gion of the gel where the interferon activity was expected. Each slice was crushed into small pieces with a plastic rod and extracted into 0.3 ml of extraction buffer (137 m*M* NaCl, 2.7 m*M* KCl, 8.1 m*M* $Na_2HPO_4$, 1.5 m*M* $KH_2PO_4$, 0.1 percent (weight to volume) SDS, 10 percent (by volume) glycerol, and 10 $\mu M$ phenylmethylsulphonyl fluoride) at room temperature overnight. The eluates were clarified by centrifugation and assayed for interferon activity by the cytopathic effect assay (*3*). The interferon-containing regions were cut from the remaining tracks and eluted essentially under the conditions described by E. Lazarides [*J. Supramol. Struct.* **5**, 531 (1976)], except that the gel pieces were immersed in running buffer and covered with a small piece of tissue paper (Kimwipe, Kimberly-Clark) rather than embedded in polyacrylamide. Following electrophoretic elution, each sample was dialyzed extensively, first against 150 m*M* NaCl, 0.1 percent (weight to volume) SDS, then by repeated dialysis against 0.02 percent (weight to volume) SDS, and finally lyophilized; about 40 to 50 percent of the protein was recovered. The approximate amounts of protein used for sequencing were: 400 pmole of interferon A, 40 pmole of interferon $b_1$, 120 pmole of interferon $b_2$, and 130 pmole of interferon C (Fig. 1).


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## Splanchnic Somatostatin: A Hormonal Regulator of Nutrient Homeostasis


Abstract. *Free (~ 1600 daltons) somatostatin-like immunoreactivity was identified in arterial plasma of dogs that had received a test meal. Neutralization of circulating somatostatin while the dogs were consuming a fatty meal increased the plasma concentrations of triglycerides, gastrin, pancreatic polypeptide, and insulin after the meal. It is concluded that, in the dog, somatostatin is a true hormone that regulates the movement of nutrients from the gut to the internal environment.*


The physiologic role of the somatostatin-containing D cells of the islets of Langerhans, stomach, and gut (*1*) is unknown. Juxtaposition of D cells to certain of the known target cells of somatostatin is consistent with a local or "paracrine" role for the tetradecapeptide (*2*), but it is not known if somatostatin has hormonal functions, that is, if it circulates in plasma in a biologically active form. In this report we present evidence that somatostatin is a true hormone (*3*) which, like two other islet cell hormones, insulin and glucagon, plays a role in the homeostasis of ingested nutrients.

Previously we reported that somatostatin-like immunoreactivity (SLI) of pancreatic and gastrointestinal origin increases in the venous plasma of dogs after they have consumed various meals (*4*); this would be expected for a hormone with the postulated homeostatic role. But gel filtration at *p*H 7.4 to 8.8 of venous plasma from fasted dogs and dogs that had just eaten a meal revealed no measurable SLI in the range (~ 1600 daltons) of somatostatin; rather, all SLI eluted in the ~ 150,000-dalton range (*5*). Although this ~ 150,000-dalton SLI represents 1600-dalton SLI bound to a component of large molecular weight from which it can be dissociated at acidic *p*H (*5*), its biologic activity under physiologic conditions is uncertain. We therefore examined the arterial plasma of dogs to determine if any free, potentially biologically active SLI could be detected at *p*H 7.4 after they had eaten a meal. We applied 3-ml samples of pre- and postprandial arterial plasma obtained from five normal dogs to a jacketed Biogel P-6 column (1.6 by 35 cm) equilibrated at 37°C with a buffer (*p*H 7.4) containing 0.9 percent NaCl, 0.1*M* EDTA (ethylenediaminetetraacetic acid), 0.5 percent bovine serum albumin, 0.02 $NaN_3$, and 100 kallikrein inactivator units of aprotinin per milliliter. We measured the SLI in 2.7-ml fractions by a modification (*6*) of previously described methods (*7*) using an antibody (80C) directed against the central portion of the somatostatin. In arterial plasma from fasting dogs we detected no SLI in fractions coeluting with the somatostatin marker. However, in arterial plasma obtained from five dogs that had received an intragastric protein-acid load (250 ml of liver, followed by 200 ml of 1*N* HCl), which stimulated plasma SLI to 606 ± 112 pg/ml (mean ± S.E.M.; $N = 5$), we recovered 352 ± 58 pg of SLI per milliliter in the 1600-dalton zone. To our knowledge this is the first demonstration at physiologic *p*H of free endogenous SLI in post-hepatic plasma. We observed higher concentrations of free endogenous SLI in the portal vein plasma (*8*).

If, as we have proposed (*9*), a meal-stimulated increase in plasma somatostatin restrains the rate at which ingested nutrients enter the circulation, a somatostatin-deficient state should be accompanied by increased concentrations of nutrients in the circulation after meals. Therefore, we induced by passive immunization a somatostatin deficiency in a group of eight fasting dogs weighing from 10 to 12.5 kg. Fifteen minutes before a meal of 600 g of meat (60 g of fat and 60 g of protein) and again 60 and 120 minutes after the meal, we injected via a leg vein 20 ml of either nonimmune sheep serum as a control, or, 2 days later, 20 ml of sheep antiserum to somatostatin (*10*). We measured the concentrations of triglycerides in venous plasma by the meth-

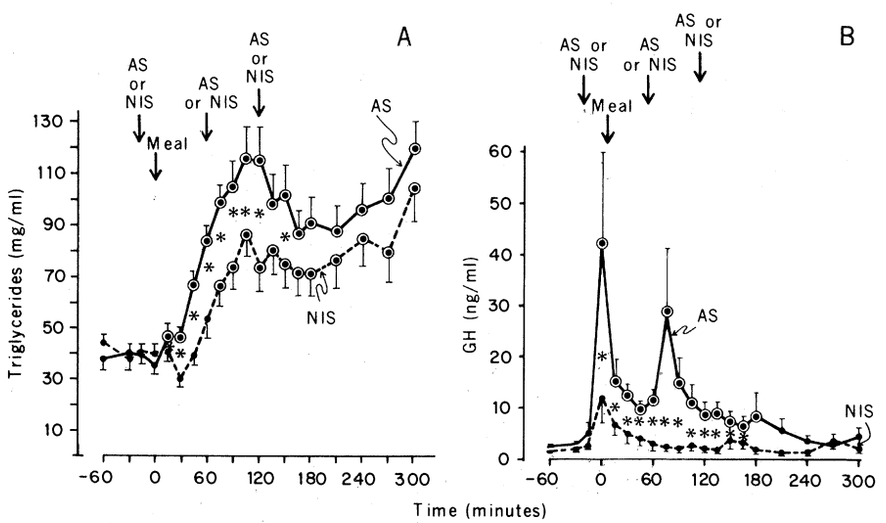


Fig. 1. (A) Comparison of the effect on plasma triglyceride levels of 20 ml of antiserum to somatostatin (*AS*) or of nonimmune sheep serum (*NIS*) injected 30 minutes before and 60 and 120 minutes after the ingestion of a fat-protein meal. Statistically significant ($P < .01$) differences are indicated by asterisks. (B) Measurements of canine growth hormone made during the foregoing experiments as evidence of neutralization of a biological action of circulating somatostatin.



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od of Eggstein and Kreutz (*11*) and, to prove that we had induced hyposomatostatinemia, we measured canine growth hormone (cGH) as previously described (*12*). For statistical analyses we used the Student *t*-test for paired data.

The triglyceride concentrations after the antiserum injections were significantly higher than control values at all but one point during the first 150 minutes ($P < .01$ to .001) (Fig. 1A). The cGH levels were also significantly greater after antiserum treatment ($P < .05$) (Fig. 1B), which is strong evidence of a hyposomatostatinemic state.

The higher postprandial triglyceride levels observed during neutralization of somatostatin could have been the consequence of either more rapid entry or of slower removal of triglycerides. Somatostatin has no known effect on chylomicron clearance, but it profoundly inhibits many gastroentero-pancreatic functions (*13*) that determine the rate at which ingested nutrients reach the circulation. Therefore, accelerated triglyceride entry seemed the more probable explanation for our findings. To establish that neutralization of somatostatin can, in fact, enhance a somatostatin-responsive function of the stomach, gut, or pancreas, we measured by previously described methods (*14*) the concentrations of a gastric hormone, gastrin, two intestinal hormones, gastric inhibitory peptide (GIP) and motilin, and three pancreatic hormones, insulin, glucagon, and pancreatic polypeptide (PP). All of these polypeptides are released after a meal and are suppressed by somatostatin (*13*). The postprandial increase in gastrin, insulin (not explained by differences in plasma glucose levels), and PP was greater during passive immunization than in the control state at most points after the first injection ($P < .05$ to .005) (Fig. 2). Glucagon, which in the dog is derived both from the islets and the gastric fundus, was not changed by passive immunization. We noted no difference in GIP or motilin levels; however, having previously observed a marked increase in enteroglucagon after a larger dose of the same antiserum (*12*), we cannot exclude an effect of somatostatin neutralization on the gut. The greater insulin and PP response could reflect a loss of somatostatin-mediated inhibition of the islets or of the islet-stimulating hormones of gut or pituitary, or both.

Although the foregoing effects of anti-somatostatin serum suggest an important biological action of endogenous somatostatin on gastrointestinal and perhaps pancreatic tissues, they do not distinguish between effects of the somatostatin released in the circulation and local actions within the tissues of origin. To determine if injected antibody can rapidly reach the interstitial spaces to activate local somatostatin of the stomach, pancreas, or intestine, we compared the tissue concentration of intraarterially injected $^{125}$I-labeled γ-globulin (125 ng) with that of $^{125}$I (as NaI), which becomes distributed throughout the extracellular space. One hour later, the pancreas and antral mucosa were excised and rinsed, and the radioactivity was counted. An hour after $^{125}$I injection (3,000,000 cpm/ml) the blood contained 2084 cpm/ml, and the pancreas and antral mucosa contained 180 and 1074 cpm/ml, respectively. However, 1 hour after the injection of labeled globulin (3,000,000 cpm/ml), at which time blood contained from 1837 to 2216 cpm/ml, we detected no radioactivity in either the pancreas or the antral mucosa of three dogs, indicating that γ-globulin does not become distributed as readily as $^{125}$I through the extracellular space of the pancreas and antrum. If one assumes that the distribution of the labeled γ-globulin reflects the distribution of somatostatin antibodies and that the islets are not more permeable to antibodies than the exocrine pancreas, it is unlikely that the antiserum used in this study neutralized "paracrine" actions of somatostatin.

The study reveals, first, that free SLI is present in the arterial plasma of dogs that have received a protein meal; and, second, that neutralization of circulating somatostatin is accompanied by higher postprandial levels of plasma triglycerides and enhanced functional activity of endocrine cells in the stomach and pancreas, the organs that determine the rate of nutrient entry. These results, together with the previous demonstration (*15*) that the infusion of synthetic somatostatin at a rate approximating the postprandial increase in endogenous plasma SLI markedly lowers postprandial triglyceride levels, provide evidence that circulating somatostatin influences the rate at which nutrients such as triglycerides move from the external to the internal environment. Through coordination with neighboring insulin-secreting cells, the somatostatin-secreting cells of the islets may contribute to the constancy of nutrient concentrations during meals by equilibrating the rates of nutrient influx and nutrient disposal. The D

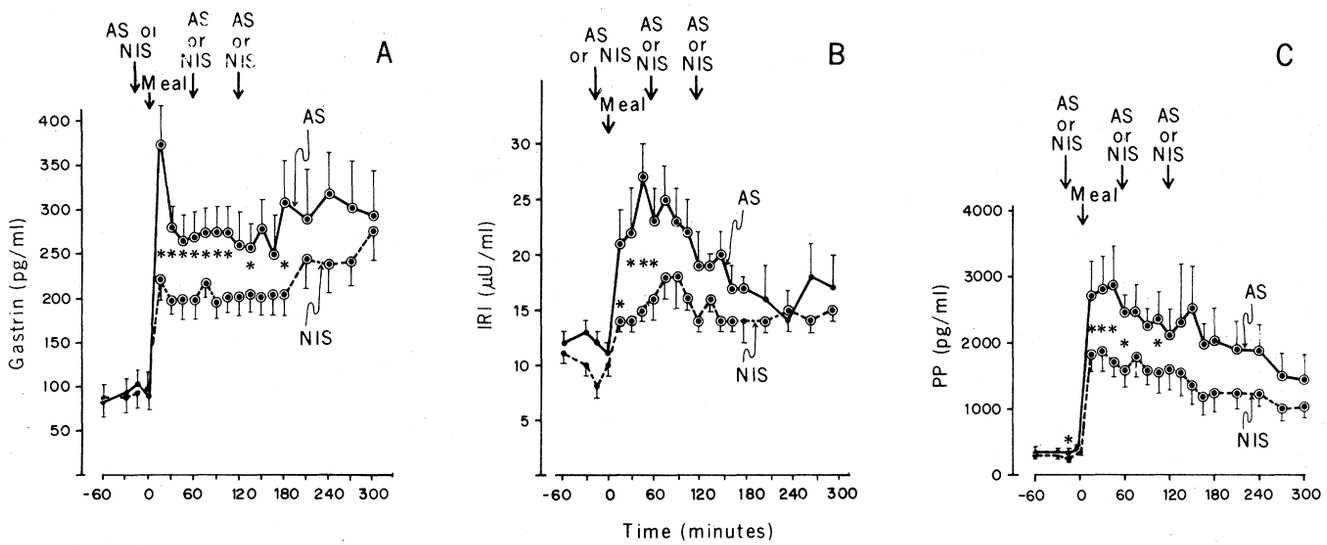


Fig. 2. Comparison of the effects of antiserum to somatostatin (*AS*) or nonimmune serum (*NIS*) on the plasma concentrations of (A) gastrin, (B) insulin (*IRI*), and (C) pancreatic polypeptide (*PP*) before and after a fat-protein meal. Statistically significant ($P < .05$) differences between time points are marked by asterisks.

cells in the gastrointestinal tract may also have important local or hormonal influence on nutrient entry. The site or sites at which somatostatin exerts its influence on the entry of ingested nutrients have not been identified, but its many inhibitory actions on rate-limiting gastric, intestinal, and pancreatic functions (*13*), suggest that any one or more of these loci may be involved.

In view of these results it seems reasonable to regard splanchnic somatostatin as a true hormone with a regulatory role in the homeostasis of ingested nutrients.

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## Laminar Organization of Thalamic Projections to the Rat Neocortex

Abstract. *Nerve fibers transmitting information from the thalamus to the cerebral cortex may be classified according to their major cortical layers of termination. (i) One class consists of inputs from thalamic relay nuclei for vision, audition, and somesthesis to layer IV, layer III, or both. In contrast, autoradiographic studies of projections from other thalamic nuclei reveal strikingly different patterns of termination: (ii) layer VI (or layer V, or both) is the target of fibers from the intralaminar nuclei, and (iii) layer I is the target for fibers from the ventromedial and magnocellular medial geniculate nuclei. (iv) The remaining class is typified by termination both in layer I and in additional layers that depend on the cortical area in which the terminations are found. The data demonstrate that convergent thalamic inputs to a given cortical area are usually not confluent within a layer and provide a new framework for categorizing thalamic nuclei.*

Lorente de Nó provided the classic description of thalamic afferent fibers in the rodent neocortex (*1*). His Golgi material displayed two fundamentally distinct laminar distributions of terminal arborizations: the "specific," which is densely aggregated in cortical layers III and IV, and the "unspecific," which is sparsely distributed throughout all cortical layers but appears predominantly in layers I and VI. Subsequent electrophysiological evidence (*2*) buttressed the consensus (*2, 3*) that within the thalamus there are (i) a layer IV projection system including the "specific" sensory relay nuclei and (ii) a "nonspecific" layer I projection system epitomized by the intralaminar nuclei but also including a number of adjacent nuclei (*4*).

In the three decades that followed the initial categorization of thalamic nuclei into specific and nonspecific domains, very little anatomical evidence has supported the dual thalamic projection system concept. Anterograde fiber-tracing data have shown the existence of the specific projection to layers III and IV arising from several thalamic nuclei (*5*) but have largely failed to support (*6*) the contention that the intralaminar nuclei have projections terminating in layer I (*3*). Instead, layer I projections have been found to arise from a posterior site termed the "central intralaminar nucleus" in the hedgehog and the opossum thalamus (*7*), from the magnocellular medial geniculate nucleus in both the rat and the monkey (*8*), and from the ventromedial nucleus in the rat (*9*).

The autoradiographic studies reported here were designed to examine the cortical projections of individual thalamic nuclei in the rat. The first goal was to determine the laminar distributions of nuclei representing specific and nonspecific domains. After stereotaxic injections of tritiated amino acids into thalamic loci, the animals were allowed to survive for periods of either 1 day or 10 to 12 days. Their brains, perfused in formalin (10 percent in 0.9 percent saline), were sectioned and processed according to usual autoradiographic procedures (*9, 10*). The successful restriction of the anterograde


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