CYCLIC AMP-DEPENDENT STIMULATION OF SOMATOSTATIN SECRETION BY ISOLATED RAT ISLETS OF LANGERHANS

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SUMMARY. Immunoreactive somatostatin is released from islets of Langerhans, isolated from rat pancreas by collagenase digestion, when incubated in an in vitro system. The rate of somatostatin secretion is independent of extracellular glucose concentration, but is stimulated by addition of 8-Br-cyclic AMP or theophylline.

Somatostatin has been shown to inhibit the secretion not only of growth hormone (1), but also the secretion of several other hormones including insulin (2,3) and glucagon (4). The more recent discovery of the presence of somatostatin in islets of Langerhans (5, 6) suggests that this hormone may well have a physiological role in the regulation of pancreatic islet function. However, nothing is known of the mechanisms that regulate the secretion of pancreatic somatostatin. We have developed a radioimmunoassay specific for somatostatin and used this technique to measure the somatostatin content of isolated rat islets of Langerhans and to study the secretion of pancreatic somatostatin. We report here, for the first time, stimulation of somatostatin release from isolated islets of Langerhans by cyclic AMP-dependent mechanisms.

MATERIALS AND METHODS

Preparation and incubation of islets of Langerhans

Islets of Langerhans were isolated from male Sprague-Dawley rat pancreas by the collagenase digestion technique of Lacy and Kostianovsky (7). Islets were separated from acinar debris by centrifugation in discontinuous density gradients of dialyzed Ficoll (8). Groups of 50 islets, randomly selected, were incubated in 0.5 ml of Krebs-Ringer bicarbonate medium containing 0.1% gelatin and 1000 Kallikrein Inactivating Units/ml of Trasylol, under an atmosphere of O_2: CO_2 (95.5). Following incubation, medium was removed, rapidly frozen and stored at -20° until assayed for somatostatin content. Islets were homogenized and samples of the homogenate taken for determination of somatostatin content. Results are expressed as pg of somatostatin released into the incubation medium. Statistical analysis of results was obtained using a Students "T" test.

Radioimmunoassay of somatostatin

Somatostatin was assayed by a modified technique of Arimura et al (9) using rabbit anti-thyroglobulin-somatostatin and ^{125}I-labelled Tyr-1-somatostatin or ^{125}I-labelled N-Tyr-somatostatin. The diluent for all reagents was 0.01M phosphate buffer pH 7.4, containing 0.14 M NaCl, 0.025 M ethylene-dinitrilotetracetic acid and 0.1%gelatin. Following incubation at 4° for 16 h, free and antibody bound hormone were separated by centrifugation after addition of dextran-coated charcoal.

RESULTS

Incubation of isolated rat islets of Langerhans in Krebs-Ringer bicarbonate buffer for a period of 30 min results in the release into the medium of immunoreactive somatostatin. The release of somatostatin is independent of the extracellular glucose concentration. A concentration of glucose (300 mg/100 ml) which stimulates the secretion of insulin from islets of Langerhans has no effect on the release of somatostatin (table 1), when compared to a non-stimulating concentration of 100 mg/100 ml of glucose. However, addition of 8-Br-cyclic AMP (Table 1) caused a 2-3 fold increase in somatostatin release. The 8-Br-cyclic

TABLE 1. Effect of 8-Br-cyclic AMP and theophylline on somatostatin release

Assay Conditions	Glucose Concentration [a]	Somatostatin release/islet/30 min Percentage of control [b]	P
No addition	100 mg/100 ml	100	
No addition	300 mg/100 ml	100 ± 11.3	N.S.
8-Br-cyclic AMP	100 mg/100 ml	279 ± 21.7	< 0.02
8-Br-cyclic AMP	300 mg/100 ml	260 ± 41.2	< 0.05
Theophylline	100 mg/100 ml	285 ± 3.1	< 0.01

a) Groups of 50 islets were preincubated in Krebs-Ringer bicarbonate medium containing 0.1% gelatin, 1000 U/ml Trasylol and 100 mg/100 ml glucose for 15 minutes. The medium was replaced with fresh medium containing, in addition, 8-Br-cyclic AMP (2 mM), theophylline (10 mM) and glucose (300 mg/100 ml) as indicated. After incubation for 30 min, triplicate samples of the incubation medium were assayed for somatostatin content.

b) Results are the mean ± S.E.M. of two separate experiments. 9.7 ± 2.3 pg of somatostatin/islet/30 min was released in control incubations.

AMP-dependent release of somatostatin was not changed by variations in the extracellular glucose concentration. The phosphodiesterase inhibitor, theophylline, when added to pancreatic islet incubations also caused a marked stimulation of somatostatin release (Table 1). Over a period of 90 min, there was a small but continuous release of somatostatin into the medium of control incubations. Addition of 8-Br-cyclic AMP caused a rapid stimulation of somatostatin release and this was sustained over the full 90 min period of incubation (Fig. 1).

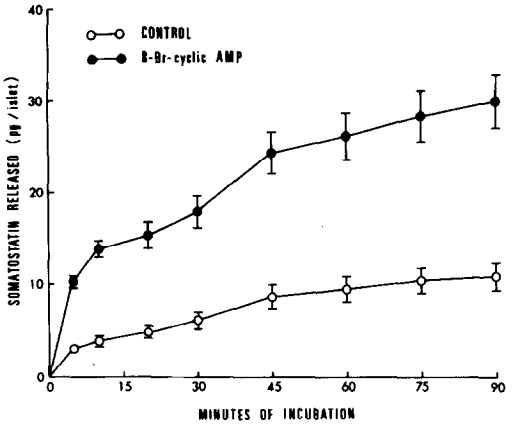

Figure 1. Time course of 8-Br-cyclic AMP-dependent somatostatin release. Following preincubation of groups of 100 islets for 15 min, incubation was continued in the presence or absence of 2 mM-8-Br-cyclic AMP. The incubation medium was removed for somatostatin assay and replaced with fresh medium at all time intervals shown. Results show the cumulative release of somatostatin per islet.

DISCUSSION

The recent discovery of pancreatic somatostatin (5, 6) suggests that this inhibitor of hormone secretion may have a physiological role in the regulation of insulin and glucagon secretion by islets of Langerhans. One approach to resolution of this question is a study of somatostatin release from islets of Langerhans and the regulation of this process. Glucose, the major regulator of insulin release has no effect on the liberation of somatostatin from pancreatic islets incubated *in vitro*. This suggests, also, that somatostatin release is unlikely to be influenced by the concentrations of insulin and, possibly, glucagon. Glucose has been shown to increase the cyclic AMP concent of islet cells (10, 11). Addition of exogenous dibutyryl cyclic AMP to pancreatic islets failed to increased insulin release,

except in the presence of stimulatory concentrations of glucose (12). 8-Br-cyclic AMP does however, stimulate the release of somatostatin from islets of Langerhans. It is thus possible that somatostatin liberated by 8-Br-cyclic AMP may inhibit any direct cyclic AMP-dependent stimulation of insulin release.

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