Inhibition of Pancreatic Glucagon Responses to Arginine by Somatostatin in Normal Man and in Insulin-Dependent Diabetics

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SUMMARY

Somatostatin, a recently synthesized hypothalamic peptide thought to represent growth hormone-release-inhibiting factor, has been previously shown to inhibit pancreatic glucagon secretion in species other than man. To determine whether somatostatin also inhibits human glucagon secretion, plasma glucagon responses during intravenous infusion of arginine alone (250 mg. per kilogram over twenty-five minutes) and in combination with synthetic cyclic somatostatin (20 μg. per minute) were compared in six normal and six insulin-dependent diabetic subjects.

Somatostatin abolished glucagon responses to arginine in both groups, with plasma glucagon declining transiently below basal levels; this occurred despite the fact that the diabetic subjects had fasting hyperglucagonemia and exaggerated glucagon responses during control arginine infusions. In both groups plasma glucose rises seen after arginine were diminished during somatostatin infusions, and, in the normal subjects, insulin responses were also inhibited.

These results demonstrate that somatostatin is a potent inhibitor of glucagon secretion in man. Accordingly, it may prove useful as an adjunct to insulin therapy in treatment of insulin-requiring diabetes mellitus. DIABETES *23*:876-80, November, 1974.

Current evidence indicates that diabetes mellitus may be a bi-hormonal disorder characterized by excessive glucagon secretion as well as diminished insulin release.[1,2] Since glucagon at physiologic concentrations is a potent glycogenolytic,[3] lipolytic,[4] and gluconeogenic[5] agent whose actions oppose those of insulin,[6] glucagon excess such as is seen in poorly controlled juvenile and adult-onset diabetics[7-9] may accentuate the consequences of insulin deficiency and lead to deterioration in glucose homeostasis. Acute administration of large amounts of exogenous insulin to diabetics has failed to restore appropriate fasting plasma glucagon levels[10] or to normalize glucagon responses during meals.[1,11] Although long-term optimal insulin replacement might possibly overcome the deleterious effects of glucagon, such ideal control is seldom attained.[12] Indeed, even under conditions of intensive insulin therapy, wide fluctuations of plasma glucose levels still occur frequently in diabetics.[12] Accordingly, some pharmacologic agent capable of inhibiting glucagon secretion might be of value in improving diabetic control. Recently somatostatin, a hypothalamic peptide[13] that inhibits human growth hormone,[14-16] TSH,[15] and insulin[17] secretion has been shown to be a potent inhibitor of glucagon secretion in vitro[18,19] and in vivo[20] in species other than man. The present investigation was therefore undertaken to determine whether somatostatin inhibits glucagon secretion in man and, particularly, whether it is also effective in subjects with diabetes mellitus.

MATERIALS AND METHODS

Subjects. Informed consent was obtained from six normal and six insulin-dependent subjects with diabetes. The normal subjects (aged twenty-one to twenty-nine years) were not obese and had no family history of diabetes. The diabetic subjects (sixteen to forty-five years) were ambulatory patients free of acute illness or ketosis at the time of study. All were nonobese and had had insulin-dependent diabetes from two to twenty years.

Experimental protocol. All studies were performed between 7 and 10 a.m. after an overnight fast. Insulin

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therapy was omitted on the morning of the test in the diabetic subjects. In most instances a mixture of 5 to 10 units Regular and 5 to 10 units NPH insulin had been taken subcutaneously fourteen to sixteen hours before. After the start of intravenous saline (.9 per cent) infusions in each arm (one for infusion of drugs, the other for blood sampling), a thirty-minute equilibration period was allowed before initial basal samples (-thirty minutes) were collected. Arginine hydrochloride (5 per cent) was infused over twenty-five minutes at a rate of 10 mg. per kilogram per minute. On a subsequent day repeat arginine infusions were performed during which synthetic cyclic somatostatin[21] (provided by Dr. Roger Guillemin, Salk Institute, San Diego) was infused in saline (.9 per cent) solution. An initial intravenous bolus of 250 μg. somatostatin was administered concurrently with the initiation of the arginine infusion, followed by a sustained infusion of 20 μg. per minute via a Harvard pump lasting for twenty-five minutes in normal subjects and extended for sixty minutes in the diabetic patients.

Plasma glucose, insulin, pancreatic glucagon, and serum growth hormone responses were determined by methods previously described.[22]

RESULTS

Normal subjects. Infusion of arginine caused modest elevations of plasma glucose, insulin, and pancreatic glucagon levels in normal subjects (figure 1, left ●—●). After stopping the arginine, plasma glucose declined transiently below baseline, whereas insulin and pancreatic glucagon concentrations returned gradually to preinfusion values. Mean serum growth hormone levels (table 1) rose slightly after arginine administration. Somatostatin virtually abolished insulin and glucagon responses to arginine with mean plasma glucagon levels declining temporarily below baseline (figure 1, left 0—0). After stopping the arginine and somatostatin infusions, plasma insulin remained unchanged, whereas plasma glucagon rose transiently above preinfusion levels. Plasma glucose responses to arginine were also diminished by somatostatin. However, after cessation of arginine and somatostatin infusions, plasma glucose rose to levels that exceeded those seen during control arginine infusions. Mean serum growth hormone responses (table 1), initially low, were not significantly different during somatostatin infusion from those seen with arginine alone.

Diabetic patients. The diabetic patients studied had

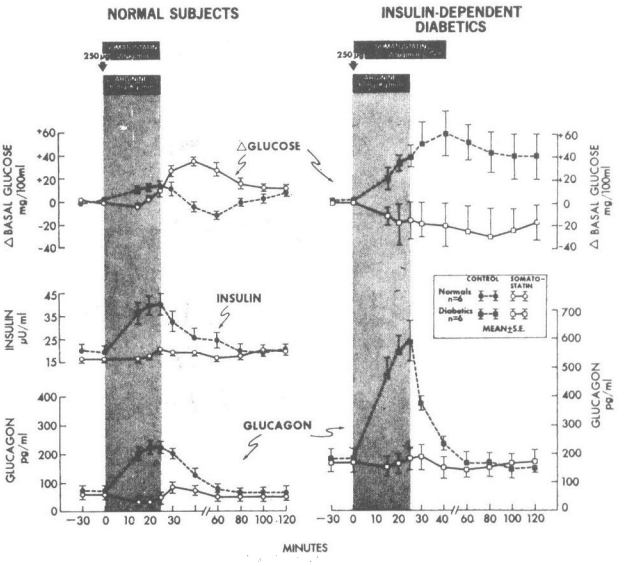

FIG. 1. Effect of somatostatin on plasma glucose, insulin and glucagon responses to intravenous arginine in six normal subjects (left) and in six insulin-dependent diabetic patients (right), mean ± S.E.

fasting hyperglycemia (table 1) and hyperglucagonemia (171 ± 27 versus 63 ± 19 pg. per milliliter in the normal subjects, $p < .01$). Rises in plasma glucose and glucagon levels after control arginine administration markedly exceeded those observed in the normal subjects (figure 1, right ■—■). Serum growth hormone responses to arginine, available in three patients, were variable (table 1). Somatostatin abolished plasma glucagon and glucose responses to arginine in diabetic patients (figure 1, right □—□). In fact, mean plasma glucose levels fell slightly during infusion of somatostatin. Serum growth hormone responses (table 1) were comparable to those seen in normal subjects both with and without somatostatin.

Adverse effects. Transient nausea and flushing lasting less than five minutes occurred almost immediately after the intravenous somatostatin bolus in both normal and diabetic subjects. Minor elevation of blood pressure (5 to 10 mM. Hg.) and decrease in pulse rate (5 to 10 beats per minute) also occurred. These lasted about five minutes despite continued infusion of somatostatin. No other acute adverse effects were noted in either group.

DISCUSSION

The present study demonstrates that somatostatin inhibits glucagon responses to arginine in normal

TABLE 1

Effect of somatostatin on plasma glucose and serum growth hormone responses to arginine in normal subjects and in insulin-dependent diabetics.

	—30	0	15	20	25	30	40	60	80	100	120
						Minutes					
						Glucose (mg./100 ml.)					
Subjects											
Normals (N=6)											
Control ($\overline{X}$)	88.2	88.5	98.5	99.0	101.8	103.7	82.6	78.0	87.2	91.8	97.4
SE	3.5	2.6	4.5	5.2	6.3	5.4	6.9	3.8	2.2	1.3	2.9
Somatostatin ($\overline{X}$)	86.3	85.6	85.5	89.6	96.6	114.0	122.8	116.2	103.0	100.8	101.0
SE	2.8	3.1	4.1	3.7	5.4	6.1	5.2	6.0	5.5	3.1	4.8
Diabetics (N=6)											
Control ($\overline{X}$)	200.1	201.3	215.6	230.2	234.2	250.2	260.7	252.1	241.6	242.1	241.5
SE	30.1	30.2	28.3	27.4	28.0	43.2	39.6	45.0	43.2	42.2	44.0
Somatostatin ($\overline{X}$)	248.3	248.2	237.5	230.2	230.5	229.5	229.2	224.5	220.0	221.6	232.2
SE	33.9	35.0	36.0	40.6	41.3	41.6	42.4	43.2	44.9	41.6	38.5
						Growth Hormone (ng/ml.)					
Normals (N=6)											
Control ($\overline{X}$)	1.5	1.4	1.4	1.4	1.8	3.4	3.8	4.3	4.3	4.0	3.4
SE	.3	.3	.2	.2	.4	1.2	1.4	1.8	2.3	2.5	1.9
Somatostatin ($\overline{X}$)	2.6	2.7	2.0	1.7	1.4	1.9	5.3	8.2	5.9	3.6	1.9
SE	.9	.7	.5	.4	.3	.3	3.1	4.6	2.8	1.3	.6
Diabetics											
Control (N=3)											
R.T.	2.4	2.7	3.0	3.3	3.2	3.6	4.5	3.8	3.5	3.2	4.2
R.W.	3.0	2.9	2.6	2.4	4.1	6.4	15.4	22.8	25.1	14.5	14.5
G.H.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Somatostatin (N=6)											
R.T.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.2	1.0	1.0
R.W.	3.1	2.1	2.6	2.6	1.9	2.8	3.2	2.5	2.7	2.7	5.0
G.H.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	6.2	3.4	1.5
R.K.	5.3	1.6	1.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
W.C.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	3.2	6.3	1.7
M.R.	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.0	4.4	3.4	1.0
X	2.1	1.3	1.3	1.3	1.2	1.3	1.4	1.3	3.1	3.9	1.9
SE	.7	.2	.3	.3	.2	.2	.4	.3	.8	.9	.6

human subjects and in insulin-dependent diabetic patients. Moreover, the transient decrements of plasma glucagon below preinfusion levels despite concomitant arginine stimulation suggest that somatostatin also inhibits basal glucagon secretion in man. Koerker et al. have shown that infusion of somatostatin not only lowers basal plasma glucagon, glucose, and insulin levels in the baboon but also inhibits their glucagon and insulin responses to arginine.[20] Indeed, more recent studies in our laboratory[23] clearly indicate that somatostatin also inhibits human basal glucagon secretion and causes rapid lowering of fasting plasma glucose levels in normal subjects as well as in insulin-dependent diabetics.

These observations suggest that glucagon may play an important role in determining fasting plasma glucose levels and point out the potential usefulness of somatostatin as an investigative tool and therapeutic agent.

The mechanism through which somatostatin inhibits hormone secretion has not been defined. Since it is a potent in vitro inhibitor of glucagon and insulin responses to various stimuli in isolated perfused canine[17,18] and rat[19] pancreas systems, somatostatin probably acts directly on pancreatic alpha and beta cells. However, it seems doubtful that somatostatin is a physiologic regulator of pancreatic endocrine function. Although no method presently exists for measuring plasma levels of somatostatin, the minimal concentrations of somatostatin effective in in vitro and in vivo studies[19] are probably several orders of magnitude in excess of peripheral levels, if indeed this hypothalamic peptide enters the general circulation at all. Thus, the effects of somatostatin on pancreatic glucagon and insulin secretion observed in the present study are most likely of a pharmacologic nature.

The present studies were designed to evaluate the effect of somatostatin on arginine-induced glucagon secretion. The 15 to 18 gm. arginine infusions employed produced variable growth hormone responses with mean peak serum levels less than 10 ng. per milliliter compared with those usually in excess of 20

ng. per milliliter found during standard (30 gm.) arginine infusions.[24] Accordingly, arginine infusions used in the present study were inadequate to generate growth hormone responses of sufficient magnitude to detect inhibition by somatostatin.

In normal subjects during the present somatostatin experiments a rebound hyperglycemia occurred after termination of arginine infusions, whereas no rebound occurred in the diabetic patients and even a slight fall was seen. The reasons for this phenomenon are unclear but may be related to the fact that in normal subjects the effects of slight rises in plasma glucagon and growth hormone after termination of somatostatin may have outweighed those of the still-reduced circulating insulin levels. Since the diabetics had previously received exogenous insulin, and since somatostatin itself has no direct effect on the liver,[20] the lack of hyperglycemic rebound in this group could have resulted from more prolonged suppression of glucagon and growth hormone secretion accompanying the longer infusion of somatostatin. Such an explanation might account for the different plasma glucose responses observed in the normal and diabetic subjects, especially if glucagon and growth hormone were important in sustaining hyperglycemia in diabetics. Indeed, on this basis somatostatin should prove useful as an adjunct to insulin therapy in the management of insulin-dependent diabetics.

In summary, the present studies indicate that synthetic somatostatin is a potent inhibitor of pancreatic glucagon secretion in man. The ability of this hypothalamic peptide to suppress glucagon secretion makes it a useful investigative tool for studying human pancreatic alpha-cell function. Moreover, its ability not only to diminish plasma growth hormone and glucagon but also to lower plasma glucose levels makes it a potentially useful adjunct to insulin therapy in the treatment of insulin-dependent diabetes mellitus.

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REFERENCES

[1]Unger, R. H.: Glucagon physiology and pathophysiology. N. Engl. J. Med. *285*:443-49, 1971.

[2]Gerich, J. E., Langlois, M., Noacco, C., Karam, J. H., and Forsham, P. H.: Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha-cell defect. Science *182*:171-73, 1973.

[3]Sutherland, E. W., and de Duve, C.: Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas. J. Biol. Chem. *175*:663-74, 1948.

[4]Lefebvre, P.: The physiological effect of glucagon on fat-mobilisation. Diabetologia *2*:130-32, 1966.

[5]Exton, J. H., and Park, C. R.: Control of gluconeogenesis in liver. Effects of glucagon, catecholamines, and adenosine 3′,5′-monophosphate on gluconeogenesis in the perfused rat liver. J. Biol. Chem. *243*:4189-96, 1968.

[6]Mackrell, D. J., and Sokal, J. E.: Antagonism between the effects of insulin and glucagon on the isolated liver. Diabetes *18*:724-32, 1969.

[7]Unger, R. H., Aguilar-Parada, E., Müller, W. A., and Eisentraut, A. M.: Studies of pancreatic alpha-cell function in normal and diabetic subjects. J. Clin. Invest. *49*:837-48, 1970.

[8]Assan, R., Rosselin, G., and Dolais, J.: Effets sur la glucagonemie des perfusions et ingestions d'acides aminés. Journ. Ann. Diabetol. Hotel Dieu 7:25-41, 1967.

[9]Müller, W. A., Faloona, G. R., and Unger, R. H.: Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance. Am. J. Med. *54*:52-57, 1973.

[10]Unger, R. H., Madison, L. L., and Müller, W. A.: Abnormal alpha-cell function in diabetics. Response to insulin. Diabetes *21*:301-07, 1972.

[11]Müller, W. A., Faloona G. R., Aguilar-Parada, E., and Unger, R. H.: Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. N. Engl. J. Med. *283*:109-15, 1970.

[12]Service, F. J., Molnar, G. D., Rosevear, J. W., Ackerman, E., Gatewood, L. C., and Taylor, W. F.: Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes *19*:644-55, 1970.

[13]Burgus, R., Ling, N., Butcher, M., and Guillemin, R.: Primary structure of somatostatin, a hypothalamic peptide that inhibits the secretion of pituitary growth hormone. Proc. Natl. Acad. Sci. *70*:684-88, 1973.

[14]Siler, T. M., VandenBerg, G., and Yen, S. S. C.: Inhibition of growth hormone release in humans by somatostatin. J. Clin. Endocrinol. Metab. *37*:632-34, 1973.

[15]Hall, R., Besser, G. M., Schally, A. V., Coy, D. H., Evered, D., Goldie, D. J., Kastin, A. J., McNeilly, A. S., Mortimer, C. H., Phenekos, C., Tunbridge, W. M. G., and Weightman, D.: Action of growth-hormone-release inhibiting hormone in healthy men and in acromegaly. Lancet *2*:581-84, 1973.

[16]Hansen, Aa. P., Orskov, H., Seyer-Hansen, K., and Lundbaek, K.: Some actions of growth hormone-release-inhibiting factor. Brit. Med. J. *3*:523-24, 1973.

[17]Alberti, K. G. M. M., Christensen, N. J., Christensen, S. E., Hansen, Aa. P., Iversen, J., Lundbaek, K., Seyer-Hansen,

K., and Orskov, H.: Inhibition of insulin secretion by somatostatin. Lancet *2*:1299-1301, 1973.

[18]Weig, G., Knowlton, S., and Martin, D.: Somatostatin inhibition of epinephrine-induced glucagon secretion. J. Clin. Endocrinol. Metab. (in press).

[19]Gerich, J., Lovinger, R., and Grodsky, G.: Inhibition of glucagon and insulin release from the in vitro perfused rat pancreas by somatostatin. Program of the 56th Annual Meeting of the Endocrine Society, 1974, p. A-190.

[20]Koerker, D., Ruch, W., Chideckel, E. et al.: Somatostatin: hypothalamic inhibitor of the endocrine pancreas. Science *184*:482-84, 1974.

[21]Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R.: Hypothalamic peptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science *179*:77-79, 1973.

[22]Gerich, J., Schneider, V., Dippe, S., Langlois, M., Noacco, C., Karam, J. H., and Forsham, P. H.: Characterization of the glucagon response to hypoglycemia in man. J. Clin. Endocrinol. Metab. *38*:77-82, 1974.

[23]Gerich, J., Lorenzi, M., Schneider, V., Guillemin, R., and Forsham, P. H.: Suppression of plasma glucose and glucagon levels in normal man and in insulin-dependent diabetics. (In preparation).

[24]Eddy, R. L., Gilliland, P. F., Ibarra, J. D., Jr., McMurry, J. F., Jr., and Thompson, J. Q.: Human growth hormone release. Comparison of provocative test procedures. Am. J. Med. *56*:179-85, 1974.