Evidence for the Hormonal Status of Somatostatin in Man

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SUMMARY

To determine the hormonal status of somatostatin in man, plasma levels of ~1600-dalton somatostatin-like immunoreactivity (SLI) were measured before and after a mixed meal. Plasma was subjected to gel filtration and the fractions coeluting with somatostatin were concentrated ninefold before radioimmunoassay. In this system the recovery of synthetic somatostatin added to plasma averaged 71 ± 4.6% (mean ± SE, N = 5). Synthetic somatostatin infused into normal volunteers gave a dose-related increase in the measured SLI value. Fasting SLI in 13 normal volunteers was 8.0 ± 0.7 pg/ml (mean ± SE) and rose to 18.6 ± 1.5 pg/ml and 20.3 ± 2.0 pg/ml at 60 and 120 min, respectively, after the meal ($P < 0.001$). In seven type I diabetics, the fasting level was 11.5 ± 1.6 pg/ml and the 60- and 120-min postprandial levels were 21.4 ± 2.5 and 22.3 ± 2.9 pg/ml, respectively. The meal-induced rise in ~1600 M.W. SLI approximated that produced by infusing somatostatin at 2 μg/h, a rate that significantly suppressed glucagon levels. These results are therefore consistent with a hormonal role for somatostatin in man. DIABETES *30*:883–886, October 1981.

Somatostatin is widely distributed throughout the central nervous system where it is generally believed to act as a neurotransmitter and to have a neurohormonal role in the regulation of growth hormone release. However, the physiologic role of the somatostatin secreted by the D-cells located in the pancreatic islets and in the gastrointestinal tract is far more controversial. The proximity of these D-cells to target cells of somatostatin favors a local or paracrine function[1] and has fostered doubts concerning its hormonal status. Yet, studies in dogs have demonstrated that ~1600 M.W. somatostatin-like immunoreactivity (SLI) is released into the circulation from the stomach and pancreas after the ingestion of a meal,[2–4] raising the possibility that it may play a hormonal role in the homeostasis of ingested nutrients.[5,6] The well-established inhibitory actions of somatostatin upon digestive events,[7–14] coupled with direct studies of the effects of experimentally induced hypersomatostatinemia and hyposomatostatinemia on the rate at which ingested nutrients enter the circulation,[9,10] tend to support the hypothesis[15] that the role of splanchnic somatostatin may be to restrain the rate at which ingested nutrients cross from the gut into the circulation.

In man the hormonal status of somatostatin has been more difficult to prove, largely because of technical problems involving its measurement in human plasma. Kronheim et al.[16] and Mackes et al.[17] have identified ~1600 M.W. SLI in fasting human plasma, but they did not demonstrate that this presumably biologically active fraction increases in response to food ingestion. On the other hand, both Wass et al.[18] and Vinik et al.[19] have reported increments in total SLI in response to a meal, but in neither of these studies was the meal-induced increase in SLI shown to be the consequence of a rise in the ~1600-dalton component, rather than of the high molecular weight component. The latter constitutes a major portion of SLI and is unlikely to have biologic activity. The present study was designed to determine in man if the ~1600 M.W. SLI rises in response to a mixed meal.

METHODS

Studies were performed in 13 normal, nonobese, healthy volunteers, six females and seven males, ranging from 21 to 51 yr, and 7 insulin-dependent diabetics, all male, ranging from 24 to 60 yr. The study was approved by the Human Research Committee of the University of Texas Health Science Center at Dallas and all subjects gave informed consent before participating. Insulin was administered as usual to the diabetic group on the day before the study, but the morning dose was omitted on the day of the study. All subjects were

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fasted overnight. A standard mixed meal containing 45 g protein, 94 g carbohydrate, and 45 g fat (a total of 1000 calories) was ingested and venous blood samples were obtained before and at 60 and 120 min thereafter. Blood samples were immediately transferred from the syringe into chilled tubes containing 6 mg disodium EDTA and 500 KIU aprotinin (Trasylol) per ml and placed in an ice bath. After 10–20 min, they were centrifuged at 4°C, and the plasma stored at −20°C. During the subsequent procedures plasma temperature was maintained to 4°C after thawing.

In two normal volunteers who had given informed consent, synthetic somatostatin (kindly provided by Clin-Midy, Montpellier, France, and tested for use in human subjects at the Salk Institute, La Jolla, California) was mixed in normal saline containing 0.5% HSA and infused at progressively increasing rates of 2, 6, and 10 μg/h for 30 min per dose. Plasma samples were collected at 15-min intervals, beginning with the initial saline infusion. In another 11 normal volunteers, synthetic somatostatin was infused at increasing rates of 2, 6, 10, 15, 30, and 60 μg/h for 30 min per dose. The response in basal levels of insulin and glucagon was measured every 15 min, also beginning with the initial saline infusion.

Aliquots (9 ml) of plasma were chromatographed on Biogel P-4 columns (100–200 mesh; 1.6 × 27 cm) equilibrated in 0.05 M NH_4/HCO_3 pH 7.7 containing 0.05% BSA and NaN_3. Fraction volumes were 2.5 ml. Fractions coeluting with somatostatin were pooled and lyophilized. To eliminate the NH_4HCO_3, they were reconstituted with H_2O and lyophilized four times more. The lyophilized material was then reconstituted with 1.0 ml of H_2O for radioimmunoassay, thus concentrating it ninefold. The somatostatin peak overlapped with a portion of the salt peak, but it was determined that the salt peak did not affect the radioimmunoassay.

SLI was determined by a previously described radioimmunoassay[20] employing antibody 80C, which is directed against the central part of somatostatin in a dilution of 1:80,000. The assay diluent was 0.2 M glycine buffer with 0.25% HSA and 1% normal sheep serum at pH 8.8. Tyr^{11}-somatostatin (prepared by Dr. Jean Rivier, Salk Institute, La Jolla, California) was labeled with ^{125}I by a modification of the Greenwood-Hunter method.[21]

Assay tubes, prepared by mixing 0.4 ml assay diluent, 0.1 ml diluted antiserum, 0.1 ml ^{125}I tyr^{11}-somatostatin (3000 cpm), and 0.1 ml of either a reference standard or chromatographic eluant, were incubated at 4°C for 72 h. Free and antibody-bound label were separated with 0.5 ml of a suspension of 0.1% charcoal (Norit A) coated with 0.25% dextran T-70 in buffer, and 0.1 ml of normal sheep serum was added to the tube.

The lower limit of sensitivity of the assay was 45 pg/ml. The within- and between-assay coefficient of variation were 10.8% and 13.9%, respectively. Recovery of 30 pg/ml of synthetic somatostatin added to 3% Norit A charcoal-treated plasma was 71% ± 4.6% (mean ± SE) in five experiments. Measured values were not corrected for recovery, but are given corrected for ninefold concentration.

Insulin and glucagon were assayed by previously described methods.[22,23] Statistical analysis was performed by the two-way analysis of variance and the Newman-Keuls multiple range test.

TABLE 1
~1600 M.W. SLI (pg/ml) in plasma of two subjects during the infusion of increasing doses of synthetic somatostatin

Infusion rate (μg/h)	0	2	6	10	0
Subject 1	17	46	60	71	23
Subject 2	16	31	72	> 10*	12

* Exceeds upper limit of assay.

RESULTS

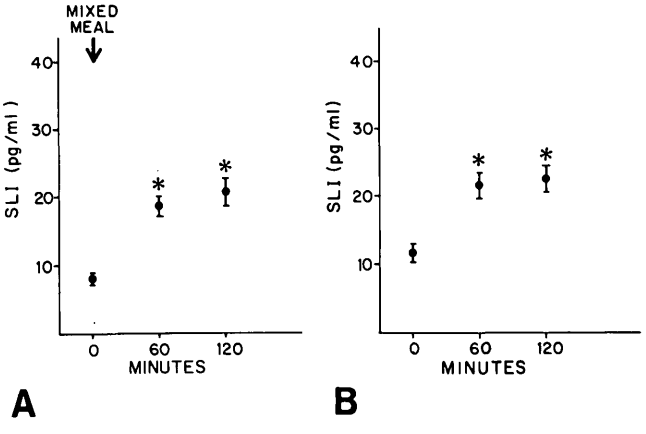
Recovery of infused synthetic somatostatin. As shown in Table 1, the infusion of synthetic somatostatin in two normal subjects at progressively increasing rates from 2 to 10 μg/h was accompanied by a progressive increase in SLI in plasma specimens obtained at the end of each 30-min infusion period. SLI returned to the baseline value within 30 min of cessation of the infusion.

Fasting and postprandial levels of normal and type I diabetic subjects. As indicated in Figure 1A, the mean SLI concentration of the 1600-dalton fraction of 13 fasting plasma specimens obtained from normal volunteers was 8.0 ± 0.7 pg/ml of unconcentrated eluate (mean ± SE). After the ingestion of a mixed meal, it increased to 18.6 ± 1.5 and 20.3 ± 2.0 pg/ml of unconcentrated eluate at 60 and 120 min, respectively ($P < 0.001$).

In type I diabetics, the fasting, 60-min, and 120-min postprandial SLI levels were, respectively, 11.5 ± 1.6, 21.4 ± 2.5, and 22.3 ± 2.9 pg/ml of unconcentrated eluate, respectively (Figure 1A). Both postprandial values were significantly greater than the fasting value ($P < 0.001$). The fasting value in diabetics was significantly greater than that of nondiabetics ($P < 0.05$). However, since the interassay coefficient of variation is 14%, the biologic significance of this small difference is questionable.

Evidence that the postprandial SLI rise may have biologic activity. The postprandial increment in ~1600 M.W. SLI approximated the increment observed at the end of a 30-min infusion of 2 μg/h of synthetic somatostatin. Figure 2 shows that somatostatin infused at a rate of 2 μg/h has biologic activity, that suppresses glucagon and, at one point only, the insulin levels.

FIGURE 1. (A) The mean (± SEM) levels of ~1600 M.W. somatostatin-like immunoreactivity in eluates of plasma obtained from 13 normal volunteers in the fasting state, at 1 and 2 h after a mixed meal. (B) The same in seven type I diabetic volunteers.



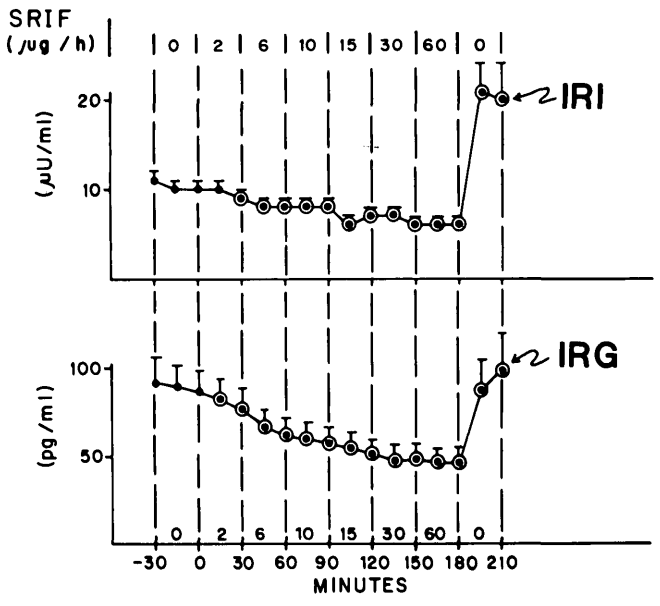


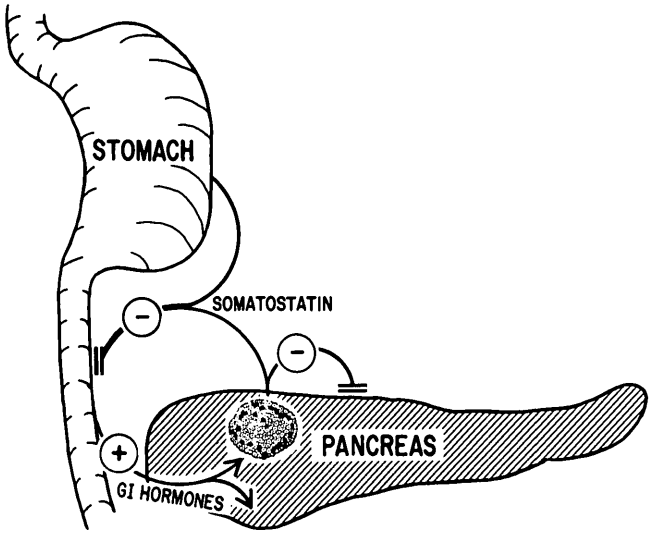
FIGURE 2. The effect of various somatostatin infusion rates upon insulin and glucagon levels in a group of 11 healthy volunteer subjects (mean ± SEM). Circled points represent a statistically significant difference from the baseline value ($P < 0.05$).

DISCUSSION

The finding of ~1600-dalton SLI in plasma obtained from fasted nondiabetic and diabetic subjects confirms earlier demonstrations of its presence under steady-state conditions.[16,17] However, this is the first demonstration in man that 1600-dalton SLI rises after a mixed meal. Such a rise has been observed in dogs[2-4] and led to the suggestion that somatostatin is a true hormone that may play a role in the digestion and absorption of food.[5,6] Passive immunization against somatostatin was shown to accelerate the rate at which triglycerides pass from the gut into the circulation,[6] while infusion of somatostatin at a rate that simulates the postprandial rise in endogenous SLI greatly retards the entry of ingested triglycerides.[5] Inasmuch as gut hormones stimulate the secretion of somatostatin by the D-cells of the islets and the stomach,[24-26] and are, in turn, inhibited by somatostatin,[7-9,12,13] the concept of a positive-negative gut hormone-somatostatin feedback (Figure 3) which regulates the rates of digestive events, and hence the rates of nutrient entry from the gut, was proposed.[15]

In the present studies, endogenous SLI increased by an average of 12 pg/ml after ingestion of a meal. This rise is not far below the increments produced by the infusion of 2 μg/h of synthetic somatostatin. Also, the infusion of 2 μg/h caused small but statistically significant lowering in glucagon and, less strikingly, insulin levels. Moreover, Johansson et al.[27] have reported that 3 μg/h of somatostatin inhibits pancreatic, exocrine, and gall bladder function. This observation is of particular relevance to the putative role of splanchnic somatostatin in regulating the influx of ingested food. Thus, the postprandial increment in ~1600 SLI, which was undoubtedly underestimated in this study, may be capable of having biologic activity in humans. These results provide the first evidence consistent with a hormonal role for somatostatin in man.

FIGURE 3. Schematization of a postulated physiologic role for splanchnic somatostatin. Somatostatin from the stomach and islets is released during a meal, and is in a large part stimulated by gut hormones. The somatostatin secreted by the splanchnic D-cells would, in turn, regulate the rate of nutrient entry from the gastrointestinal tract by restraining the secretion of gastrointestinal hormones and of the digestive events they stimulate.



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