EFFECT OF SOMATOSTATIN ON GLUCOSE INDUCED INSULIN RELEASE IN ISOLATED PERFUSED RAT PANCREAS AND ISOLATED RAT PANCREATIC ISLETS

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1. Introduction

Recently, a growth hormone (GH) release inhibiting factor, somatostatin, was isolated from sheep hypothalamus [1]. The same authors demonstrated that, in man, somatostatin, besides inhibiting GH release, lowered basal plasma insulin levels [2]. Furthermore, somatostatin suppressed glucose-induced insulin release in man [3] and in the isolated dog pancreas [3].

The present report deals with the effect of somatostatin on glucose induced insulin release from the isolated perfused rat pancreas and isolated islets from rat pancreas.

2. Materials and methods

For the preparation of the perfused isolated pancreas Sprague–Dawley rats weighing 200–250 g, and fasted for 24 hr were used. They were anesthetized by intraperitoneal injection of 50 mg of Pentobarbital, and the pancreas isolated by a slight modification of the technique of Loubatières [4]. The gland was perfused with a Krebs–Ringer bicarbonate solution [5], to which was added 1.5 g/l of glucose and 20 g/l of beef albumin. The final solution was adjusted to pH 7.4 with 0.1 N HCl, and was continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. The perfusate was administered into the coeliac artery and run into the prepared pancreas by an open circuit 'non-recycling perfusion system'. Flow rates were kept around 2.5 ml/min by making minor changes in arterial pressure.

Preparation and incubation of pancreatic islets were performed according to a recently reported technique [6].

Crude collagenase was purchased from Worthington Co., bovine albumin (fraction V) from Armour Co., and insulin reagent kits from the Radiochemical Centre, Amersham.

Somatostatin (linear and cyclized) and rat insulin were generous gifts of Dr. R. Guillemin, Salk Institute, La Jolla, California and Dr. J. Schlichtkrull, NOVO Research Institute, Copenhagen, respectively.

The effect of somatostatin on insulin release was calculated by comparison of the areas under the insulin curves. The initial phase of insulin release was taken from 0–7 min after the start of the infusion, while the second phase comprised the period between 7–17 min.

3. Results

Increase in glucose concentration in the perfusate from 1.5–3.0 mg/ml was accompanied by considerable insulin release from the pancreas (fig. 1). A typical biphasic insulin curve was obtained. The insulin level increased sharply, a peak value of 1300 μU/min being reached one min after the increase in glucose concentration in the perfusate. Insulin fell to a nadir at about 6 min, and then rose again during the remaining part of the infusion.

Linear somatostatin in a concentration as low as one ng/ml, when infused for 10 min prior to and then during the infusion with 3.0 mg/ml of glucose, indu-

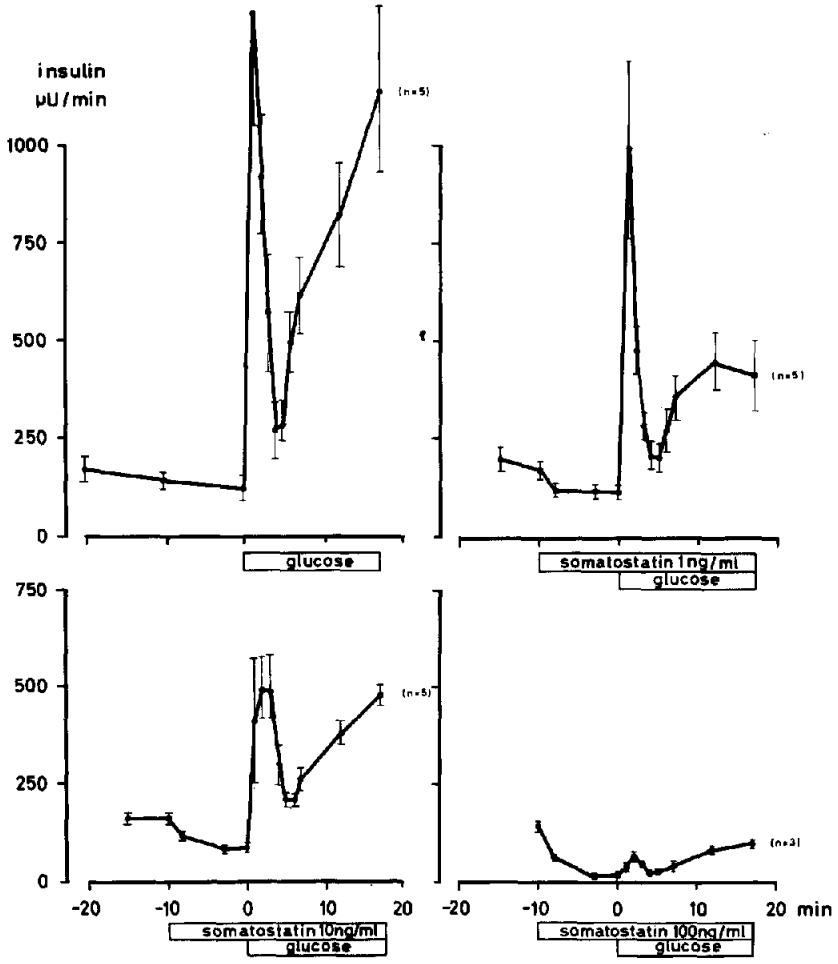

Fig. 1. Effect of linear somatostatin (1-10-100-ng/ml of perfusate) on glucose (3.0 mg/ml) induced insulin release from the isolated perfused rat pancreas. Following isolation the pancreases were equilibrated for 30 min with 1.5 mg/ml of glucose in the perfusate. The glucose stimulus was applied between 0 and 17 min, somatostatin 10 min prior and during the glucose stimulation. Results are expressed as the mean ± S. E. M. of five experiments.

Table 1
Effect of somatostatin on glucose induced insulin release from isolated perfused rat pancreas*

Additions to perfusate	Insulin release in µU			
	0–7 min	p**	7–17	p**
Glucose (3.0 mg/ml)	3233 ± 534		7261 ± 1234	
Glucose (3.0 mg/ml) + somatostatin (1 ng/ml)	1866 ± 262	< 0.05	3020 ± 271	< 0.01
Glucose (3.0 mg/ml) + somatostatin (10 ng/ml)	1785 ± 206	< 0.025	2867 ± 306	< 0.01

* Mean ± S.E.M. of five experiments

** p values refer to the significance of difference of experiments with and without somatostatin

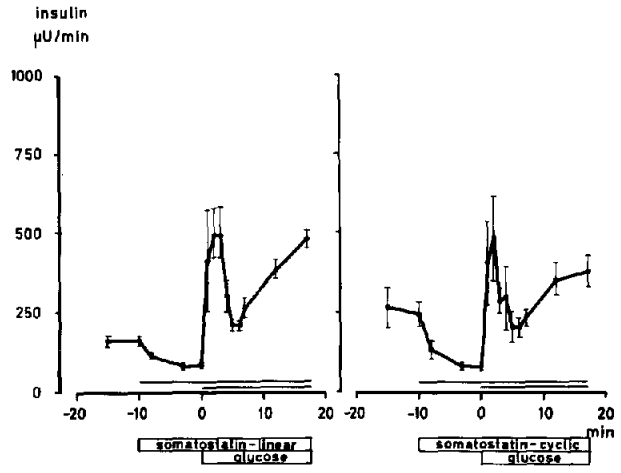

Fig. 2. Comparison of the effects of linear and cyclized somatostatin (10 ng/ml) on glucose induced insulin release from isolated perfused rat pancreas. For legend, see fig. 1.

ced a significant suppression of glucose induced insulin release (fig. 1, table 1). This effect was most prominent on the second peak of the insulin curve. With 10 ng/ml of somatostatin even the initial peak was clearly inhibited, and with 100 ng an almost complete inhibition of glucose induced insulin release was obtained.

As seen in fig. 2, linear and cyclized somatostatins in a dose of 10 ng/ml of perfusate were equally effective in suppressing glucose induced insulin release.

When tested on isolated islets from rat pancreas, linear somatostatin in concentrations up to 200 ng/ml of incubation medium, had no effect on the release of insulin induced by 1.5 mg/ml of glucose (table 2).

Table 2
Effect of somatostatin (200 ng/ml) on glucose induced insulin release in isolated rat islets*

Additions to medium	Insulin, μU/islet
Glucose (0.6 mg/ml)	2.80 ± 0.22
Glucose (1.5 mg/ml)	28.1 ± 3.6
Glucose (1.5 mg/ml) + somatostatin (200 ng/ml)	30.4 ± 6.1

* Mean ± S.E.M. of duplicates from five experiments. Incubations were carried out for 60 min.

4. Discussion

Some recent studies have suggested that the central nervous system plays a role in the control of insulin release from the pancreas. Thus, it has been demonstrated that perfusion of the hypothalamus in baboons with the alfa-adrenergic blocking agent, phentolamine, induced an enhancement of basal insulin secretion when administered in a concentration which had no effect when given to the systematic circulation [7]. Furthermore, lesions applied to the ventromedial nuclei of the hypothalamus were accompanied by hyperinsulinemia [8]. It is still an open question whether these effects on insulin secretion were mediated by nervous routes or by humoral factors.

In the present study it has been clearly demonstrated that a newly discovered hypothalamic hormone, somatostatin, did indeed inhibit insulin secretion from the isolated perfused rat pancreas in a concentration as low as one ng/ml of perfusate. This should be compared with the dose required for inhibition of GH secretion from rat pituitary cells in monolayer culture, 1.8 ng/ml [1]. The fact that such a low dose of somatostatin was that effective in our perfusion system might suggest that this hormone might be of physiological importance in the regulation of insulin secretion.

The effect of somatostatin can hardly be attributed to impurities since synthesized linear as well as cyclized somatostatin were equally effective as inhibitors of insulin secretion.

A surprising finding was that somatostatin did not inhibit the release of insulin from isolated rat pancreatic islets. One explanation might be that the preparation of the islets with collagenase damages some receptor sites for somatostatin on the beta-cell membranes. This possibility is being evaluated at present.

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