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The Effect of Somatostatin on Glucose Stimulated Adenosine 3′–5′, Monophosphate Accumulation and Glucose Oxidation by Isolated Rat Islets of Langerhans[1, 3] (40225)

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Somatostatin or somatotropin-release inhibitor factor is a tetradecapeptide found originally in the hypothalamus (1), but now shown to be present in other tissues such as pancreas (2) and gut (3). When administered in pharmacological amounts to man or experimental animals, somatostatin will inhibit the secretion of hormones from the anterior pituitary (1, 4–6), pancreas (7, 8) and gut (9). Although the mechanism(s) by which somatostatin exerts these inhibitory effects is not understood, studies *in vitro* have shown that somatostatin inhibits prostaglandin-stimulated accumulation of cyclic AMP by rat anterior pituitary glands (10) and calcium ion flux by isolated rat islets of Langerhans (11, 12). Further studies using isolated islets and analogues of cyclic AMP, phosphodiesterase inhibitors and labelled ATP have provided indirect evidence that somatostatin interferes with the adenylyl cyclase–cyclic AMP system (13). Because the secretory functions of the islet not only depend upon the cyclic AMP system, but also have metabolic fuel requirements, the present study was undertaken to determine the effect of somatostatin on cyclic AMP and glucose utilisation in isolated rat islets of Langerhans.

Materials and methods. Islets were isolated from fed male Sprague–Dawley rats weighing 250–300 g, using a collagenase digestion procedure previously described (14). Batches of islets (20–50) were incubated in siliconized counting vials containing Krebs bicarbonate buffered medium (pH 7.4), supplemented with 0.2 g% bovine serum albumin (Sigma Chemicals, St. Louis, MO) and glucose 1.67 m*M*. Incubations were carried out at 37° in a metabolic shaker set at 90 oscillations per minute for 20 min in cyclic AMP studies and 120 min in glucose oxidation studies. The islets were stimulated by addition of glucose (16.7 m*M*) in the presence or absence of cyclic somatostatin (1 μg or 10 μg/ml; Ayerst Laboratories, Montreal, Quebec, Canada), mannoheptulose (20 m*M*), epinephrine (0.1 mM) and theophylline (2 m*M*; Sigma Chemicals, St. Louis, MO).

In each experiment, islets from the same preparation were simultaneously incubated in the absence and presence of somatostatin, mannoheptulose or epinephrine.

Glucose oxidation studies. Either Cl or C6 D-[^{14}C]glucose (5 μCi; Radiochemical Centre, Amersham, Bucks, U.K.) was added to 2.0 ml of medium bathing the islets. At the end of the incubation period an aliquot (0.10 ml) of the medium was withdrawn into a Hamilton syringe via a needle penetrating the rubber septum capping the vial. $^{14}CO_2$ was released from the medium by addition of 0.10 ml perchloric acid and trapped by Hyamine solution (0.10 ml; Packard Industries Freehold, New Jersey), contained in a small plastic well (Kontes, Vineland, New Jersey), suspended above the medium. Entrapment of $^{14}CO_2$ was carried out for one hour at 37° at the end of which all the Hyamine solution was removed and placed into scintillation fluid and counted in a Packard Liquid Scintillation Spectrometer. Glucose oxidation rates were calculated by the method of Ashcroft and Randle (15).

Cyclic AMP Studies. Islets were incubated in a small siliconized glass tube (10 × 40 mm) containing 0.5 ml of medium supplemented with the phosphodiesterase inhibitor, theophylline, in a final concentration of 2 m*M*. After a 20-min period of stimulation with glucose (16.7 m*M*), a 50 μl aliquot was taken for insulin determination. The islets were then disrupted by 10 sec of sonication, using

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a Sonifier Cell Disruptor (Ultrasonics Inc., Plainview, NY), and the lowest setting. All tubes were stoppered and heated to 92°–96° for 5-min before being assayed for cyclic AMP by a protein binding assay procedure described by Moxley and Allen (16). Standard curves were run in media treated in the same manner as the sample extracts.

Insulin secretion. The insulin content of the media was determined using the method of Makulu *et al.* (17).

Evaluation of results. The statistical significance of the results was determined using Student's *t* test.

Results. Glucose oxidation studies. As shown in Fig. 1, increasing the glucose concentration in incubation medium from 1.67 m*M* to 16.7 m*M* caused a six- to sevenfold increase in oxidation of both D-(1-[^{14}C]) and D-6-[^{14}C] glucose. Addition of somatostatin at a final concentration of 10 μg or 1 μg/ml had no significant effect on the oxidation rates of either type of glucose, while mannoheptulose (20 m*M*) significantly inhibited oxidation of both. Epinephrine had a variable effect causing a slight but significant increase in D-(1-[^{14}C]) and no change in D-(6-[^{14}C]) glucose oxidation. Increasing glucose concentration from 1.67 m*M* to 16.7 m*M* caused a significant increase in insulin release, which was inhibited significantly by somatostatin, mannoheptulose and epinephrine (*P* values are shown in Fig. 1).

Cyclic AMP Studies. As shown by Table I, increasing the glucose concentration from 1.67 m*M* to 16.7 m*M* caused a significant increase in cAMP accumulation and insulin secretion. The addition of either somatostatin at a final concentration of 1 μg or 10 μg/ml, mannoheptulose or epinephrine to islets stimulated by glucose, caused significant reductions in both cAMP accumulation and insulin secretion. (*P* values in Table 1).

Discussion. Results obtained from these studies have shown that in isolated rat islets of Langerhans, somatostatin, whilst having no effect on glucose oxidation, did inhibit insulin release and cyclic AMP accumulation

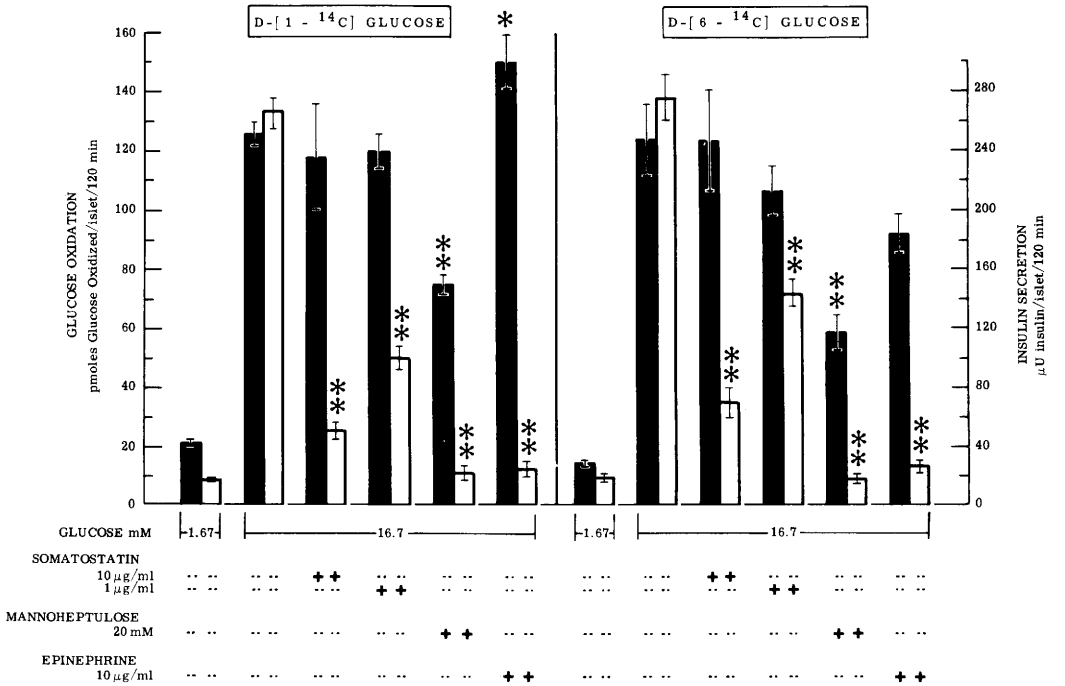

FIG. 1. Vertical bars represent the mean ± (SEM) for 16–34 observations obtained from six to ten islet preparations. Solid bars represent pico-moles of 1-[^{14}C] or 6-[^{14}C] labeled glucose oxidized and the open bars insulin secretion by islets during a 120-min incubation. The effects of somatostatin, mannoheptulose and epinephrine in the presence of glucose (16.7 m*M*) were compared with glucose (16.7 m*M*) alone. * represents $P < .05$ and ** represents $P < .001$.

TABLE I. INSULIN SECRETION AND ACCUMULATION OF CYCLIC AMP BY ISOLATED ISLETS.

No. of observation	Additions to medium: Glucose (m*M*)	Somatostatin (μg/ml)	Mannoheptulose (m*M*)	Epinephrine (μg/ml)	Cyclic AMP pmoles/200 islet/20 min	Insulin μU/islet/20 min
(20)	1.67	—	—	—	4.07 ± 0.36	52 ± 8
(30)	16.7	—	—	—	6.51 ± 0.39*	103 ± 8*
(21)	16.7	1.0	—	—	4.23 ± 0.37*	68 ± 8*
(26)	16.7	10.0	—	—	4.50 ± 0.42*	79 ± 6*
(19)	16.7	—	20	—	3.85 ± 0.52*	57 ± 7*
(18)	16.7	—	—	10	2.56 ± 0.33*	50 ± 8*

[a] Values shown represent mean ± SEM for cyclic AMP results. * Represents $P < 0.001$.

in response to a glucose stimulus. The effects of epinephrine and mannoheptulose on glucose stimulated insulin release, cyclic AMP accumulation and glucose oxidation by isolated islets confirm earlier findings by other investigators (15, 18).

In the studies presented in this communication, glucose labelled in either the C_1 or C_6 positions were used to determine whether the pentose shunt or Embden Meyerhoff pathways, respectively may have been preferentially affected by somatostatin. The results obtained however, clearly indicate that somatostatin does not affect glucose utilization by either pathway. Contrary to our findings on glucose oxidation are those of Hahn and Gottschling (19), who demonstrated inhibition of oxidation by somatostatin of D-U-[^{14}C]labelled glucose. This difference cannot readily be explained, but perhaps it may be related to different sources of somatostatin used in the two studies.

The finding that somatostatin inhibits glucose stimulated accumulation of cyclic AMP by islets is consistent with the observed effects of this polypeptide on the cyclic AMP system in isolated hepatocytes (20) and the anterior pituitary of the rat (10, 21). Efendic and Luft (13), in studies designed to measure conversion of ^{3}H adenine to ^{3}H cyclic AMP by isolated islets, were able to demonstrate inhibition by somatostatin of cyclic AMP accumulation in response to a submaximal 8.3 m*M* glucose stimulus. However, they failed to demonstrate a similar effect at a higher glucose concentration (27.8 m*M*). They concluded that glucose in high concentration is able to overcome the blockade of glucose stimulated cyclic AMP accumulation by somatostatin.

Whilst the exact mechanism by which somatostatin affects islet secretory functions is not known, data reported in this communication along with data reported from other laboratories would suggest that somatostatin affects cellular functions by a mechanism involving the calcium–cyclic AMP system rather than by interference with cellular glucose utilization.

Summary. Somatostatin at a final concentration of 1 μg or 10 μg/ml did not affect glucose stimulated oxidation of either D-[^{14}C]$_1$ or D-[^{14}C]$_6$ glucose, but inhibited both insulin release and cyclic AMP accumulation by isolated rat islets of Langerhans.

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