INHIBITION BY SOMATOSTATIN OF GLUCOSE INDUCED 3′:5′-MONOPHOSPHATE (CYCLIC AMP) ACCUMULATION AND INSULIN RELEASE IN ISOLATED PANCREATIC ISLETS OF THE RAT

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1. Introduction

It has been demonstrated that the hypothalamic growth hormone (GH) release inhibiting factor, somatostatin, also suppresses glucose induced insulin release in man [1–3] and in the isolated perfused pancreas from the dog [1] and rat [4,5]. In the latter preparation the lowest effective dose of somatostatin was 1 ng/ml of perfusate. By increasing the dose to 100 ng/ml, an almost complete inhibition of basal as well as stimulated insulin release was observed. On the other hand, somatostatin in concentrations up to 200 ng/ml did not influence glucose stimulated insulin release from isolated rat islets [4].

The present study demonstrates that somatostatin in very high concentrations suppressed the effect of glucose on insulin release. Furthermore, the accumulation of cyclic AMP, shown in previous studies to occur in response to the hexose [6], was concomitantly diminished.

2. Materials and methods

Pancreatic islets were isolated from fed male Sprague-Dawley rats (150–200g) by a modified collagenase technique [6]. After isolation under a stereomicroscope the islets were preincubated in Krebs-Henseleit bicarbonate buffer (KHB) containing 2 mg/ml of bovine albumin, 0.6 mg/ml of glucose, and 100 μCi of [^{3}H]2-adenine for 60 min at 37°C under continuous gasing with CO_2:O_2 (5:95%). After the preincubation period the islets were washed four times. Groups of 15 islets each were selected under the microscope and transferred to incubation tubes containing KHB. The incubations were started by the addition of the test agents. The final volume of incubations was 1.0 ml. Incubations were carried out in duplicates or triplicates. The phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), was included in a concentration of 0.1 mM in all incubations. At the end of the incubation period a portion of the media was removed, after which the incubations were terminated by immersing the tubes in boiling water for 3 min. [^{3}H]cyclic AMP was purified by ion-exchange chromatography and barium sulfate precipitation [7]. Insulin was measured radioimmunologically using a double antibody system [8].

Crude collagenase was purchased from Worthington Biochemical Corporation (Freehold, N. J., USA), bovine albumin (Fraction V) from Armour Co. (Eastbourne, UK), IBMX from Aldrich Co. (Melwauker, Wis., USA). Cyclized somatostatin and rat insulin were generous gifts of Dr R. Guillemin, The Salk Institute (La Jolla, Calif., USA) and Dr J. Schlichtkrull, Novo Research Institute (Copenhagen, Denmark). [^{3}H]2-adenine with the specific acitivty of 31.7 Ci/mmol was obtained from New England Nuclear (Dreieckenhein, West Germany).

Table 1
Effect of somatostatin on glucose induced insulin release from isolated rat islets after 60 min of incubation (μU insulin/islet; mean ± S.E.M. of 7 complete experiments)

Glucose (mg/ml)	Somatostatin (ng/ml)		
	0	500	1 250
0.6	2.1 ± 0.4	3.8 ± 0.7	–
1.5	20.7 ± 7.3	11.2 ± 4.7*	–
5.0	111.4 ± 15.8	108.0 ± 13.0	91.0 ± 9.0

* $p < 0.02$ (Students t-test for paired differences)

3. Results

As seen in table 1, somatostatin (500 ng/ml) significantly inhibited only insulin release induced by the intermediate concentration of glucose used, 1.5 mg/ml, and not that induced by 5.0 mg/ml. The decrease in insulin release was about 45% ($p<0.02$). Insulin release to the highest concentration of glucose was not influenced even by as high a concentration of somatostatin as 1250 ng/ml.

The corresponding results concerning [^{3}H]cyclic AMP accumulation in the incubation media and in the islets are presented in table 2. The medium content of the cyclic nucleotide was increased 2.5- and 6-fold by glucose in the concentrations 1.5 and 5.0 ng/ml, respectively. A lesser effect was obtained with these amounts of glucose on the cyclic AMP content of the islets, the stimulation now amounting to 1.6- and 2.5-fold. The addition of somatostatin (500 ng/ml) – as in the case of insulin release – was accompanied by a significant inhibition of cyclic AMP accumulation in the medium only in the presence of 1.5 mg/ml of glucose. This inhibition amounted to 40% ($p<0.02$). No significant effect was noted on the tissue level of the nucleotide. Again, 1250 ng/ml of somatostatin had no effect on cyclic AMP in the medium or islets in the presence of 5.0 mg/ml of glucose.

4. Discussion

There is ample evidence that somatostatin inhibits insulin release induced by glucose [1–5], glucagon [9], isoproterenol [10], theophylline [10] and tolbutamide [9]. All these insulinogogues have in common stimulation of production of cyclic AMP in the islets. Therefore, it could be suggested that somatostatin exerts its effect on insulin secretion by way of a decrease in the intracellular level of the nucleotide. This idea is strongly supported by the present finding that somatostatin parallelly inhibited glucose stimulated insulin release and the accumulation of [^{3}H]cyclic AMP in the incubation medium of rat islets.

No significant effect was obtained with somatostatin on the content of [^{3}H]cyclic AMP in the islets. This difference in the level of cyclic AMP in the medium and the tissue under the influence of somatostatin most likely has to do with the time of incubation. During prolonged incubation – as in the present experiments – changes in cyclic AMP in the medium reflect the activity of the adenyl cyclase–cyclic AMP system during the entire incubation period. In contrast.

Table 2
Effect of somatostatin on glucose induced accumulation of [^{3}H]cyclic AMP in isolated rat islets and their incubation media after 60 min of incubation (dpm/islet; mean ± S.E.M. of 7 complete experiments)

Glucose mg/ml	Islet			Medium		
	Somatostatin ng/ml			Somatostatin ng/ml		
	0	500	1 250	0	500	1 250
0.6	13.4 ± 2.1	12.6 ± 2.7	–	9.9 ± 4.4	6.9 ± 1.8	–
1.5	22.7 ± 4.4	16.5 ± 2.0	–	25.5 ± 6.9	15.2 ± 4.6*	–
5.0	32.9 ± 6.9	21.7 ± 3.0	20.7 ± 2.6	56.9 ± 10.1	43.4 ± 6.9	40.4 ± 2.8

* $p < 0.02$ (Students t-test for paired differences).

the accumulation of cyclic AMP in the islets only reflects short time events, since the turnover of the nucleotide in the islets is very rapid [6].

In the present study, the accumulation of [^{3}H]-cyclic AMP was measured after a prelabelling procedure. However, it has been demonstrated previously that this measurement corresponds to the total amount of the nucleotide when this is determined by the protein kinase binding method [6].

In a previous paper we were unable to inhibit glucose stimulated insulin release from rat islets with 200 ng/ml of somatostatin [4]. In the present experiments, the administration of 500 ng/ml was accompanied by a definite inhibition of insulin release. In both sets of experiment the glucose dose was 1.5 mg/ml. Somatostatin in the dosages 500 and 1250 ng/ml exerted no significant effect on either insulin release or cyclic AMP accumulation in the presence of as high a glucose concentration as 5.0 mg/ml. This favors the idea, suggested earlier [5], that somatostatin inhibits glucose induced insulin release in a competitive way.

We have recently demonstrated, by use of immunohistochemical technique, that considerable amounts of somatostatin or a somatostatin-like peptide are accumulated in the islets of Langerhans of rats and guinea-pigs [11]. Against this background, the present finding that somatostatin inhibits glucose induced accumulation of cyclic AMP indicates a physiological role for somatostatin in the regulation of cyclic AMP in the endocrine pancreas. In this context it is of interest that somatostatin also inhibits cyclic AMP accumulation in isolated cells of the hypophysis [12, 13].

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