, substance P, luteinizing, and oxytocin. The antigenic specificity of the antibodies was determined by using somatostatin analogues (11). Phosphate buffer (0.04 M, pH 7.4) containing 1% bovine serum albumin was used as the diluent for all components in this radioimmunoassay. Incubations were for 48 hr at 4°C. Separation of bound from free tracer was on dextran-coated charcoal (12). Dilution of perfusates demonstrated binding parallel to that of the synthetic standard. The sensitivity of the assay was 5 pg/ml (13).

RESULTS

Effect of Glucose Concentration on Responsiveness to Glibenclamide by Beta, Alpha, and Delta Cells of the Pancreatic Islets. After isolation, each pancreas was equilibrated for 10 min with a medium containing 3.3 mM glucose and then perfused with either 0, 1.1, 3.3, or 4.4 mM glucose. Twenty minutes after the start of these infusions, glibenclamide at 1 µg/ml was added to the infusion medium (Fig. 1). Before the addition of glibenclamide, the amount of insulin and somatostatin in the perfusate increased with increasing glucose concentrations, whereas the amount of glucagon decreased (Fig. 1).

The insulin release by glibenclamide was considerably more pronounced at glucose concentrations of 3.3 and 4.4 mM than 0 and 1.1 mM. In contrast, the release of somatostatin after glibenclamide addition was more pronounced at glucose concentrations of 0–3.3 mM than at 4.4 mM.

The elevated levels of glucagon appearing at glucose concentrations of 0 and 1.1 mM were markedly inhibited by glibenclamide.

Table 1. Effect of interaction of glibenclamide and glucose on release of insulin and somatostatin

<table>
<thead>
<tr>
<th>Hormone released</th>
<th>Glucose load, mM</th>
<th>Glibenclamide (1 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, µU min⁻¹ ml⁻¹</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td>Sorbitol, µU min⁻¹ ml⁻¹</td>
<td>6.7</td>
<td>1.260 ± 147</td>
</tr>
<tr>
<td>Somatostatin, pg min⁻¹ ml⁻¹</td>
<td>16.7</td>
<td>12,965 ± 1129</td>
</tr>
<tr>
<td>Somatostatin, pg min⁻¹ ml⁻¹</td>
<td>33.3</td>
<td>20,649 ± 2176</td>
</tr>
</tbody>
</table>

* Insulin and somatostatin responses are expressed as incremental areas from 0 to 20 min (see Fig. 2). Values are given as mean ± SEM.
Effect of glibenclamide (1 μg/ml) on the release of insulin, glucagon, and somatostatin from the isolated rat pancreas perfused with 0 (n = 6), 1.1 (n = 8), 3.3 (n = 9), and 4.4 (n = 5) mM glucose (solid line). Hatched areas between the curves denote control experiments without glibenclamide. Values are given as mean ± SEM. U, unit.


benclamide. This latter effect become apparent 4–5 min after the glibenclamide infusion was started. In addition, glibenclamide seemed to exert a stimulatory effect on glucagon release during the first 2 min.

Effect of Glibenclamide on Release of Insulin and Somatostatin with Increasing Glucose Loads. In these experiments, the pancreas was equilibrated for 20 min with 3.3 mM glucose and then stimulated with increasing amounts of glucose in the absence or presence of glibenclamide (Fig. 2, Table 1). The lowest glucose load applied, 6.7 mM, clearly stimulated insulin release. This was even more pronounced with 16.7 mM glucose, whereas infusion of 33.3 mM glucose was accompanied by only a minor further increase. Somatostatin release was not affected by 6.7 mM glucose whereas glucose at 16.7 and 33.3 mM was stimulatory.

Infusion of 1 μg of glibenclamide per ml with 6.7 mM glucose produced an additive effect on insulin release. The drug had no effect on the insulin release stimulated by 16.7 and 33.3 mM glucose.

The increases in somatostatin release by glibenclamide was markedly inhibited by 6.7 mM glucose (P < 0.005). The inhibition was less pronounced at 16.7 mM glucose (P > 0.05) and almost absent at 33.3 mM glucose (P > 0.05) (Table 1). This complex interaction between glucose concentration, glibenclamide, and the release of insulin and somatostatin was confirmed by another series of experiments (Fig. 3). Again, glibenclamide enhanced the insulinogetic effect of 6.7 mM but not of 16.7 mM glucose. Furthermore, 6.7 mM glucose itself had no effect on somatostatin release but markedly inhibited glibenclamide-induced release of somatostatin. Conversely, 16.7 mM glucose stimulated somatostatin release while somewhat inhibiting the effect of glibenclamide on somatostatin release.
**DISCUSSION**

The insulin-releasing effect of the sulfonylureas is well recognized. The present study demonstrates that one such drug, glibenclamide, also influences the release of two other islet hormones, glucagon and somatostatin, and that the prevailing glucose concentration is of importance in this connection.

As expected, glibenclamide was a more potent insulin releaser under normoglycemic than under hypoglycemic conditions. Somatostatin release also increased with increasing glucose concentrations, but the response of somatostatin to glibenclamide was lowest at the highest basal glucose concentration.

The effect of glibenclamide on glucagon release at the two lowest glucose concentrations occurred in two phases: an initial stimulation lasting for about 3 min followed by marked inhibition. This observation probably explains previous reports suggesting either a stimulatory or inhibitory effect of sulfonylureas on glucagon release (6–8). The inhibition of glucagon release by glibenclamide, which was parallel to the phase of rapid increase in somatostatin release, implies that somatostatin may have mediated the inhibition of glucagon release. In such a case, the only possible explanation is a paracrine action of somatostatin on the glucagon-producing (alpha) cells of the islets. This is supported by our finding that glibenclamide (3) as well as another hypoglycemic agent (HB 699) (unpublished results) enhanced arginine-induced somatostatin release and inhibited arginine-stimulated glucagon release from the perfused rat pancreas. The close proximity of delta and alpha cells in the rat pancreas favors this hypothesis. Finally, the enhanced glucagon release induced by somatostatin antibodies in isolated rat islets further supports this idea (14).

It is not now possible to infer whether the marked increase in somatostatin release induced by glibenclamide also participates in the regulation of insulin release from the rat pancreas. However, the finding that a moderate glucose load enhances the effect of glibenclamide on insulin release while inhibiting its effect on somatostatin release provides support for such an idea. Moreover, high glucose loads, themselves agonists of so-
somatostatin release, did not suppress glibenclamide-stimulated somatostatin release and the insulinogenic effects of those glucose loads were not enhanced by glibenclamide. A paracrine interrelationship between the delta and beta cells has been suggested (15, 16).

It thus appears that sulfonylureas (glibenclamide) stimulate the alpha and beta as well as delta cells of the pancreas and that the prevailing glucose concentration plays an important role in the action of the drug on the three cell types. The studies also imply that a paracrine interaction of somatostatin with the alpha and beta cells is important in determining the net effect of the drug on insulin and glucagon release.

So far, it cannot be concluded that similar interrelationships among the islet cells also exist in humans. However, the development of a drug that, by stimulating somatostatin release, inhibits the release of glucagon in humans is not unlikely. Such a drug would be of value in the treatment of insulin-requiring diabetics. Another possibility implicit in these results is the development of drugs that stimulate pancreatic insulin release without interfering with somatostatin release. Such a drug, in principle, could be of value in the treatment of non-insulin-requiring diabetics.

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