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Somatostatin: Hypothalamic Inhibitor of the Endocrine Pancreas

Abstract. *Somatostatin, a hypothalamic peptide that inhibits the secretion of pituitary growth hormone, inhibits basal insulin secretion in fasted cats and rats. In fasted baboons both basal and arginine-stimulated secretion of insulin and glucagon are inhibited. Somatostatin appears to act directly on the endocrine pancreas. The action is dose-related, rapid in onset, and readily reversed.*

Somatotropin release inhibiting factor (somatostatin, SRIF) has been shown to inhibit both basal (*1*) and stimulated growth hormone release (*1–4*) in a variety of in vivo and in vitro systems, with the use of rats, dogs, and humans. In addition, the increased thyrotropin (TSH) secretion caused by thyrotropin releasing hormone (TRH) is inhibited by SRIF, while the prolactin response to TRH was unaffected (*5–7*). The SRIF does not affect luteinizing hormone releasing hormone (LH-RH) stimulation of LH or follicle stimulating hormone (FSH) (*2, 4, 7*), nor does it alter the adrenocorticotropic hormone (ACTH)–corticosteroid response to hypoglycemia (*4*) in these studies. Basal secretion of pituitary hormones other than growth hormone has been unaffected.

While extending these studies in conscious, overnight-fasted, chair-restrained male baboons with permanently installed venous catheters, we noted that plasma glucose consistently fell during the intravenous administration of synthetic SRIF (the linear peptide form) and promptly returned to control levels upon termination of the infusion.

In order to determine whether this fall in glucose was attributable to a decrease in glucose production or an increase in glucose utilization, we performed an isotope dilution study (*8*) with uniformly labeled [^{14}C]glucose. In three studies the average steady-state glucose production rate was calculated to be 3.5 mg/kg · min (range 2.3 to 4.7). During a 30-minute SRIF infusion the specific activity of plasma glucose increased by an average of 22 percent (range, 18 to 26 percent), indicating a decrease in hepatic glucose production. If we assume that glucose utilization remains constant at the rate observed during the control period and calculate the magnitude by which the glucose concentration would fall in 30 minutes if production were completely inhibited, the value obtained is close to that observed experimentally. This implies that the observed hypoglycemia was largely accounted for by the inhibition of hepatic glucose production and that any change in glucose utilization must be small.

At this stage of fasting, factors which could rapidly reduce hepatic glucose production include (i) an increase in insulin secretion (*9*), (ii) an increase in parasympathetic activation of hepatic glycogen synthetase (*10*), (iii) a decrease in pancreatic glucagon secretion, or (iv) a decrease in adrenergic stimulation of hepatic phosphorylase (*11*). Alternatively, SRIF may act directly on the liver.

Preliminary attempts to look for direct effects of SRIF on hepatic glucose production in vitro were inconclusive. We therefore measured the plasma concentration of three of the above potential hormonal mediators during hypoglycemia caused by SRIF.

Concentrations of norepinephrine in the plasma (*12*) did not fall during SRIF-induced hypoglycemia in two animals. In contrast, plasma immunoreactive glucagon (*13, 14*) and insulin (*15*) changed during SRIF administration. Figure 1 shows the results obtained in four animals that had been given an intravenous loading dose of SRIF (25.0 μg/kg) which was followed by a 30-minute infusion of 0.83 μg/kg·min, a maximally effective hypoglycemic dose. Within 10 minutes venous plasma concentrations of both insulin and glucagon fell to less than 15 percent of pre-infusion values, while glucose had only fallen to 90 percent of pre-infusion values. Subsequently, insulin and glucagon could no longer be detected in three of the four animals studied, whereas glucose fell to 70 percent of the pre-infusion value at the end of the 30-minute SRIF infusion. When the somatostatin infusion was stopped, glucagon rebounded promptly to concentrations above those prior to infusion. This may be related to the lower glucose concentration acting as a stimulus to glucagon release once SRIF has been stopped. Glucose returned to normal within 30 minutes, while insulin recovered much more slowly—again perhaps reflecting the negative stimulus of hypoglycemia. With SRIF infusions of longer duration (up to 2 hours), insulin and glucagon remained very low until the end of the infusion. When added in vitro to standard hormone solutions, SRIF had no effect on the immunoassay systems.

The fall in basal insulin secretion could be either secondary to the fall in glucose concentration or could be due to a direct effect of somatostatin on the β cells of the pancreatic islets. The latter alternative is supported by the following observations: (i) At 3 and 6 minutes after SRIF was administered to baboons, insulin had fallen by 39 and 66 percent, respectively, whereas glucose remained unchanged. (ii) Soma-

tostatin when infused directly into dog pancreas promptly and completely inhibited insulin secretion. (iii) In rats and cats insulin secretion is inhibited, but glucose remains unchanged. Yen *et al.* (*5*) have also shown that in humans insulin falls before glucose.

The pituitary-adrenal axis is apparently not affected by the administration of SRIF at this dose in that cortisol rises as would be expected in the presence of a falling glucose. Plasma free fatty acids (FFA) did not change during infusion of SRIF. The lowering of basal insulin might have been expected to result in a dramatic rise in FFA (*16*). This apparent independence of lipolysis and basal insulin may reflect the operation of other control systems during early fasting (*17*).

The changes in glucose and insulin were dose-related when the ratio of the loading dose (μg/kg) to the infusion dose (μg/kg · min) was from 4.54/0.15 to 90.8/3.0. Inhibition of glucagon secretion was essentially complete at all doses studied.

The observed failure of glucose production and subsequent hypoglycemia cannot be accounted for either by a fall in sympathetic activity, at least as reflected in the unchanging plasma norepinephrine, or by an increase in plasma insulin. The fall in plasma glucagon is an obvious possible explanation for the observed phenomenon. The fact that hypoglycemia occurred in spite of a marked reduction in insulin indicates that whatever the mechanism may be, it is not counteracted by the simultaneous lowering of basal insulin. These data suggest the working hypothesis that glucagon is a major determinant of glucose homeostasis in the fasted baboon.

Since basal secretion and stimulated secretion of hormones are often regulated by different mechanisms we attempted to ascertain whether stimulated insulin and glucagon secretion could also be blocked by somatostatin. Arginine was selected as a stimulus because it affects both insulin and glucagon. As is seen in Fig. 2, SRIF reduced arginine-stimulated insulin and glucagon release (four of five animals showed complete inhibition of the response to arginine while the fifth showed a much reduced response).

In these and additional studies with SRIF in baboons, both basal and stimulated growth hormone secretion is reduced (*18*), as has been reported in other species. As with glucagon, a rebound of growth hormone is generally

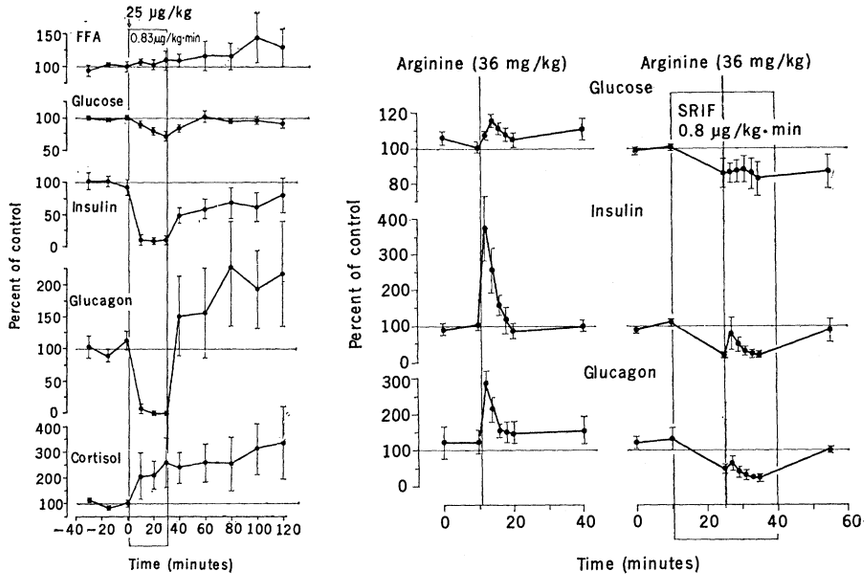

Fig. 1 (left). The effect of somatostatin (SRIF) on fuel and hormonal parameters in baboons fasted overnight. SRIF (0.83 μg kg^{-1} min^{-1}) was infused intravenously (four animals) for 30 minutes after a loading dose (25 μg/kg) was administered. The control values for free fatty acids (FFA) were 1.032 ± 0.096 μeq/ml; for glucose, 91 ± 2 mg/100 ml; for insulin, 64 ± 10 μunit/ml; for glucagon, 156 + 15 pg/ml; for cortisol, 19 ± 3 μg/100 ml. The values represent in each case the mean ± S.E.M. Fig. 2 (right). The effect of somatostatin on arginine-stimulated insulin and glucagon release. All data are presented as mean ± S.E.M. The arginine pulse alone and the arginine pulse plus SRIF were administered to each animal on the same day. A recovery time of 1 hour was allowed between the arginine stimuli. The order of presentation had no effect. Repeated arginine doses produced identical insulin and glucagon responses. The number of animals on arginine alone was six; the number on arginine and SRIF was five, because of infusion pump failure. At the time of arginine infusion the following conditions existed:

	Glucose (mg/100 ml)	Insulin (μunit/ml)	Glucagon (pg/ml)
Arginine	73 ± 9	72 ± 23	299 ± 80
Arginine + SRIF	65 ± 10	9 ± 3	134 ± 44

seen upon completion of a SRIF infusion and may be related to the presence of hypoglycemia. TRH-stimulated TSH release is also inhibited, whereas the prolactin response is unaffected (*18*).

The physiological significance of these observations is unclear. Although a hypothalamic influence over pancreatic hormone secretion has been reported, it has usually been attributed to efferent innervation of the islets (*19*). However, a recent report (*20*) suggests that humoral substances derived from the hypothalamus also have this potential. Our studies have demonstrated that somatostatin is a potential glucoregulatory hormone derived from the hypothalamus, although it has not yet been found in the peripheral circulation.

Whatever its physiological role, somatostatin should be useful for investigating interactions between various glucoregulatory systems. Among known inhibitors of insulin or glucagon secretion SRIF appears to be unique because it inhibits both hormones, whereas others such as glucose or catecholamines inhibit selectively. Since the effects are apparently nontoxic (we observed no cardiovascular or behavioral changes), abrupt in onset and rapidly reversed, the actions of SRIF simulate those of an acute reversible endocrine pancreatectomy.

Finally, the apparent ability of somatostatin to inhibit release of several peptide hormones in two different endocrine structures raises the possibility that this molecule inhibits some common step in endocrine secretory systems.

Note added in proof: Since this report was submitted for publication, a similar inhibition of insulin secretion by somatostatin has been reported (*21*).

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Vagotomy: Effect on Electrically Elicited Eating and Self-Stimulation in the Lateral Hypothalamus

Abstract. *A subdiaphragmatic vagotomy markedly inhibits eating and self-stimulation produced in rats by lateral hypothalamic stimulation. The stomach is known to be affected by hypothalamic stimulation via the vagus, and afferents from the stomach can influence the hypothalamus via the same nerve. Consequently, this result suggests that eating and self-stimulation may be partly controlled by hypothalamic influences on the stomach which, in turn, affects hypothalamic sensitivity.*

Rats with electrodes implanted in the lateral hypothalamus exhibit self-stimulation behavior and will also eat during continuous stimulation (*1*). On the other hand, studies show that stimulation in, or close to, the lateral hypothalamus affects the stomach, increasing gastric motility and acidity, while prolonged stimulation may produce hemorrhaging and ulceration (*2*). It is tempting to look for a connection between these two sets of data since the stomach has been implicated in theories of eating for many years.

Since lateral hypothalamic stimulation produces stomach conditions which in some respects mimic those found in the hungry animal, electrically elicited eating may be partly a function of these hypothalamic influences on the stomach. If this is so, severing the neural connection between the lateral hypothalamus and the stomach should attenuate this behavior. The major connection between the central nervous system and the gastrointestinal tract is the vagus nerve. It is already known that severing this nerve prevents lateral hypothalamic stimulation from influencing the stomach (*3*). In this study I severed this nerve to see if it prevented lateral hypothalamic stimulation from eliciting eating as well as the closely related phenomenon, self-stimulation.

Eight male Sprague-Dawley rats from the Charles River Breeding Laboratories were implanted with electrodes in the lateral hypothalamus and the lateral septal area (*4*). These septal electrodes were to serve as a check on the condition of the animals after the vagotomy operation. As septal stimulation does not produce eating, I intended to use any decrement in performance on this electrode as an indicator of debilitation due to the vagotomy.

After the animals had recovered from the implant operation, I determined their thresholds for eating and self-stimulation. Using a constant current stimulator which delivered a 1-msec negatively going pulse every 10 msec, I determined the amount of current needed on the lateral hypothalamic electrode to produce consistent eating of wet mash in a 10-second stimulation period, presented every 30 seconds. Later in the day, I measured the threshold current necessary to sustain self-stimulation behavior for a period of 2 minutes on the same electrode. The animals were further tested for septal self-stimulation thresholds. In all these determinations I used the psychophysical method of minimal changes. Each animal was tested for 20 minutes a day on each test for at least 2 weeks. Because some of the electrodes failed to elicit consistent behavior, and because of deaths following the vagotomy operation, only five animals completed the study.

Once highly repeatable performances had been obtained on all the appropriate measures, the vagal nerves were cut below the diaphragm in each of the rats. A section of nerve, at least 5 mm long, was removed from the side of the esophagus immediately above the stomach. The esophagus was carefully cleared of all visible fibers, and the animal was allowed to recover for several days before retesting. As animals tend to overeat immediately after vagotomy, they were deprived of food for 24 hours. After this time, they became somewhat anorexic for the next few days, confirming earlier observations (*5*). They seemed to have difficulty swallowing, which may have been due to possible damage to the esophageal musculature during the operation. Some failed to recover from the anorexia and died. However, the majority eventually started eating and gaining weight normally.

Eight days after the vagotomy the threshold currents for eliciting eating and self-stimulation were rechecked. Each animal was retested for 50 days after the operation. Figure 1 shows the results of the experiment. Median threshold scores are plotted for the five animals, beginning just before and continuing for 50 days after vagotomy. The vagotomy had its largest effect on the threshold for feeding, raising it by 150 percent. It had a similar, but slightly smaller, effect on the lateral hypothalamic self-stimulation threshold,