The Effect of Somatostatin on Glucose Uptake and Production by Rat Tissues in Vitro

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SUMMARY

Somatostatin has recently been used as a tool to investigate the roles of insulin and glucagon in the regulation of glucose metabolism in vivo. The alterations in glucose uptake and production that somatostatin causes have been attributed to the modifications it induces in pancreatic hormone levels rather than to effects of the peptide per se, although the direct effects of the latter on glucose turnover have not been clearly assessed. The aim of the present study therefore was to determine whether or not somatostatin directly affects either the rate of glucose uptake by fat and muscle or the rate of glucose production by the liver.

Glucose uptake by the perfused rat hindquarter (skeletal muscle) was unaltered by somatostatin (10-1,000 ng./ml.) whether or not the process was stimulated by insulin (250 μU./ml.). Basal and insulin-(5-40 μU./ml.)-stimulated glucose oxidation (uptake) by isolated rat adipocytes was also unaffected by somatostatin (5-500 ng./ml.). In addition, the peptide had no effect on basal or glucagon- (10^{-10}–10^{-9} M)-stimulated glucose output by isolated rat liver cells. Somatostatin also failed to modify the actions of these submaximally effective levels of glucagon on either the intracellular level of cAMP or the activity of cAMP-dependent protein kinase. The present studies support the hypothesis that the acute changes in the level and turnover of glucose induced by somatostatin are attributable to its effect on the endocrine system rather than to its direct effects on glucose metabolism. DIABETES *26*:740-48, August, 1977.

The ability of somatostatin to inhibit the release of insulin and glucagon from the pancreas[1,2] has led to its use in studies investigating the metabolic importance of the pancreatic hormones in the normal[3-7] and diabetic state.[8-12] It has been possible, with somatostatin, to show that glucagon plays a role in the regulation of basal glycogenolysis[3] and gluconeogenesis.[4] In addition, it has been possible to show that glucagon has a marked effect on ketogenesis,[8,9] suggesting a potential role for this hormone in the development of diabetic ketoacidosis.

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Despite the widespread use of somatostatin as a research tool and the diversity of its effects on the endocrine system, little is known about its nonendocrine actions. Somtatostatin has been reported to have an effect on the exocrine pancreas[12,13] and an action on platelet aggregation,[14,15] but few studies have been carried out to investigate its direct effects on glucose metabolism. The peptide has been reported to be incapable of modifying glucose uptake by muscle; however, measurements were made either in the absence of insulin[16] or in a preparation (rat hemidiaphragm) that was relatively insensitive to the latter hormone.[7] No data are currently available regarding the effect of somatostatin on glucose uptake by fat cells. Lastly, studies carried out to assess the direct effects of the peptide on glucose production by the liver have led to conflicting results.[17,18]

In view of the important conclusions being drawn from studies utilizing somatostatin to assess the hormonal regulation of glucose metabolism, it is essential to determine whether or not the peptide exerts direct effects on the net flux of glucose into fat and muscle or out of the liver. This has been accomplished in the present study of perfused rat hindquarters,[19] isolated rat adipocytes,[20] and isolated rat hepatocytes.[21] The effect of somatostatin on glucose uptake or production was assessed when the process in question was basally or submaximally stimulated. In addition, the effect of somatostatin on the glucagon-induced elevation of cAMP and activation of cAMP-dependent protein kinase in hepatocytes was determined.

MATERIALS AND METHODS

Surgical Procedures and Animals

Experiments were carried out on male Sprague-Dawley rats (140-200 gm.). Animals used for hindlimb perfusions were fasted overnight prior to use while those used for in-vivo experiments were fasted

six hours. Rats used for liver and fat-cell experiments had free access to Purina laboratory chow.

(i) Isolated adipocytes were prepared from rat epididymal tissue by the procedure of Rodbell.[20] Incubations were performed at 37° in a Krebs Henseleit bicarbonate buffer with 1 per cent dialyzed albumin, equilibrated with O_2:CO_2, 95 per cent:5 per cent, pH 7.4. Each incubation vessel contained approximately 2×10^5 adipocytes suspended in 2.5 ml. of buffer, with 1 mM glucose and 0.222 μCi. glucose-U-^{14}C per ml. Experiments were started by the addition of cells to the experimental flask. Details of the method have been reported previously.[22-23]

(ii) Isolated rat liver cells were prepared from Nembutal-anesthetized (50 mg./kg. IP.) rats by a modification[23] of the method of Berry and Friend.[21] Incubations were carried out at 37° in Krebs Henseleit bicarbonate buffer containing 1.5 per cent gelatin equilibrated with O_2:CO_2, 95 per cent:5 per cent, pH 7.4. Each incubation vessel contained approximately 200 mg. (wet weight) of cells in 5 ml. of buffer. Cells were preincubated for 10 minutes prior to any additions, and experiments were started by hormone addition.

(iii) The perfused-hindquarter experiments were carried out on Nembutal-anesthetized (50 mg./kg. IP.) animals prepared with a modification* of the procedure of Ruderman et al.[19] Hindlimbs were perfused with continuously oxygenated Krebs Henseleit buffer (pH 7.4) containing 4 per cent dialyzed albumin, 11 mM glucose, and 30 per cent washed, aged human red cells, at a flow rate of approximately 10 ml./min. in a recirculating system. A five-minute flowthrough period was employed prior to recirculation. Each experiment was started by the addition of the required hormone to the perfusate.

(iv) The in-vivo experiments were carried out on urethane- (1 gm./kg. IP.) anesthetized rats with a polyethylene catheter in the jugular vein. This catheter was used for somatostatin injection and subsequent blood sampling.

Experimental Design

(i) Adipocytes were incubated in closed pregassed vials for 60 minutes at 37° with continuous agitation in the presence of insulin (0-160 μU./ml.) ± somatostatin (5, 50, or 500 ng./ml.). Samples were taken after one hour.

(ii) In one set of experiments, hepatocytes were incubated in closed pregassed vials for 15 minutes at 37° with continuous agitation in the presence of glucagon ($0\text{-}5 \times 10^{-10}$ M) ± somatostatin (10, 100, 1,000 ng./ml.). Aliquots of cells were removed for determination of cAMP levels and cAMP-dependent protein kinase activity after one minute and for assessment of glucose production after 15 minutes. In another set of experiments, liver cells were preincubated with somatostatin (1,000 ng./ml.) or saline for 15 minutes prior to the addition of saline or glucagon (10^{-9} M) with or without somatostatin and samples were taken at 0, 1, 3, 15, and 30 minutes for determination of protein kinase and phosphorylase activity and at 0, 15, and 30 minutes for measurement of glucose levels in the medium.

(iii) The perfused-hindquarter experiments were started by the addition of insulin (0-2,500 μU./ml.) with or without somatostatin (0, 10, 100, 1,000 ng./ml.) to the perfusate reservoir. Samples for glucose determination were withdrawn from the arterial and venous lines every 15 minutes for the next hour.

(iv) The experiments on intact rats employed a 15-minute control period, a somatostatin injection (100 μg.), and a subsequent 15-minute test period. Blood samples were drawn at -15, 0, 7, and 15 minutes.

Processing of Samples

(i) At the end of the fat-cell experiments. 0.2 ml. of 1 N H_2SO_4 was added to the incubation medium to stop the reaction and liberate CO_2. The $^{14}CO_2$ was trapped in 0.2 ml. of 10 × hyamine hydroxide added to the center well of the incubation vessel. The radioactivity of the samples was determined by liquid scintillation spectroscopy. The $^{14}CO_2$ production was used as index of glucose metabolism and as a reflection of glucose uptake.

(ii) Glucose concentrations were determined with the Beckman Glucose Analyzer.

(iii) Intracellular cyclic AMP levels were determined by the Gilman binding assay[25] following removal of the liver-cell incubation medium.

(iv) Hepatic cAMP-dependent protein kinase was measured with a modification of the method of Corbin and Reimann[26] described in detail elsewhere.[27] In brief, 1 ml. of liver cell suspension was removed from the incubation flask and immediately spun (50 × g) for 10 seconds. After aspiration of the incubation medium, the cells were resuspended in 2.0 ml. ice-cold buffer containing 10 mM potassium phosphate pH 6.8, 10 mM Na EDTA, 0.5 mM 1-methyl-3-isobutylxanthine (MIX), and 150 mM KCl and homogenized in an Ultraturrax homogenizer (8 sec-

*M. D. Caldwell—unpublished data.

onds per 40 mg. tissue). Extracts were stable for at least 60 minutes at 0° but were always assayed within one hour. The assay mixture (70 μl.) consisted of 10 mM Mg acetate, 10 mM NaF, 0.2 mM ATP, 10 mg./ml. histone (Sigma, type II-A), and 0.1 mM MIX with or without 5 μM cAMP. The assay was started by adding 20 μl. of liver homogenate (4 mg. wet wt./assay tube). Incorporation of ^{32}P from ATP into histone was linear under the assay conditions (8 minutes; 30° C.) in the presence or absence of exogenous cAMP. The reaction was stopped by spotting 50 μl. of the assay mixture on Whatman no. 2 filter paper and transferring the filter paper to 10 per cent trichloracetic acid. The amount of ^{32}P incorporated was determined by liquid scintillation spectroscopy. Phosphorylation of non-cAMP dependent kinases represented less than 10 per cent of the ^{32}P incorporation observed.

(iv) Phosphorylase *a* activity was measured by the method of Stalmans and Hers[28] after preparation of cell homogenates according to Hutson et al.[23]

Materials

U-^{14}C glucose was obtained from Amersham/Searle Corp. and used without further purification. Linear and cyclic somatostatin were obtained from Bachem, Inc., while insulin and glucagon were obtained from Eli Lilly Laboratories. Gelatin was purchased from Difco Laboratories and bovine serum albumin, fraction V, from Sigma Biochemicals. The latter was dialyzed three times against saline prior to use. Collagenase (type II) was bought from Worthington Biochemical Corp., and histone (type II-a) was bought from Sigma Biochemicals.

RESULTS

Injection of 100 μg. of somatostatin into the rat in vivo caused the plasma concentrations of immunoreactive insulin and glucagon to fall by 70 ± 12 and 54 ± 14 per cent, respectively, after 15 minutes. These findings are consistent with those made in dogs with the same somatostatin[5,6] and thus established the biologic effectiveness of the peptide.

Figure 1 shows the dose-response relationship between insulin and glucose clearance by the perfused rat hindquarter. Physiologic increments in the concentration of the hormone (25-100 μU./ml.) significantly ($p < 0.05$) stimulated glucose uptake. Figure 2 demonstrates that linear somatostatin (10, 100, or 1,000 ng./ml.) failed to alter the process whether or not it was insulin-stimulated. While the dose of insulin (250 μU./ml.) used in these experiments tripled glucose clearance, it was still clearly submaximal (figure 1). In another experiment (data not shown), a higher dose of somatostatin (5,000 ng./ml.) was also without effect on basal or insulin-stimulated glucose uptake.

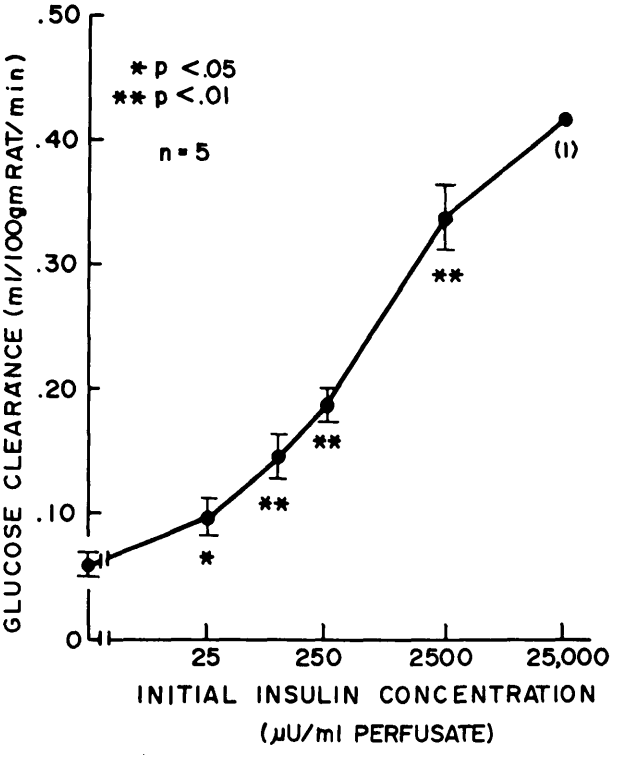

FIG. 1. Effect of insulin on glucose clearance in isolated perfused rat hindquarters. The value shown represents the mean rate for the entire hour of perfusion. Results are mean ± S.E.M. of separate perfusions; p values (unpaired *t* test) are relative to the zero insulin value.

Figure 3 shows the dose-response relationship between glucose oxidation by isolated fat cells and the insulin level in the incubation medium. The basal rate of glucose oxidation was significantly stimulated by very low levels of insulin (5 μU./ml.) and was virtually saturated at a dose of 10-fold higher. The effect of linear somatostatin (5, 50, 500 ng./ml.) was examined in the absence of insulin and in the presence of various submaximally effective doses of the hormone. As shown in figure 4, the peptide had no effect; glucose oxidation was unaltered whether or not it was stimulated by insulin.

The ability of glucagon to elevate cAMP, to activate cAMP-dependent protein kinase, and to stimulate glucose production by isolated rat liver parenchymal cells is illustrated in figure 5 and table 1.

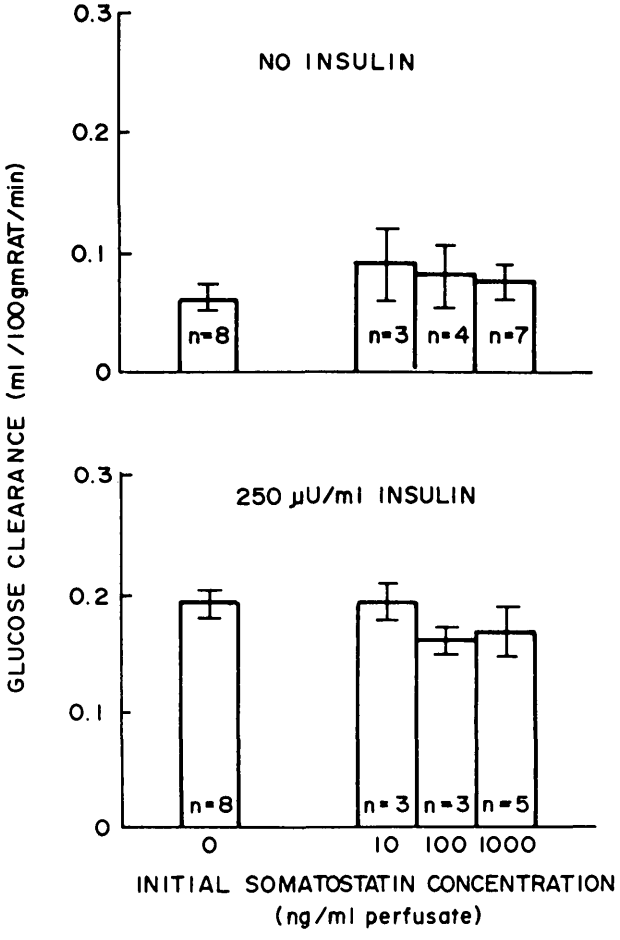

FIG. 2. Effect of linear somatostatin on basal and insulin-stimulated glucose clearance by the isolated perfused rat-hindquarter preparation. The value shown represents the mean rate for the entire hour of perfusion. Results are mean ± S.E.M. of separate perfusions; no values were significantly different from those obtained when somatostatin was absent.

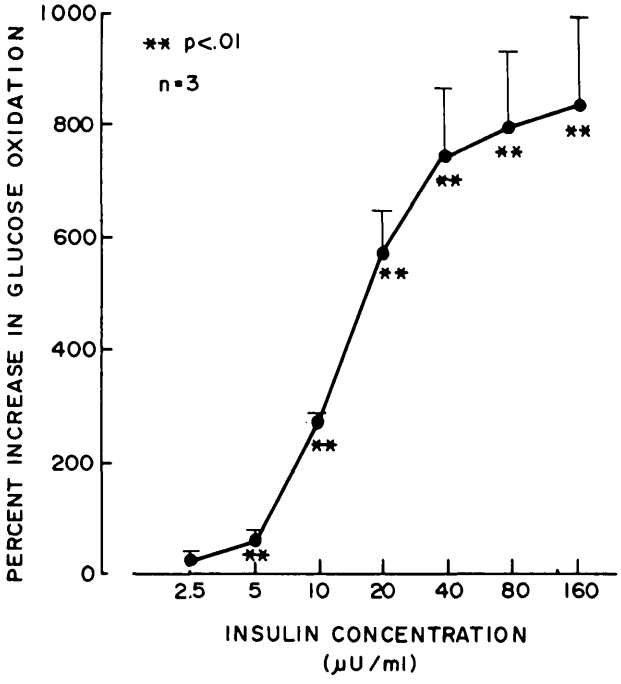

FIG. 3. Effect of insulin on glucose oxidation over one hour in isolated rat adipocytes. Values are mean ± S.E.M. of three experiments on different cell batches; p values (paired *t* test) are relative to the zero insulin value.

Basal glucose production (34 ± 3 nmol./hr./mg. wet weight) was associated with a basal protein kinase activity ratio (-cAMP/+cAMP) of 0.32 ± 0.02. Glucagon at 10^{-10} M doubled glucose output and caused an increase of 0.11 in the protein kinase activity ratio one minute after exposure to the hormone. Samples were drawn at that time because previous studies had shown that the peak effects of glucagon on cAMP accumulation and protein kinase activation occurred one to two minutes after hormone addition.[27,29] Figure 6 and table 1 show that neither the basal nor the glucagon-stimulated level of cAMP or the ensuing activation of the kinase was altered by linear somatostatin (10-1,000 ng./ml.). In addition, neither the basal nor glucagon-stimulated rates of glucose production were affected by the peptide. Table 2 shows that the ability of 10^{-9} M glucagon to elevate cAMP, to activate protein kinase, and to simulate glucose production was unchanged even when hepatocytes were preincubated with linear somatostatin (1,000 ng./ml.).

Somatostatin exists in two forms (cyclic or linear), and since Oliver and Wagle[18] used the cyclic form in their study, a series of experiments was undertaken using the cyclized rather than linear peptide. Table 3 shows that cyclic somatostatin was also without significant effect on basal or glucagon (5×10^{-10}M) stimulated glucose output. In addition, the data in this table demonstrate that glucagon's effect on phosphorylase *a* activity was virtually unchanged by somatostatin even when liver cells were preincubated with the peptide (1,000 ng./ml.) and then exposed to a second dose of somatostatin (1,000 ng./ml.) simultaneously with exposure to glucagon.

DISCUSSION

The present experiments indicate that somatostatin per se does not interfere with basal or insulin-stimulated glucose uptake by fat or skeletal muscle.

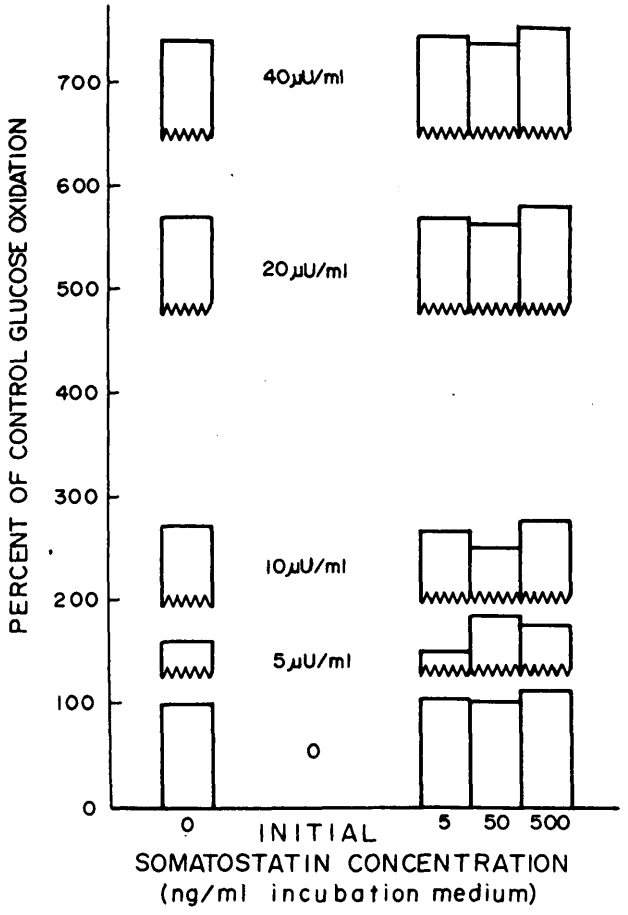

FIG. 4. Effect of linear somatostatin on basal and insulin-stimulated glucose oxidation by isolated rat adipocytes. Each dose of somatostatin was tested with a different batch of cells and a control was run in each case. Incubations were for one hour. The insulin concentration used is listed in the middle of the figure (μU./ml.).

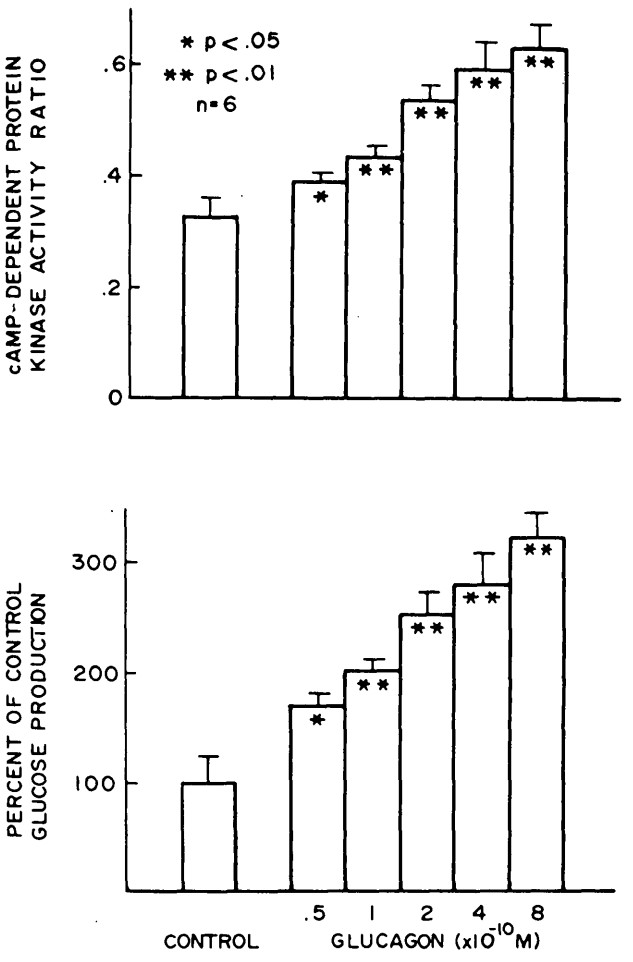

FIG. 5. Effect of glucagon on the cAMP-dependent protein kinase activity ratio (-cAMP/+cAMP) and glucose production in isolated rat liver parenchymal cells. Protein kinase activity was determined at one minute and glucose output after 15 minutes. Values are mean ± S.E.M. of six experiments carried out on different cell batches; p values (paired *t* test) are relative to the control value.

Furthermore, they indicate that the peptide does not affect basal or glucagon-stimulated hepatic glucose production. Taken together, these findings suggest that the acute alterations in the blood glucose level[6,17,30] and the rate of glucose turnover[3,5] that accompany somatostatin infusion in vivo are a result of the endocrine disturbances that the peptide induces.

The doses of somatostatin used in the present studies were chosen so that they were in excess of those reported to inhibit the secretion of insulin from isolated islets of Langerhans[31-33] and perfused pancreas,[1] of glucagon from perfused pancreas,[2] of growth hormone from isolated pituitaries,[34] and of gastrin from isolated sections of rat stomach.[35] Since it was not possible to assay the perfusion medium for somatostat-

TABLE 1

Effects of linear somatostatin on glucagon-induced changes in cAMP in isolated rat liver cells

Glucagon (M)	0				10^{-10}				5×10^{-10}			
Somatostatin (ng./ml.)	0	10	100	1,000	0	10	100	1,000	0	10	100	1,000
cAMP (pmol/mg. wet wt.)	0.41	0.42	0.39	0.41	0.69	0.62	0.67	0.73	1.31	1.29	1.38	1.32
S.E.M.*	0.01	0.03	0.04	0.05	0.06	0.03	0.05	0.06	0.04	0.07	0.05	0.14

*Samples from four separate sets of liver cells were used for each value.

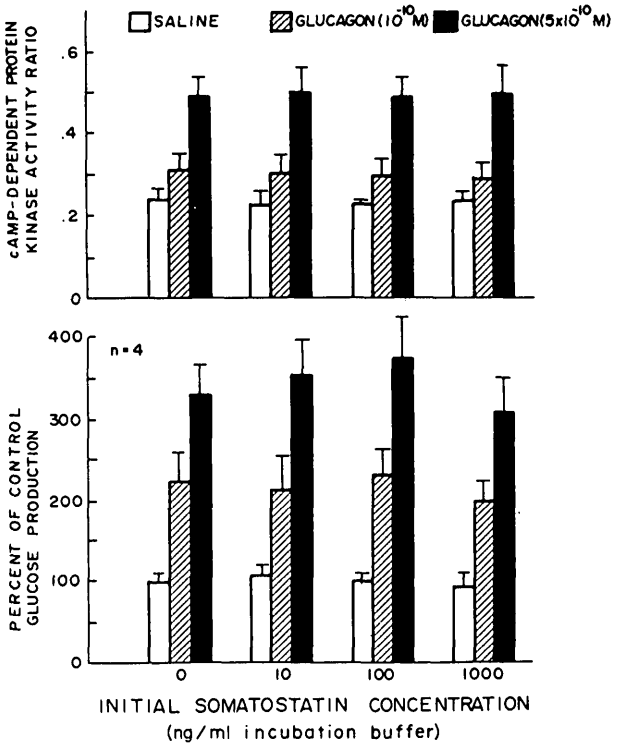

FIG. 6. Effect of linear somatostatin on basal and glucagon-stimulated protein kinase activity and glucose production by isolated rat liver cells. Samples were taken as in figure 5. Values are mean ± S.E.M. of four experiments carried out on different cell batches. There was no significant effect of somatostatin on either parameter whether or not glucagon was present.

in and since its site of degradation is not known, it was not possible to estimate the extent to which breakdown of the peptide occurred. Somatostatin's half-life is reported to be approximately four minutes in vivo.[36] Thus, even if one were to assume the unlikely possibility that the tissue in question (fat, muscle, or liver) is the sole site of its degradation, the concentration of somatostatin in the medium would still have remained above that reported to affect hormone secretion for the entire duration of the experiment when a high dose (500-2,000 ng./ml.) of the peptide was given originally. Furthermore, in vivo a maximally effective infusion of somatostatin (0.5 μg./kg.-min.) would result in plasma levels of the peptide of only 375 ng./ml. after one hour of infusion even if one assumes that the peptide neither was destroyed nor escaped the vascular compartment. Since neither of these assumptions can be true, the actual somatostatin levels obtained in such experiments must be quite low, probably less than 10 ng./ml., and well within the range of the levels used in the present study.

The isolated perfused rat-hindquarter preparation has been used successfully in recent years to study the metabolism of skeletal muscle.[37] One of its advantages, as shown in the present study, is that it is sensitive to levels of insulin similar to those found in vivo. Insulin at an initial concentration of 25 μU./ml. caused a doubling of glucose uptake by the rat

TABLE 2

Effect of linear somatostatin on glucagon-induced increases in cAMP, cAMP-dependent kinase, and glucose output in rat liver cells (n = 1)

Pretreatment (15 min.)	Treatment	Measured parameter	Time after treatment 0	1	3	15	30
Saline	Saline	Protein kinase ratio*	0.25	0.20	0.22	0.26	0.22
		cAMP†	0.35	0.28	0.37	0.36	0.27
		Glucose output‡			49.8		
Saline	Somatostatin (1,000 ng./ml.)	Protein kinase ratio*	0.22	0.23	0.22	0.24	0.26
		cAMP†	0.33	0.31	0.36	0.35	0.30
		Glucose output‡			47.6		
Saline	Glucagon (10^{-9} M)	Protein kinase ratio*	0.19	0.60	0.57	0.38	0.43
		cAMP†	0.32	1.04	0.93	0.74	0.47
		Glucose output‡			154.2		
Saline	Glucagon (10^{-9} M) + Somatostatin (1,000 ng./ml.)	Protein kinase ratio*	0.19	0.66	0.62	0.51	0.40
		cAMP†	0.34	1.15	1.07	0.78	0.51
		Glucose output‡			144.0		
Somatostatin (1,000 ng./ml.)	Glucagon (10^{-9} M) + Saline	Protein kinase ratio*	0.23	0.58	0.56	0.51	0.35
		cAMP†	0.41	0.98	0.94	0.82	0.48
		Glucose output‡			149.4		

*(-cAMP/+cAMP)
†(pmol/mg. wet wt.)
‡(nmol/hr./mg. wet wt.)

TABLE 3

Effect of cyclic somatostatin on glucagon-induced increases in cAMP-dependent protein kinase, phosphorylase, and glucose output in rat liver cells (n = 4)

Pretreatment (15 min.)	Treatment	Measured parameter	Time after treatment 0	1	3	15	30
Saline	Saline	Protein kinase ratio*	0.34 ± 0.03	0.35 ± 0.04	0.34 ± 0.03	0.35 ± 0.04	0.39 ± 0.05
		Phosphorylase activity†	8.7 ± 0.6	9.0 ± 0.8	8.7 ± 0.8	8.5 ± 0.8	8.3 ± 0.5
		Glucose output‡			39.1 ± 7.6		
Saline	Somatostatin (1,000 ng./ml.)	Protein kinase ratio*	0.35 ± 0.02	0.34 ± 0.03	0.34 ± 0.04	0.35 ± 0.03	0.37 ± 0.03
		Phosphorylase activity†	8.6 ± 0.5	8.8 ± 0.9	8.3 ± 0.7	8.2 ± 0.5	8.0 ± 0.4
		Glucose output‡			37.4 ± 6.5		
Saline	Glucagon (5×10^{-10} M)	Protein kinase ratio*	0.36 ± 0.03	0.57 ± 0.03	0.53 ± 0.05	0.45 ± 0.05	0.36 ± 0.04
		Phosphorylase activity†	8.6 ± 0.9	12.6 ± 1.7	13.2 ± 1.4	10.4 ± 1.7	8.1 ± 0.4
		Glucose output‡			77.1 ± 10.4		
Saline	Glucagon (5×10^{-10} M) + Somatostatin (1,000 ng./ml.)	Protein kinase ratio*	0.35 ± 0.03	0.60 ± 0.03	0.57 ± 0.04	0.45 ± 0.05	0.39 ± 0.03
		Phosphorylase activity†	8.8 ± 0.7	12.5 ± 1.2	13.2 ± 1.2	10.4 ± 1.1	8.3 ± 0.5
		Glucose output‡			73.6 ± 9.8		
Somatostatin (1,000 ng./ml.)	Glucagon (5×10^{-10} M) + Somatostatin (1,000 ng./ml.)	Protein kinase ratio*	0.37 ± 0.03	0.59 ± 0.02	0.55 ± 0.05	0.47 ± 0.03	0.40 ± 0.04
		Phosphorylase activity†	8.5 ± 0.9	11.7 ± 1.2	11.8 ± 1.1	9.6 ± 1.0	8.0 ± 0.5
		Glucose output‡			67.4 ± 8.2		

*(-cAMP/+cAMP) †(units/mg. wet wt.) ‡(nmol/hr./mg. wet wt.)

hindquarter. Since the concentration of insulin in the medium declined by 35 per cent during the hour of perfusion,† the average level of the hormone in the perfusate was below 25 μU./ml. The sensitivity of rat adipocytes to insulin is well established[20,22,23] and is clearly demonstrated in the present study. Insulin at 5 μU./ml. significantly stimulated glucose oxidation. Since glucose transport is thought to be rate-limiting when low levels of exogenous glucose are present, glucose oxidation is considered to reflect glucose uptake.[20,22,23] The degradation of insulin by fat cells incubated under conditions similar to those used here has previously been reported to be negligible.[23] Clearly both (muscle and fat) preparations were very sensitive to insulin and provided an excellent system in which to assess the effects of somatostatin on glucose uptake.

The inability of somatostatin to modify glucose uptake by skeletal muscle is shown clearly in the present study. At initial concentrations ranging from 10 to 1,000 ng./ml., the hormone was without effect on basal or submaximally stimulated glucose uptake. The present findings extend those of Gerich et al.,[7] who reported that somatostatin (1,000 ng./ml.) was without effect on glucose uptake by the rat hemidiaphragm, whether or not insulin (1,000 μU./ml.) was present in the incubation medium, although the insulin sensitivity of the preparation was poor. The present experiments also demonstrate that the oxidation of glucose by isolated fat cells is unaffected by somatostatin, thus suggesting that glucose uptake by this tissue is also unaltered by the peptide.

The isolated rat liver cells used in the present study were sensitive to levels of glucagon (5×10^{-11} M) as low as those known to occur postabsorptively in the portal vein.[38] The glucagon-induced increase in the level of cAMP was closely correlated with increases in cAMP-dependent protein kinase activity and a stimulation of glucose output. Since glucagon increased the -cAMP/+cAMP activity ratio of the kinase without affecting the total activity of the enzyme, it appears to act by dissociating the holoenzyme. In liver, as in fat and muscle, somatostatin was again virtually without effect. The hypothalamic peptide failed to alter either the basal level of cAMP, basal cAMP-dependent protein kinase activity, basal phosphorylase *a* activity, or the basal rate of glucose output in saline-treated liver cells. Similarly, it failed to significantly alter glucagon-induced changes in any of the above parameters. Preincubation of liver cells with somatostatin (1,000 ng./ml.) and reexposure to a second dose of the peptide (1,000 ng./ml.) concurrently with glucagon (5×10^{-10} M) resulted in a small, albeit nonsignificant, reduction of the effect of glucagon on phosphorylase activity and glucose output in the absence of a change in glucagon's effect on protein kinase. It is possible that high doses of somatostatin might antagonize the action of glucagon slightly via a

†Unpublished observation.

non-cAMP-dependent mechanism, but clearly only at levels one to two orders of magnitude higher than those obtained in vivo during somatostatin infusion. These findings are in agreement with those reported by Chideckel et al.,[17] who used liver slices, and Gerich et al.,[39] who used perfused liver to show that somatostatin was without effect on basal or glucagon-stimulated glucose output. They differ, however, from those of Oliver and Wagle,[18] who reported that somatostatin (70-7,000 ng./ml.) could interfere with glucagon-stimulated glucose output by liver cells. These authors used cyclic somatostatin, but it is unlikely that this contributed to the discrepant findings since, despite one report to the contrary,[40] several studies have shown that there is no detectable difference between the biologic activity of the two forms of the peptide[41,42] and both forms were found to be impotent in the present experiments. The studies of Oliver and Wagle are unusual in that they observed increasing inhibition of glucagon action with decreasing concentrations of somatostatin. It should be emphasized that in the present study the peptide (10-2,000 ng./ml.) now only failed to alter glucose output by isolated liver cells but also failed to induce significant changes in the cAMP level, the activity of cAMP-dependent protein kinase, or phosphorylase *a* activity.

The present studies thus indicate that somatostatin produces its effects on glucose homeostasis in vivo by altering hormone secretion rates rather than through direct effects on glucose uptake or output. The peptide thus provides a unique means by which the glucoregulatory feedback loops involving the pancreas can be broken in vivo without major surgery. It thereby makes possible well-controlled acute studies of the regulation of carbohydrate metabolism in vivo.

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