STUDIES ON THE INHIBITION OF INSULIN RELEASE, GLYCOGENOLYSIS AND GLUCONEOGENESIS BY SOMATOSTATIN IN THE RAT ISLETS OF LANGERHANS AND ISOLATED HEPATOCYTES*

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SUMMARY

The effect of somatostatin on insulin release, glycogenolysis and gluconeogenesis was studied in isolated islets of Langerhans and hepatocytes. Addition of somatostatin (0.2 μg - 100 μg) to isolated islets of Langerhans inhibited insulin release from 30 to 90 percent. Studies with isolated hepatocytes showed that somatostatin inhibited both glucagon-stimulated glycogenolysis and gluconeogenesis by 40-50 percent, whereas it had no effect on epinephrine-stimulated glycogenolysis.

Somatostatin, the hypothalamic inhibitor, has been shown to inhibit the secretion of a number of hormones such as growth hormone (1), insulin (2, 3), glucagon (4) and TSH (5). However, its in vitro effects on liver metabolism have not been reported. In this communication we present the first of such studies on the inhibition of insulin release in isolated islets of Langerhans and also the inhibition of glucagon-stimulated glycogenolysis and gluconeogenesis in the isolated hepatocytes by somatostatin.

MATERIALS AND METHODS

Male, well-fed, or 18-24 hr fasted Cox rats (200-300 g) were used throughout these studies. Islets of Langerhans were isolated from the pancreas by the procedure of Lacy and Kostianovsky (6). Approximately 10 islets were transferred to a flask containing 2.0 ml of Krebs bicarbonate buffer with 0.20 g percent bovine serum albumin, Fraction V, (Sigma Chemicals, St. Louis, Missouri), glucose 300 mg percent and various concentrations of somatostatin. The flasks were gassed with a 95 percent O_2 : 5 percent CO_2 gas mixture and incubated at 37°C for 90 min. Insulin released into the medium was assayed by radioimmuno-

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TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF SOMATOSTATIN ON INSULIN RELEASE BY ISOLATED ISLETS OF LANGERHANS

Conditions of Incubation	μUnits Insulin Released in the Medium per Islet per 90 min.
Complete System* (CS)	244.9 ± 24
CS + 0.002 μg Somatostatin	219 ± 20
CS + 0.02 μg Somatostatin	226 ± 32
CS + 0.2 μg Somatostatin	162 ± 43
CS + 2.0 μg Somatostatin	92 ± 11
CS + 5.0 μg Somatostatin	114 ± 16
CS + 10.0 μg Somatostatin	43 ± 32
CS + 100 μg Somatostatin	25 ± 2
Basal + 30 mg percent Glucose	16 ± 3
Basal + 30 mg percent Glucose + 2 μg Somatostatin	10 ± 2

*Approximately 10 islets were incubated in 2 ml of Krebs bicarbonate buffer containing 0.20 g percent bovine serum albumin and 300 mg percent glucose and with the various concentrations of somatostatin. Insulin released in the medium was assayed. Values are Mean ± SEM of six observations.

assay using alcohol precipitation of the insulin antibody complex (7). Hepatocytes were isolated by collagenase perfusion technique (8). Approximately 50-70 mg of cells were incubated in 3 ml of Umbreit Ringer 25mM bicarbonate buffer (9) with various concentrations of hormones at 37°C and at 90 oscillations per min as described previously (10). At the end of the incubation, the vial contents were placed in ice cold centrifuge tubes and centrifuged at 2000 rpm in an International Centrifuge for 10 min. The supernatant medium was assayed for glucose by the glucose oxidase method and radioactive glucose was isolated as the phenyl osazone as described previously (11). The synthetic cyclic form (12) of somatostatin (Lot No. AY-24,910) was a generous gift from Dr. R. Deghenghi, Ayerst Labs, Montreal, Quebec. Porcine glucagon was supplied by Eli Lilly and Company, Indianapolis, Indiana and epinephrine was obtained from Sigma Chemicals, St. Louis, Missouri.

TABLE II

EFFECT OF SOMATOSTATIN ON GLUCAGON AND EPINEPHRINE-STIMULATED GLYCOGENOLYSIS IN ISOLATED HEPATOCYTES OBTAINED FROM FED RATS

Conditions of Incubation	Glucose Released in the Medium μmoles per g per hour
Complete System* (CS)	59.4 ± 5.3
CS + Somatostatin (20 μg)	58.5 ± 7.6
CS + Glucagon (10^{-10}M)	122.0 ± 10.0
CS + Glucagon (10^{-10}M) + Somatostatin (0.2 μg)	62.0 ± 8.0
CS + Glucagon (10^{-10}M) + Somatostatin (2.0 μg)	87.5 ± 7.3
CS + Glucagon (10^{-10}M) + Somatostatin (20 μg)	90.8 ± 9.5
CS + Epinephrine (10^{-6}M)	94.0 ± 7.5
CS + Epinephrine (10^{-6}M) + Somatostatin (2.0 μg)	98.0 ± 8.5
CS + Epinephrine (10^{-6}M) + Somatostatin (20 μg)	105.0 ± 10.6

*Complete systems contained approximately 50 to 70 mg of hepatocytes incubated for 1 hour at 37°C in 3 ml of Umbreit Ringer 25mM bicarbonate (9) buffer with no substrate and containing various concentrations of glucagon, epinephrine or somatostatin. Isolated hepatocytes had initial glycogen levels of 180 ± 35 μmoles glucose per g of cells. Values are Mean ± SEM of six observations.

RESULTS AND DISCUSSION

Results on insulin release with concentrations of somatostatin ranging from 0.002 - 100 μg in the presence of 300 mg percent glucose are summarized in Table I. It can be seen from this table that low concentrations of somatostatin (0.002 - 0.02 μg) had no effect on insulin release in the islet cell preparations. However, when the concentration of somatostatin was increased from

TABLE III

EFFECTS OF SOMATOSTATIN ON GLUCAGON-STIMULATED GLUCONEOGENESIS IN ISOLATED HEPATOCYTES OBTAINED FROM FASTED RATS

Conditions of Incubation	μmoles glucose per g cells per hour in 5mM lactate	U-^{14}C-alanine (5mM) Incorporated (c.p.m.) into glucose per g cells per hour
Complete System* (CS)	42.5 ± 7.5	32,800 ± 2,300
CS + Glucagon (10^{-8}M)	75.8 ± 11.0	68,600 ± 4,800
CS + Glucagon (10^{-8}M) + Somatostatin (2.0 μg)	56.3 ± 8.5	53,350 ± 3,800

*Complete system containing approximately 50 to 70 mg of cells were incubated in 3 ml of Umbreit Ringer 25mM bicarbonate buffer (9) containing 5mM lactate and 5mM alanine (0.5 μCi) and with various concentrations of glucagon and somatostatin. Net glucose synthesis was calculated by substracting the glucose production in the absence of substrate from that in the presence of substrate. Approximately 2 to 3 μmoles of glucose was released in the medium due to glycogenolysis in the absence of substrate. Values are Mean ± SEM of 4 observations.

0.2 μg to 100 μg a 30-90 percent inhibition of insulin release was observed. Addition of somatostatin itself had only a slight effect on basal insulin secretion. The results obtained in the present studies with isolated islet preparations are in agreement with those reported previously using perfused pancreas (3, 4).

Studies on the effect of somatostatin on glucagon-stimulated glycogenolysis are summarized in Table II. It can be seen from this table that somatostatin inhibits glucagon-stimulated glycogenolysis by 40 to 50 percent, whereas

it had no effect on epinephrine-stimulated glycogenolysis. Glucagon stimulated glycogenolysis by two-fold and addition of 2.0 μg of somatostatin inhibited this stimulation by 50 percent. An increase in somatostatin concentration by ten-fold showed no further increase in the inhibition. The results on gluconeogenesis in hepatocytes obtained from fasted rats are summarized in Table III. It can be seen from this table, that glucagon stimulated gluconeogenesis from lactate by two-fold. This stimulation was inhibited by 50 percent with the addition of 2.0 μg of somatostatin. Similar results were observed when incorporation of U-^{14}C-alanine into glucose was studied.

In vivo studies in the baboons (4) have shown that infusion of somatostatin will cause a rapid and pronounced hypoglycemia with concomitant inhibition of secretion of the glucose regulatory hormones, glucagon, insulin and growth hormone. It has been suggested by these workers (4) that this induced hypoglycemic state is due to the inhibition of pancreatic glucagon secretion. The studies presented here show that somatostatin will not only inhibit the secretion of hormone from the pancreas but will also interfere with the effect of glucagon on hepatic gluconeogenesis and glycogenolysis.

Curry and Bennett (12) have suggested that somatostatin inhibition of secretory processes may be involved with the binding or inactivation of calcium. In addition, Kaneko *et al.* (14) working with the rat pituitary have shown somatostatin lowers cyclic AMP levels and increases cyclic GMP levels. We have previously shown that glucagon (15, 16) stimulates cyclic AMP levels in isolated hepatocytes. Thus, it would be worthwhile to investigate by which of the above mechanisms somatostatin inhibits glycogenolysis and gluconeogenesis in hepatic tissue.

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