SOMATOSTATIN AND INSULIN RELEASE FROM ISOLATED RAT PANCREATIC ISLETS STIMULATED BY GLUCOSE

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1. Introduction

Somatostatin inhibits insulin release in vivo [1–5] and in vitro [6–10] after exogenous administration. This inhibitory action was discovered at a time when only the hypothalamus and some extrahypothalamic areas in the central nervous system were known to contain somatostatin-producing cells [11–12]. The suggestion that somatostatin might be of physiological importance in the regulation of insulin secretion was, therefore, based on the assumption that somatostatin is released into the peripheral circulation and transported to the B-cell membrane at concentrations high enough to block secretion of insulin.

The discovery that the D-cells of the islets of Langerhans react with antisomatostatin serum suggested local effects of the polypeptide via paracrine secretion. Somatostatin may indeed play a physiological role in the release of insulin [13–17]. We now present evidence that somatostatin is released from isolated rat pancreatic islets and that insulin and somatostatin release are interrelated.

2. Materials and methods

Fed, male Wistar rats (200–250 g) were used throughout the study. Bovine serum albumin was purchased from Behringwerke A. G. Marburg, FRG; ^{125}J-porcine insulin (specific activity 150–200 mCi/mg) from Farbwerke Hoechst A. G. Frankfurt, FRG; crystalline rat insulin from NOVO Industri A. S. Copenhagen, Denmark; cyclic somatostatin from Serono, Freibrug, FRG; collagenase from Worthington Biochemical Co., USA.

Islets were isolated from rat pancreas by collagenase 2 h after intraperitoneal administration of 0.6 ml pilocarpin hydrochloride (2% w/v), as previously described [18,19], and incubated in batches of 10 in 500 μl Krebs-Ringer bicarbonate buffer with glucose, mannoheptulose, epinephrine, rotenone and antimycin added as indicated in fig.1 and table 1.

Insulin release into the medium was determined by radioimmunoassay with rat insulin as standard. Insulin release is expressed as ng/10 islets/45 min. Somatostatin was measured by radioimmunoassay using a rabbit antiserum produced by immunisation with cyclic somatostatin coupled to bovine serum albumin and ^{125}J-tyrosilated somatostatin labelled by the chloramin-T procedure [20] and subsequently purified on CM-cellulose. The assay sensitivity is 2 pg/ml and there is no cross-reaction with insulin, proinsulin, glucagon, gastrin or secretin. Further details of the assay procedure will be presented elsewhere [21].

3. Results

The effect of glucose on somatostatin release from isolated rat pancreatic islets is shown in fig.1. In the presence of 5 mM glucose somatostatin release was 25 ± 3 pg/10 islets/45 min and increased to 46 ± 5 pg/10 islets/45 min when the glucose concentration was raised to 25 mM. The corresponding insulin release was 16.8 ± 1 and 73.1 ± 4 ng/10 islets/45 min, respectively (fig.1).

Glucose-induced (25 mM) somatostatin release significantly decreased after addition of mannoheptulose (25 or 50 mM), epinephrine (2 μg/ml) or

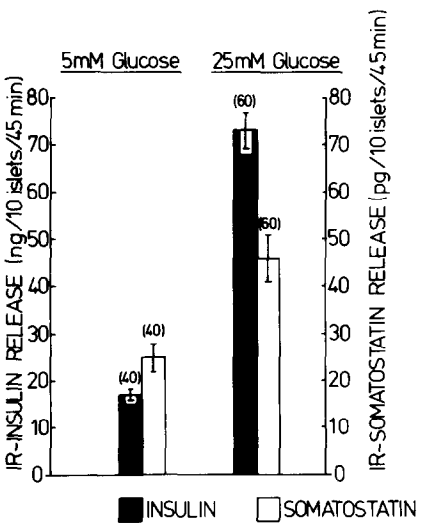

Fig.1. Effect of glucose on the release of somatostatin and insulin from isolated rat pancreatic islets. Incubations were in 500 μl Krebs–Ringer bicarbonate buffer, pH 7.4, 37°C, 72 cycles/min, 1 mg BSA/ml. Mean values ± S.E.M. are shown with the number of individual observations obtained from 10 rats in parentheses. Somatostatin release and insulin release is significantly higher in the presence of 25 mM glucose compared with 5 mM glucose ($P<0.01$). Values for P were calculated by the t-test based on non-paired comparisons.

antimycin (5 μg/ml) plus rotenone (10 μg/ml). The inhibition of insulin release induced by these agents was greater than that of somatostatin (table 1).

4. Discussion

The present data provide evidence that somatostatin is released from isolated islet tissue and that this release can be blocked. Somatostatin release, analogous to the secretion of insulin, is significantly higher in the presence of 25 mM glucose compared with 5 mM glucose (fig.1). This might indicate a direct stimulatory effect of glucose on the secretory mechanism of somatostatin containing D-cells. Alternatively, insulin might have a direct stimulatory effect on the release mechanism of D-cells. This is supported by the observation that somatostatin release is high if insulin release is high (at 25 mM glucose) and diminished if the stimulatory action of 25 mM glucose on insulin release is blocked by mannoheptulose, epinephrine or antimycin plus rotenone (fig.1, table 1).

The observation that in streptozotocin-diabetic rats the somatostatin content in D-cells is increased [24] is compatible with the above concept, i.e., the somatostatin content might be high because stimulation of its release by insulin is diminished.

Table 1

Inhibition of glucose-induced somatostatin and insulin release from isolated rat pancreatic islets by mannoheptulose, epinephrine or antimycin plus rotenone. Incubations were as indicated in the legend to fig.1. Mean values ± S.E.M. are shown with the number of individual observations obtained from 6 rats in parentheses. An asterisk indicates a significant difference from the respective control value ($P<0.05$ or less). Values for P were calculated by the t-test based on non-paired comparisons.

Glucose (mM)	Mannoheptulose (mM)	Epinephrine (μg/ml)	Antimycin + Rotenone (μg/ml)		IR-Insulin (ng/10 islets/45 min)	IR-Somatostatin (pg/10 islets/45 min)
25	--	-	-	--	68 ± 6.0 (26)	56 ± 6.5 (26)
25	25	-	-	--	20 ± 1.6 (19)*	34 ± 4.0 (19)*
25	50	-	-	--	18 ± 1.2 (20)*	31 ± 3.2 (20)*
25	--	-	-	--	62 ± 5.5 (20)	46 ± 5.5 (20)
25	--	2	-	--	14 ± 1.5 (20)*	31 ± 4.5 (20)*
25	--	-	-	--	63 ± 3.6 (40)	48 ± 6.0 (40)
25	--	-	5	10	11 ± 1.1 (40)*	20 ± 3.5 (40)*

The percent inhibition of insulin release is greater than that of somatostatin release, possibly because of a high 'basal release' of somatostatin, i.e., leakage from damaged D-cells. This is understandable because the D-cells are located in the periphery of the rat islets where damage during isolation is greatest.

The physiological significance of endogenous somatostatin release for the secretion of insulin is unclear. Our data indicate a positive correlation between somatostatin levels and insulin levels. This also applies if the kinetics of glucose-induced somatostatin and insulin release are compared.

Because of the wide distribution of somatostatin and its inhibitory effect on multiple secretory functions it has been suggested to be a candidate paracrine transmitter substance, i.e., the somatostatin containing cells are suggested to secrete their product not into the circulation but into the intracellular space, where it acts locally [23]. Somatostatin is degraded significantly during incubation with isolated rat islets and, if at all, is present at extremely low levels in the peripheral circulation (unpublished observations). This is entirely compatible with the above concept and indicates that the values for somatostatin given in this report must not be considered absolute but should be corrected for losses by enzymatic breakdown. Inhibition of the enzymes responsible for degradation during incubation is at present being investigated.

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