SOMATOSTATIN INHIBITION OF INSULIN RELEASE FROM FRESHLY ISOLATED AND ORGAN CULTURED RAT ISLETS OF LANGERHANS IN VITRO[1]

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Received February 24,1975

SUMMARY. $1x10^{-6}$M somatostatin causes a 37-44% inhibition of glucose induced insulin release from freshly isolated rat islets of Langerhans. A 81 to 95% inhibition is observed when the isolated islets are maintained in organ culture for 2 days prior to the somatostatin treatment. The dose curve of somatostatin on cultured islets shows an apparent K_I of $1.4x10^{-9}$. The tetradecapeptide also causes a reversible inhibition of the stimulation of insulin release by 5 mM theophylline and 23 mM K^+.

It has been demonstrated recently that bolus injection or I.V. infusion of a synthetic growth hormone release inhibiting hormone first isolated from ovine hypothalamus (1), and called somatostatin (1) or GH-RIH (2) also inhibits the basal and/or stimulated release of insulin from baboon (3,4), dog (5) and human (6-8) pancreas in vivo. A similar inhibition was observed using isolated dog (3,6) or rat (9-11) pancreas perfused with the tetradecapeptide under various conditions. The results of these experiments have suggested that the inhibition of insulin release by somatostatin takes place directly at the level of the B cell. However somatostatin has been reported not to affect basal, arginine nor glucose stimulated release of insulin by isolated rat pancreatic islets of Langerhans in vitro (9,11).

The present paper reports the effect of somatostatin on the stimulated release of insulin in rat pancreatic islets freshly isolated and/or maintained in organ culture for 2 days before exposure to the tetradecapeptide.

[1] Supported in part by NIH grants AM 01226 and AM 06181.
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MATERIALS AND METHODS

Isolation of islets of Langerhans: Islets of Langerhans were isolated aseptically from 250-300 grams adult male Wistar rats by the collagenase technique of Lacy and Kostianovsky (12).

Organ culture: The isolated islets were cultured for 2 days at 37°C in an atmosphere of 95% air and 5% CO_2 in Falcon organ culture dishes (No. 3010) containing the medium used previously by Kostianovsky et al (13) with 10% fetal bovine serum.

Incubation procedure: Either immediately after the isolation or after 2 days of organ culture the islets were transferred by groups of 7-10 to siliconized glass tubes and preincubated at 37°C for 30 minutes in 0.5 ml Krebs-Ringer bicarbonate buffer (14) containing 0.5 mg of glucose and 0.1 mg of bovine serum albumin per ml in a Dubnoff metabolic shaker at 100 rpm in a water-saturated atmosphere of 95% O_2 and 5% CO_2. The final incubation was carried out in the absence or in the presence of linear somatostatin (Pierce Chemical, Illinois) as indicated in the legends to the figure and tables.

Insulin assay: The insulin released in the incubation medium was measured by the ethanol back-titration radio-immunoassay procedure of Wright et al (15). Crystalline porcine insulin (E. Lilly) was used as standard, and (^{125}I) porcine insulin (Cambridge Nuclear Radio Pharmaceutical Co.) was used as the isotope tracer.

RESULTS

Table I shows the effect of somatostatin on glucose stimulation of insulin release by freshly isolated islets as compared to the insulin released under similar conditions by islets previously cultured for 2 days. Increasing the glucose concentration from 0.5 mg/ml to 3.0 mg/ml in the medium causes a 3-5 fold

Table I Effect of somatostatin on the glucose stimulation of insulin release.

Isolated islets	Insulin released (uU/islet/30 min.)			Insulin released over basal (uU/islet/30 min.)		% of inhibition
	Glucose (0.5 mg/ml)	Glucose (3 mg/ml)	Glucose (3 mg/ml) Somatostatin ($1x10^{-6}$M)	Glucose (3 mg/ml)	Glucose (3 mg/ml) Somatostatin ($1x10^{-6}$M)	
A						
Fresh	7.4 ± 0.7	35.8 ± 2.4	25.4 ± 1.8	28.4	18.0	36.7
Cultured	15.3 ± 2.8	47.5 ± 4.0	21.4 ± 2.9	32.2	6.1	81.1
B						
Fresh	13.9 ± 1.2	59.7 ± 5.7	33.9 ± 2.5	35.8	20.0	44.1
Cultured	17.9 ± 1.5	53.0 ± 1.1	19.4 ± 0.4	35.1	1.5	95.7

After preincubation, 3 groups of 7-10 islets per experimental condition were incubated in Krebs-Ringer bicarbonate buffer containing 0.5 mg/ml or 3.0 mg/ml of glucose with or without $1x10^{-6}$M somatostatin. The insulin released was measured after 30 minutes of incubation. Part A represents the combined results from 3 different preparations of freshly isolated islets and from 5 different preparations of islets cultured for 2 days. Part B shows the results of a typical experiment using the same preparation of islets, one half being used immediately after isolation and the other half after 2 days of culture. Results are expressed as the mean ± SEM.

increase in the insulin release after 30 minutes of incubation both from fresh and cultured islets. Under those conditions, $1x10^{-6}$M somatostatin inhibits the glucose stimulation of insulin released over basal by 36.7% in 3 preparations of freshly isolated islets (Table I A). When the islets are first cultured for 2 days the inhibition of the glucose stimulation of insulin release by somatostatin is found to be 81.1% in 5 combined experiments. The inhibition by somatostatin on the insulin released by the islets from a single preparation tested immediately after isolation and after 2 days of culture is 44 and 95% respectively (Table I B).

The effect of increasing concentrations of somatostatin on the glucose stimulation of insulin released by islets previously cultured for 2 days is shown in Figure 1 A. After 30 mi-

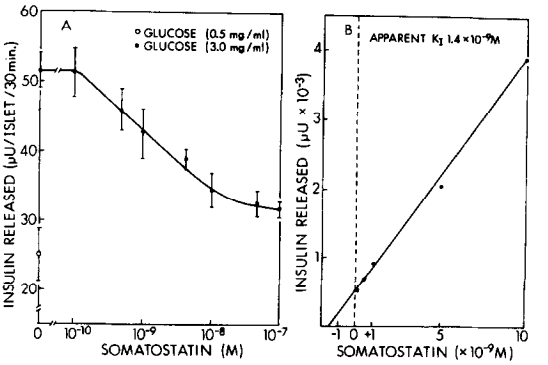

Figure 1 Effect of increasing concentrations of somatostatin on the glucose stimulation of insulin release.

After preincubation, groups of 7-8 islets were incubated in Krebs-Ringer bicarbonate buffer containing 0.5 mg/ml or 3.0 mg/ml of glucose in the absence or in the presence of increasing concentrations of somatostatin. In part A the insulin released after 30 min. is expressed as the mean ± SEM for 4-5 groups of islets per experimental conditions. In part B is shown the Dixon plot of those data.

nutes of incubation an inhibition is already apparent at $5x10^{-10}$M. Maximal inhibition is observed at $5x10^{-8}$M. The apparent K_I as measured on the Dixon plot of those data is $1.4x10^{-9}$M (Fig. 1 B).

The effect of somatostatin on the insulin released by isolated and cultured islets in response to other stimulatory agents has also been studied. When islets are first incubated for a period of 35 minutes in the presence of 5 mM theophylline or 23 mM K^+ the amounts of insulin released over control are respectively 60.0 and 59.1 uU/islet/35 min. (Table II A). Those values represent a 6-7 fold increase over basal insulin release which is 9.7 $\pm$ 2.0 uU/islet/35 min. in this experiment. Upon the addition of $1x10^{-6}$M somatostatin, the stimulation over basal levels is decreased to 22.5 and 26.5 uU/islet/35 min. representing 37.5 and 44.8% of the theophylline and high K^+ controls respectively.

The reversibility of the somatostatin effect has been studied by incubating the same islets in a new medium under identical conditions but in the absence of the tetradecapeptide. The basal insulin release for this second incubation period is 11.7 $\pm$ 1.4 uU/islet/35 min. A similar 6-7 fold stimulation of insulin release over basal is also observed both for the theophylline and high K^+ control groups. In the groups previously incubated in the presence of somatostatin the amount of insulin released over basal is increased to 54.7 and 41.8 uU/islet/35 min. now representing 88.0 and 73.7% of the theophylline and high K^+ controls respectively.

DISCUSSION

The results presented in this paper demonstrate that somatostatin inhibits glucose stimulation of insulin released by rat isolated islets of Langerhans *in vitro*. When the islets were incubated immediately after isolation, the somatostatin inhibi-

tion was consistently lower than 50%, although the usual 3-5 fold increase in the insulin released in the presence of high glucose was observed in the same islets preparations (Table I A). However after 2 days of organ culture, a much greater and almost complete inhibition was observed either on different preparations of islets (Table I A) or on the same preparation of islets tested freshly and after culture (Table I B).

Others have reported recently that somatostatin has no effect on basal, high glucose and arginine stimulation of insulin release by collagenase-isolated rat pancreatic islets (9,11). The different degrees of inhibition by somatostatin between fresh and cultured islets can possibly be explained by a susceptibility of the somatostatin response to the collagenase digestion. It has been found that recently dissociated pituitary cells respond poorly to a number of stimuli and that a period of culture as long as 72 hours was necessary to obtain a response to various secretagogues (16,17). The partial response to somatostatin immediately after enzyme treatment and the maximal response after 2 days of culture would be explained on the basis of damage to cell surface followed by the regeneration of the required surface components during the succeeding 48 hours culture period.

Cultured islets indeed are very sensitive to low concentrations of somatostatin. The apparent K_I was found to be 1.4×10^{-9}M (Fig. 1 B). These results are in agreement with those of Efendic et al (9) who have shown that comparable low amounts of somatostatin inhibits both phases of insulin release in rat perfused pancreas.

Somatostatin also inhibits the stimulation of insulin released by addition of theophylline or high K^+ concentration in the medium (Table II A). This effect of somatostatin is rapidly

Table II Effect of somatostatin on the stimulation of insulin release by theophylline and high K^+.

	Insulin released over basal (uU/islet/35 min.)			
	5 mM Theophylline	% of control	23 mM K^+	% of control
A. First incubation period				
Control	60.0	100	59.1	100
Somatostatin added	22.5	37.5	26.5	44.8
B. Second incubation period				
Control	62.1	100	56.7	100
Somatostatin removed	54.7	88.0	41.8	73.7

After preincubation, 5 groups of 7-8 islets per experimental condition were incubated in Krebs-Ringer bicarbonate buffer containing 1.0 mg/ml of glucose with or without 5 mM theophylline or 23 mM K^+ and in the absence or in the presence of 1×10^{-6} M somatostatin. After 35 min. media were removed and the islets were incubated for another 35 min. in a new and identical medium without somatostatin. Results are expressed as the insulin released over basal.

reversible. The incomplete recovery of the response of the islets is possibly due to a minimal residual amount of somatostatin in the second incubation medium. Somatostatin has also been reported recently to inhibit the theophylline stimulation of growth hormone released by rat hemipituitaries *in vitro* (18-20). Borgeat *et al* (19) have also shown that somatostatin prevents the basal and prostaglandin induced cyclic AMP accumulation in hemipituitaries. The somatostatin inhibition of the stimulation of insulin release by the phosphodiesterase inhibitor would indicate an action of this small peptide at a step following cyclic AMP formation as also suggested by Borgeat *et al* (19). The effect of somatostatin on cyclic AMP accumulation in islets has not been reported yet.

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