Tolbutamide, glucose, calcium, and somatostatin secretion

Kjeld Hermansen

Second University Clinic of Internal Medicine, Kommunehospitalet, DK-8000 Aarhus C, Denmark

Abstract. We studied the actions of tolbutamide on the release of somatostatin, insulin, and glucagon from the isolated, perfused dog pancreas. Tolbutamide (180 μM) elicited a biphasic response of all three islet hormones in the absence of glucose. In the presence of normal glucose (5.5 mM), 180 μM tolbutamide was again stimulatory, however, now the D cell response appeared uninphasic and the relative increase of somatostatin was lower than in the absence of glucose. At the highest perfusate glucose of 11 mM, no augmentation of the somatostatin output was seen to 180 μM tolbutamide, whereas the B and A cell secretion was still stimulated. In dose-response studies with tolbutamide (1.8–1800 μM) it was found that the D cell threshold to tolbutamide was 18 μM. Furthermore, that maximal D cell secretion was attained in the presence of 180 μM tolbutamide at low glucose (1.4 mM) and of 18 μM tolbutamide at normal glucose (5.5 mM), respectively. The insulin and glucagon responses showed clearcut dose-dependency over the range of tolbutamide doses applied. The B and A cell threshold to tolbutamide was 1.8 μM when the prevailing glucose level was stimulatory for the B and A cell, respectively. The finding that D and B cell responses to tolbutamide were eliminated during calcium deprivation indicates a key role of calcium in the events that proceeds to tolbutamide-mediated somatostatin and insulin release. In conclusion, the primary effect of tolbutamide on the islet cells is to stimulate hormone secretion, however, perturbations in terms of appearance and magnitude of D, B, and A cell responses depend on the balance between the concentrations of tolbutamide and glucose.

Recent findings have revealed that sulphonylureas, when administered acutely, stimulate the somatostatin secretion from pancreas (Ipp et al. 1977; Samols et al. 1978; Efendic et al. 1979, 1980). Their action on the pancreatic D cell secretion is modulated by the prevailing glucose concentration. Thus larger somatostatin responses are apparently obtained at lower rather than at higher glucose levels (Samols et al. 1978; Efendic et al. 1979). Since no dose-response studies for the sulphonylureas have been performed in these reports, the interpretation of the results is, however, difficult.

This study has been undertaken, therefore, to investigate the effects of different doses of tolbutamide upon the somatostatin secretion from the isolated dog pancreas both at low and normal glucose levels. We also looked at the role of extracellular calcium for the tolbutamide mediated somatostatin secretion. To elucidate and compare the responses of the D, B, and A cells, the results of insulin and glucagon measurements will be discussed briefly.

Material and Methods

Preparation and perfusion media

Mongrel dogs, fasted overnight, weighing 18–25 kg, were used as pancreas donors. The technique for isolation of the pancreas and the perfusion system has previously been described in detail (Iversen & Miles 1971). In brief, the preparation consisted of the pancreas and the proximal 10 cm of the attached duodenum. A non-recirculating medium, consisting of a Krebs-Ringer bicarbonate buffer containing 40 g/l dextran (MW 75 000), 2 g/l bovine albumin, glutamate, fumarate, and pyruvate, each at a concentration of 5 mM, was pumped through the splenic and coeliac arteries, and the total portal effluent was collected every minute. The ionic composition of the standard perfusion medium was as follows: (mEq/l): Na+, 140.0; K+, 4.4; Ca++, 2.6; Mg++, 1.8; Cl−, 124.0; HCO−3, 24.4; SO4−2, 1.8; and H2PO4−, 1.1.

Oxygenation of the Krebs-Ringer bicarbonate buffer
was achieved by means of a rotating roller screen in an atmosphere of 94.4% O₂ and 5.6% CO₂. During the experiments, the perfusion fluid had a constant pH of 7.4 and a temperature of 37°C. The perfusion pressure was 30–40 mmHg, and the perfusion flow was 20 ml/min.

Experimental procedure
Samples were taken every minute from the efflux. To prevent possible degradation of glucagon and somatostatin, 3 mg/ml EDTA was added to the tubes collecting the efflux. The samples were stored immediately at −18°C until assayed.

In the dose-response studies, performed both in the presence of 1.4 mM and 5 mM glucose, tolbutamide was administered at increasing concentrations to three pancreata, while in one pancreas it was infused at decreasing concentrations. Studying the influence of the prevailing glucose concentration for the islet cell responses to tolbutamide (180 μM), the three glucose concentrations (0, 5.5, and 11 mM) were randomly given to five pancreata.

RIAs
Somatostatin was measured by radioimmunoassay as previously described (Hermansen et al. 1979a,b; Hermansen 1980a,b, 1981). The perfusion buffer was used as diluent for standards. The detection limit was 2 pg/ml. The antibody employed had no cross-reactivity with other pancreatic hormones (insulin, glucagon VIP, or pancreatic polypeptide). Neither interfered tolbutamide in the somatostatin assay. Insulin and glucagon were both measured in the perfusate by specific and sensitive radioimmuassays as previously described in detail (Ørskov et al. 1968). A pancreatic glucagon specific antiguacagon serum (Lise Heding, NOVO Research Institute, Copenhagen) was used.

Calculations
The change in hormone secretion (Δ values): the mean of the 1 min hormone values during the entire tolbutamide infusion (B) is related to the average of the last five 1 min values just before the addition of the sulphonylurea (A) i.e. Δ = B−A. Statistical analysis of the dose-response relationship was made by Page’s test (Page 1963), which is a non-parametric paired test designed to test for the presence of trends in data. Otherwise Student’s t-tests were used. In all cases a 5% limit of confidence was employed to assess the significance of differences.

Results
Dynamics of tolbutamide-stimulated hormone release at zero, normal, and high glucose
The effects of 10 min infusions of tolbutamide upon D, B, and A cell secretion were studied at zero, normal (5.5 mM) and high (11 mM) glucose concentrations in five perfusion experiments (Fig. 1). At zero glucose, the basal levels of somatostatin and insulin were low and those of glucagon were high. Tolbutamide at a concentration of 180 μM elicited an immediate biphasic release of somatostatin (2P < 0.05), insulin (2P < 0.02), and glucagon (2P < 0.05) (Fig. 1, left part). When the glucose concentration was raised to 5.5 mM, the somatostatin and insulin release was augmented and glucagon was suppressed (Fig. 1, middle part). Also at this perfusate glucose concentration, tolbutamide (180 μM) stimulated the D (2P < 0.05), B (2P < 0.001) and A (2P < 0.01) cell secretion. However, the release pattern of the somatostatin response had changed. Now only the positive first phase of somatostatin release was seen. The relative increase above basal of somatostatin was significantly higher at zero than at 5.5 mM glucose (104 ± 26% vs 33 ± 8%, 2P < 0.05). At the elevated level of somatostatin appearing at a glucose concentration of 11 mM, 180 μM tolbutamide failed to cause any significant change, although a response consisting of a tiny positive phase followed by a slight suppression appeared. In contrast, tolbutamide induced a significant increase in both insulin (2P < 0.01) and glucagon (2P < 0.05) output at this high perfusate glucose.

Effects of various tolbutamide doses at low and normal glucose levels
The effects of 10 min perfusion of 1.8, 18, 180 and 1800 μM tolbutamide were studied both at a glucose concentration of 1.4 and of 5.5 mM. Individual experiments are illustrated in Fig. 2. At the glucose concentration of 1.4 mM (Fig. 2, A) tolbutamide caused biphasic somatostatin responses when infused at concentrations of 18, 180 and 1800 μM. Also at a normal glucose concentration of 5.5 mM the tolbutamide dose of 18 μM elicited a biphasic D cell response (Fig. 2, B). By increasing the sulphonylurea concentration above these concentrations at the respective perfusate glucose, a prompt but transient somatostatin output was seen. The rebound after withdrawal of tolbutamide was elicited when the second phase was absent or even consisted of a slight suppression. As expected, insulin responses to tolbutamide were biphasic. Also the A cell responses were biphasic, however, with a relatively high, sustained second phase.

Fig. 3 demonstrates the relationship between the tolbutamide doses (1.8–1800 μM) administered
Somatostatin, insulin, and glucagon secretion in response to tolbutamide (180 μM) from the isolated dog pancreas perfused with (0, 5.5, and 11 mM glucose. Results are given as mean ± SEM of 5 pancreas perfusions.

and the changes in pancreatic hormone release. At sulphonylurea doses above 1.8 μM, a significant increase in D cell secretion appeared irrespective of the glucose level. At the lowest glucose concentration of 1.4 mM maximal somatostatin secretion was seen in the presence of 180 μM tolbutamide while it
Fig. 2.
Effects of tolbutamide at concentrations of 1.8, 18, 180, and 1800 μM upon the secretion of somatostatin, insulin, and glucagon during perfusion with 1.4 mM (A) and 5.5 mM glucose (B). These representative experiments are also included in Fig. 3.
was already attained at 18 μM tolbutamide when perfusate glucose was raised to 5.5 mM. Furthermore, the highest tolbutamide concentration of 1800 μM was unable to alter the D cell secretion at this physiological glucose concentration. The results were independent of whether the sulphonylurea was administered at increasing or decreasing concentrations. Tolbutamide elicits a significant dose-dependent stimulation of insulin and glucagon secretion at both 1.4 mM glucose (2P < 0.001 for both) and 5.5 mM glucose (2P < 0.01 and 2P < 0.001, respectively). The threshold to tolbutamide was significantly lower at a stimulatory glucose level for both the B (2P < 0.05) and A cells (2P < 0.001) i.e. at glucose 5.5 mM for insulin and 1.4 mM for glucagon release.

To see whether repetition of the tolbutamide stimulation influenced the D and B cell responses, the effects of 10 min perfusion of 180 μM tolbutamide administered twice with 15 min intervals were studied at 5.5 mM glucose. It was found in three pancreata that repeated stimuli with a constant dose of tolbutamide gave rise to the same somatostatin and insulin responses, respectively. The second hormone response during the 10 min period with tolbutamide thus averaged 101 ± 13% (for somatostatin) and 94 ± 3% (for insulin) of the response obtained during the first tolbutamide stimulation (mean ± SEM, n = 3).

Effects of tolbutamide at zero calcium

The dependency on extracellular calcium of the D and B cell responses to tolbutamide was investigated at normal glucose (5.5 mM) in three pancreata. During perfusion with a calcium depleted medium 10 min infusions of tolbutamide (180 μM) had no effect on either the release of somatostatin (27 ± 3 pg/ml (control) vs 31 ± 3 pg/ml; mean ± SEM, n = 3) or the release of insulin (3 ± 1 μU/ml (control) vs 3 ± 1 μU/ml; mean ± SEM, n = 3).

Discussion

The present studies were designed to examine the acute effects of tolbutamide on pancreatic somatostatin release at different glucose and sulphonylurea concentrations. To this end we have compared different aspects of the pancreatic D, B and A cell responses to tolbutamide, the secretory pattern, the interaction with glucose, the threshold doses of the sulphonylurea.

Apart from the prompt onset of the somatostatin release, the dynamic pattern of the D cell responses differed somewhat at the individual glucose and sulphonylurea doses. Characteristically a biphasic
somatostatin response was caused by tolbutamide. However, by increasing the glucose level and/or tolbutamide level the response faded out after the first positive phase. In other words the development of the secondary phase was more prone to manifest itself at lower glucose and/or sulphonylurea doses. In contrast, the dynamic pattern of B and A cell responses appeared less dependent of the prevailing sulphonylurea and glucose levels.

Perfusion with 180 μM tolbutamide caused a higher relative stimulation of somatostatin during hypo- or aglucosia than during normogluicosia (Figs. 1 and 3), as has previously been documented under comparable conditions by Samols et al. (1978). The disparity in response-sizes may be ascribed to differences in the position of the dose-response curves obtained at the two perfusate glucose levels (Fig. 3). Maximal D cell responses are thus seen at 18 μM tolbutamide in the presence of 5.5 mM glucose and 180 μM tolbutamide at 1.4 mM glucose. A further shift in the dose-response curve may explain why the D cell response to 180 μM tolbutamide is even lacking at a glucose dose of 11 mM that elicits maximal D cell response (Hermansen 1981). Thus perturbations in terms of stimulation or no effect may be possible depending on the balance between the concentrations of glucose and tolbutamide. The effects on D cell secretion observed in response to tolbutamide concentrations between 1.8 and 180 μM may be of clinical relevance since such concentrations cover the range in treated patients (Braselton et al. 1977). At present it is, however, unknown whether these in vitro findings correlated the in vivo situations.

With respect to insulin it is well established that sulphonylureas stimulate the B cell secretion and that the stimulatory effect is closely dependent on the glucose concentration present (for review see Hellman & Tåljedal 1975), and this is confirmed in this report. In contrast, both in vivo and in vitro experiments are inconsistent regarding the effects of sulphonylureas on glucagon release. They have been reported to inhibit (Samols et al. 1969; Laube et al. 1971; Ohneda et al. 1974), to have no effect (Aguilar-Parada et al. 1969; Pek et al. 1972; Kajinuma et al. 1974), to stimulate (Loubatières et al. 1974) or to exert a dual stimulatory and inhibitory effect on glucagon secretion (Samols & Harrison 1976, 1977; Grodsky et al. 1977). Based on the latter results (Samols & Harrison 1977; Grodsky et al. 1977) it has been suggested that the primary effect of sulphonylureas on A cell is to stimulate glucagon secretion, a proposal that is corroborated by the present results. Some reasons for these disparate results in the abovementioned investigations may, however, also be differences in species, in levels of the free non-protein bound sulphonylurea concentration, and in nutritional milieus.

The mechanism of action of sulphonylureas is still incompletely understood. Recent reports, however, demonstrate that tolbutamide stimulated Ca++ influx in islet cells (Henquin 1980; Malaisse et al. 1980). Since the present evidence points to a key regulatory role for calcium in the cascade of events that proceeds to pancreatic somatostatin (Hermansen 1979, 1980a, b; Hermansen et al. 1978, 1979a, b, 1980), glucagon (Hermansen & Iversen 1977; Iversen & Hermansen 1977) and insulin secretion (Grodsky & Bennett 1966; Milner & Hales 1967), it is tempting to assume that the effects of tolbutamide are linked to the changes in Ca++ permeability which the drug produces in islet cells. This is also supported by the present experiments demonstrating that 180 μM tolbutamide was unable to elicit any D or B cell response in the absence of extracellular calcium while a clearcut response appeared at 1.3 mM calcium. The finding that sulphonylureas concomitantly induce an increased content in islet cells of cAMP (Charles et al. 1976; Grill & Cerasi 1978), a secretagogue for the pancreatic D cells (Barden et al. 1976), makes it likely that cAMP is involved in the stimulation of somatostatin release by tolbutamide. However, sulphonylureas do not increase cAMP levels in islet cells in the absence of extracellular Ca++ (Grill & Cerasi 1978), indicating that the role played by cAMP is secondary to that of Ca++. The suggestion that the sulphonylurea-mediated release of somatostatin by direct paracrine interaction has an impact on the A and/or B cells in the vicinity of the D cells (Samols & Harrison 1977; Efendić et al. 1979, 1980) remains to be elucidated. At present no method is, however, available that can substantiate the hypothesis of paracrine communication.

Acknowledgments

Cyclic somatostatin for immunization and standards was kindly donated by Normal Grant, Wyeth Laboratories, Philadelphia, Pennsylvania, and Roger Guillenmin, Salk Institute, La Jolla, California, generously gave us Tyr11 somatostatin for the iodination. Joan Hansen, Karen
Just, and Eva Seier are thanked for expert technical assistance and Anette Larsen for typing the manuscript.
This work was supported by the Danish Medical Research Council, P. Carl Petersen Foundation, and Nordic Insulin Foundation.

References


Received on January 29th, 1981.