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Review Articles

Somatostatin – Both Hormone and Neurotransmitter?*

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The role of the hypothalamus as a regulator of anterior pituitary function was definitely established by the isolations from the hypothalamus of the thyrotropin and luteinizing hormone-releasing factors or hormones. These discoveries represented major breakthroughs in the whole field of endocrinology. They were soon to be followed by another discovery of equally great impact: the isolation and synthesis of the first hypothalamic factor inhibiting pituitary function, the so-called growth hormone-release inhibiting factor (hormone), somatostatin [12]:

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

Shortly afterwards we made an interesting finding: somatostatin is also present outside the hypothalamus, in other parts of the nervous system as well as in several peripheral endocrine-like cells. Somatostatin shares this property with some other peptides such as substance P, but none of these peptides appear to have such a wide distribution with regard to endocrine-like cells. This distribution of somatostatin suggested a dual role for the peptide: as a hormone and also as a modulator of nervous functions.

This review summarizes our attempts to elucidate these two functions of somatostatin. The results suggest an inhibitory role of somatostatin both in its capacity as a hormone and as a neurotransmitter. Recently a further exciting aspect on somatostatin has been revealed. Our immunohistochemical findings indicate that somatostatin may occur in a population of peripheral sympathetic noradrenergic neurons, raising the possibility of the concomitant occurrence of two transmitters in one neuron.

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Localization of Somatostatin

The localization of somatostatin in the body has been elucidated mainly by two techniques, immunohistochemistry and radioimmunoassay (RIA). Both methods are based on the interaction of tissue somatostatin with antibodies to this hormone. The specificity of such antibodies was shown by abolition of the reactions by absorption of the antiserum with somatostatin. It must, however, be emphasized that the antiserum may cross-react with unknown somatostatin-like peptides or larger protein molecules containing somatostatin-like aminoacid sequences.

1. In Endocrine or Endocrine-Like Cells

Somatostatin has been identified in a number of *endocrine or endocrine-like cells.* In the rat *pancreas,* somatostatin containing cells were demonstrated mainly in the peripheral parts of the islets, and localized to their D-cells or A_1-cells (Fig. 1) [67, 27, 41, 46, 72, 83, 90, 38, 74]. In the *stomach,* somatostatin-positive cells were observed mainly within the lower half of the mucosa of the antrum in close connection with the gastrin producing cells [46, 83, 91, 74, 82]. Finally, such cells were demonstrated in the *thyroid* with a localization similar to that of the parafollicular cells [46, 74].

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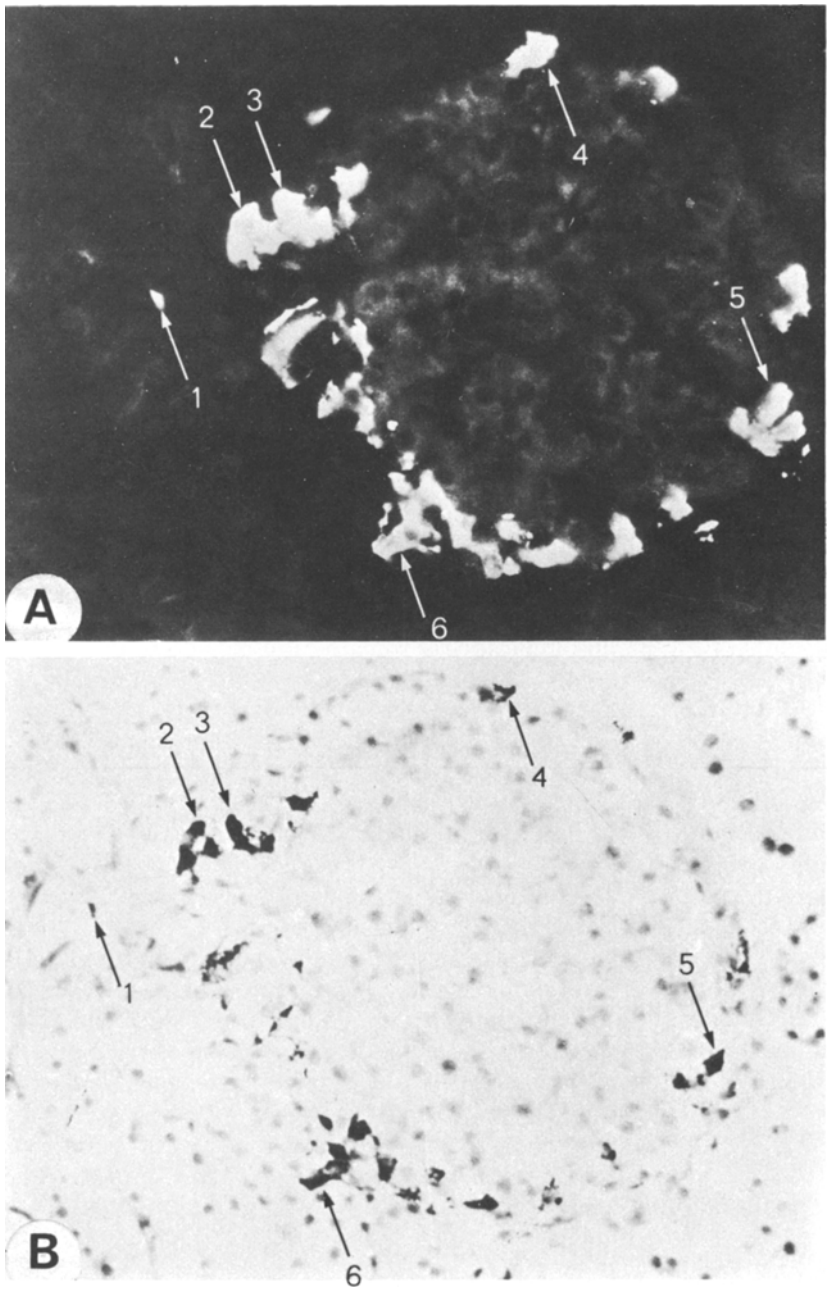


Fig. 1. The same section of pancreas incubated with antibodies to somatostatin, photographed (**A**) and subsequently processed for silver staining (**B**). Note the absolute identity between somatostatin positive and argyrophilic cells. Magnification 370×. From Hökfelt et al. [46]

2. In the Nervous System

In the hypothalamus, both somatostatin positive cell bodies [26, 37, 46, 2] and fibre-like structures are found [25, 47, 46, 50, 23, 55, 78, 96]. The neuronal nature of the fibres was definitely proven by Pelletier et al. [77] demonstrating that somatostatin was localized in granular vesicles in nerve endings. In our preparations, fluorescent cell bodies were localized in such sites as the periventricular area at the level of the suprachiasmatic nucleus extending in the caudal direction [46]. It is important that, in addition, somatostatin positive cell bodies were distributed throughout the brain, particularly the neo- and limbic cortical areas. Others have localized somatostatin cell soma to other hypothalamic nuclei, such as the paraventricular and supraoptic ones [26].

In our studies, somatostatin positive fibres were present in the external layer of the median eminence from its beginning all the way down into the stalk (Fig. 2), originating in cell bodies in the periventricular anterior hypothalamic nucleus as shown in lesion studies [36]. The presence of somatostatin innervation in the median eminence is in good agree-

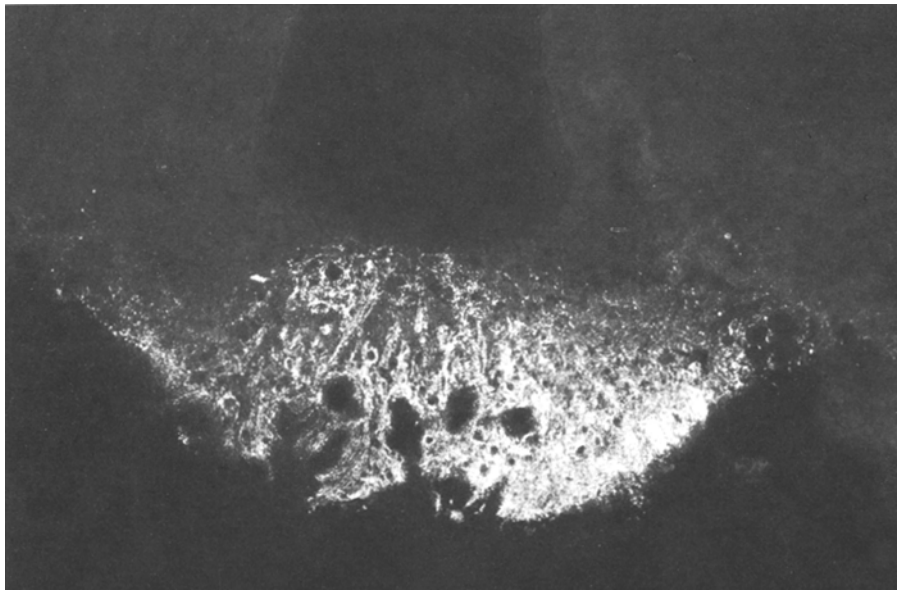


Fig. 2. Immunofluorescence micrographs of the basal hypothalamus after incubation with somatostatin antisera. A dense plexus of fluorescent fibres is observed in the median eminence, and a weak plexus also in the arcuate nucleus. Magnification 135×. From Hökfelt et al. [46]

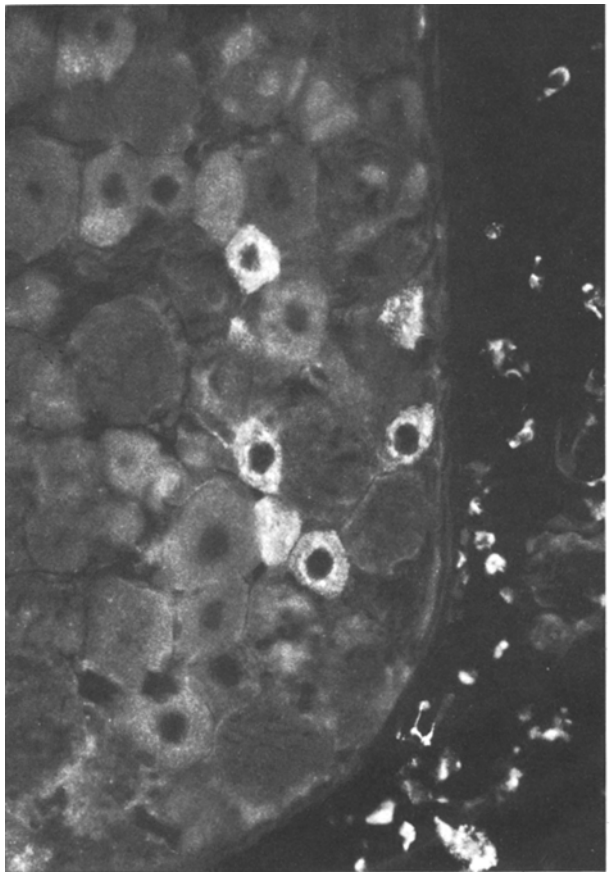


Fig. 3. Immunofluorescence micrograph of section of spinal ganglion incubated with antiserum to somatostatin. Six somatostatin positive cells are seen. Magnification 340×. From Hökfelt et al. [48]

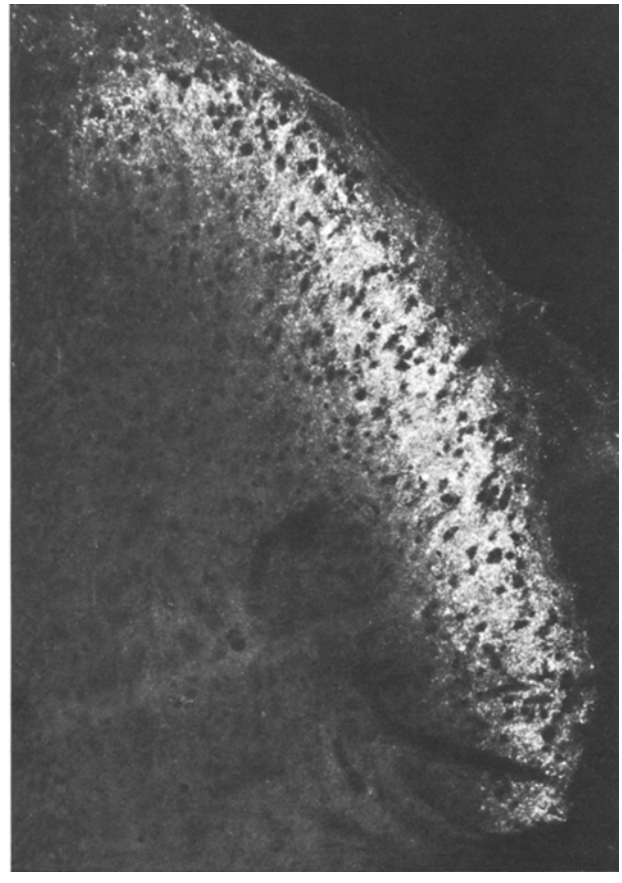
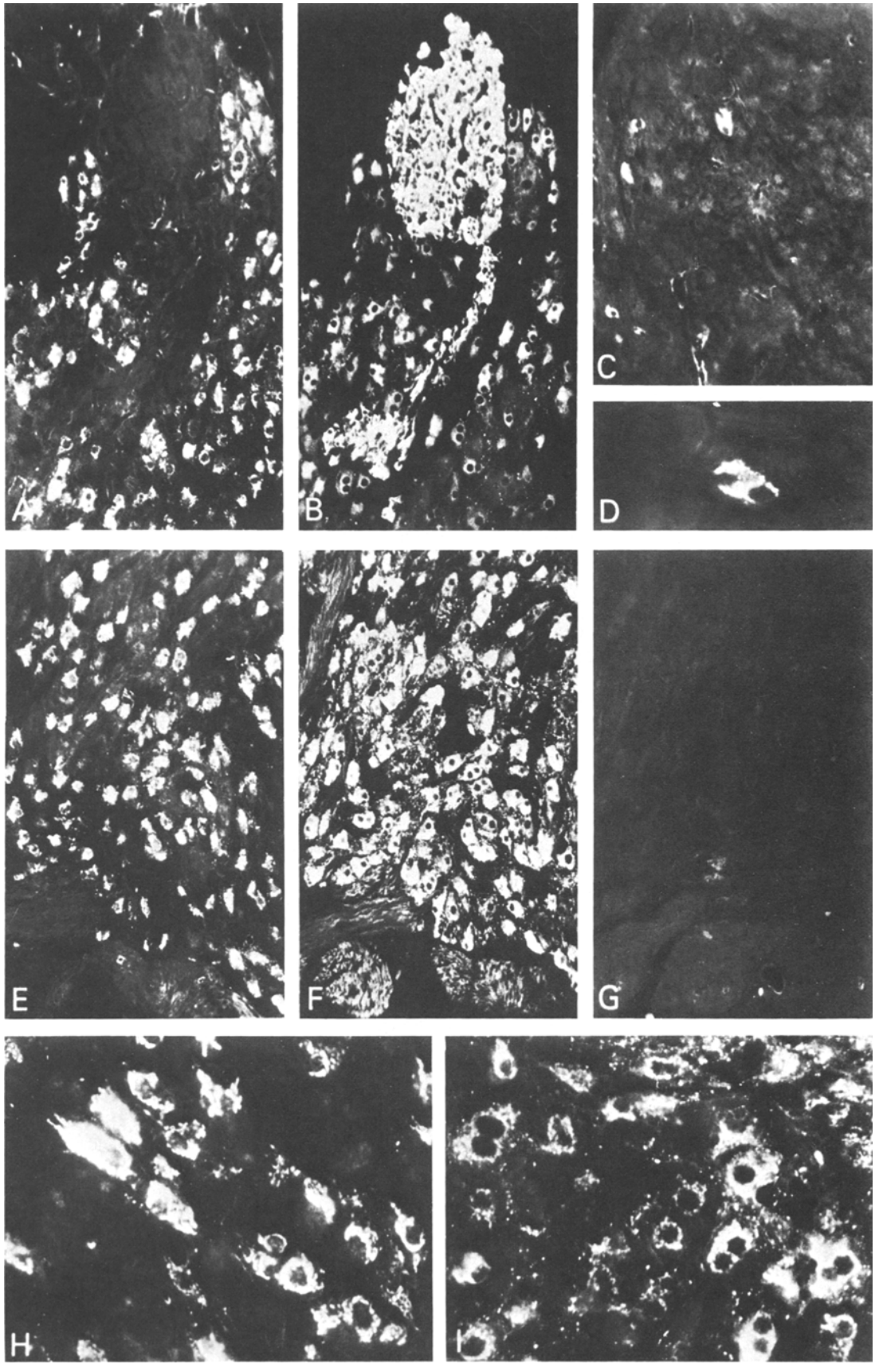


Fig. 4. Immunofluorescence micrograph of a section of the dorsal horn of the spinal cord (lumbar level) after incubation with antiserum to somatostatin. Somatostatin positive fibres are present in laminae I and II. Magnification 136×. From Hökfelt et al. [48]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ment with the inhibitory role of somatostatin on the release of pituitary hormones. In this connection, it is of interest that rather dense plexuses of positive fibres were observed in the ventromedial and the arcuate nuclei extending caudally into the ventral premammillary nucleus. The localization of these nerve endings suggests a release of somatostatin from nerve endings at synapses, and could thus indicate a transmitter or modulator role for somatostatin.

A further support for such a transmitter role is the finding of the peptide in some primary sensory neurons. Thus, firstly, somatostatin positive cell bodies were observed in spinal ganglia (Fig. 3). Secondly, a dense plexus of somatostatin fibres was seen in the substantia gelatinosa of the dorsal horns of the spinal cord (Fig. 4). Finally, in a peripheral tissue such as the gut, a sparse network of fluorescent fibres was found which may, at least in part, represent peripheral branches of sensory neurons. The functional role of these somatostatin neurons is not known.

Recent findings indicate that somatostatin is present in a further population of peripheral neurons [49]. Particularly in prevertebral ganglia of the guinea pig, the inferior mesenteric ganglion and the coeliac-superior mesenteric ganglion complex, a large proportion of all noradrenergic cell soma also contained somatostatin-like immunoreactivity, located in the Golgi complex (Fig. 5). This could be established by staining consecutive sections with antisera to somatostatin and to dopamine-β-hydroxylase, respectively, the enzyme converting dopamine to noradrenaline. These findings are of interest for at least two reasons. Firstly, this appears to be the first example of the concomitant storage of a small biologically active peptide and an amine in one and the same *neuron,* thus extending the APUD (Amine Precursor Uptake and Decarboxylation) concept of Pearse [76] from endocrine cells to neurons. Secondly, this may represent the first case of a mammalian neuron producing two putative transmitters. Such a neuron would not conform to the one neuron – one transmitter hypothesis, often referred to as Dale's principle. Evidence for neurons producing several putative transmitters has previously been obtained in studies on invertebrates [15]. The consequences of their findings and the validity of Dale's principle has recently been discussed by Burnstock [16].

In conclusion, immunohistochemistry offers numerous examples of neurons containing somatostatin thus satisfying one of the criteria for a transmitter substance.

Actions of Somatostatin

1. On Endocrine Functions

On the Release of Hormones. Since the inhibitory effect of somatostatin was primarily demonstrated in in vitro studies on rat pituitary cell cultures it is obvious that, initially, its action on the release of pituitary hormones in man and animals was of interest. As far as *growth hormone (GH) release* is concerned somatostatin has, indeed, been shown to be an effective inhibitor in a number of species (rat, dog, baboon, man) independently of the agents used for stimulation of the secretion (arginine, L-dopa, barbiturates, chlorpromazine, exercise, sleep, meals, catecholamines, insulin hypoglycemia) [12, 43, 85, 89, 98, 53, 66, 73, 79, 108, 40, 56]. This inhibitory action of somatostatin on the release of pituitary hormones seems also to include TSH [105, 98, 17].

While investigating the effect of somatostatin on GH release, several groups made the unexpected observation that the peptide also inhibited *insulin release* [1, 57, 35, 39, 69, 18, 32, 33, 62, 29, 34]. This was particularly interesting in view of the hypothesis that impaired insulin release is a primary derangement in diabetes mellitus. An endogenous inhibitor of insulin release, like somatostatin, was of obvious interest to us in this context. Therefore, a detailed study was made of the effect of somatostatin on the endocrine pancreas in rats and man. It turned out to be an extremely potent inhibitor of both insulin and glucagon release. Thus, as small a dose as 1 ng/ml of perfusate significantly inhibited

◀ **Fig. 5.** Immunofluorescence micrographs of the inferior mesenteric ganglion (**A, B, I**), the superior cervical ganglion (**C, D**), and the coeliac-superior mesenteric ganglion complex (the anterior inferior part, **E–H**) after incubation with somatostatin antiserum (**A, C–E, H**), dopamine-β-hydroxylase antiserum (**B, F**), with both somatostatin and dopamine-β-hydroxylase antiserum (**I**) or with control serum (somatostatin antiserum blocked with excess of somatostatin) (**G**). A and B on one hand and **E–G** on the other hand show consecutive sections. In the inferior mesenteric ganglion (**A**) as well as in the anterior part of the coeliac-superior mesenteric ganglion complex (**E**) the majority of the principle ganglion cells are somatostatin positive whereas the SIF cells (asterisks) lack a specific fluorescence (**A**). The superior cervical ganglion contains only few positive cells (**C, D; D** represents a higher magnification of **C**). The somatostatin immunoreactive material appears to be localized to the Golgi apparatus (**H**). Most of the principle ganglion cells as well as the SIF cells contain dopamine-β-hydroxylase (**B, F, I**) indicating that they represent catecholamine neurons. After consecutive incubations with both antisera (**I**) the fluorescence is localized both to the Golgi apparatus and to a pool in the cytoplasm. There are also many fluorescent, mainly dopamine-β-hydroxylase-positive nerve endings in the inferior mesenteric ganglion (**I**). Note lack of immunoreaction after incubation with control serum (**G**). Magnifications 95× (**A–C, E–G**) and 240× (**D, H, I**). From Hökfelt et al. [49]

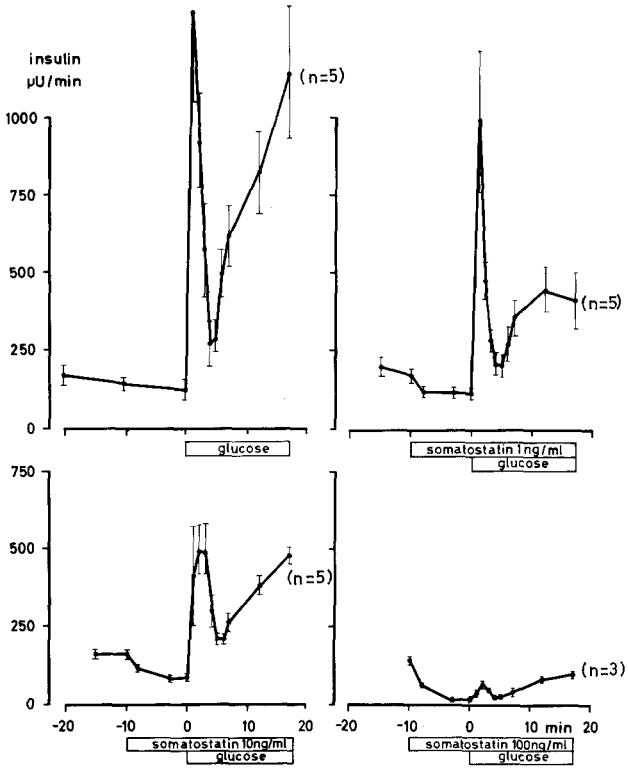

Fig. 6. Effect of linear somatostatin (1–10–100 ng/ml of perfusate) on glucose (3.0 mg/ml) induced insulin release from the isolated perfused rat pancreas. Following isolation the pancreas was equilibrated for 30 min with 1.5 mg/ml of glucose in the perfusate. The glucose stimulus was applied between 0 and 17 min, somatostatin 10 min prior to and during the glucose stimulation. The results are expressed as the mean ± SEM of five experiments. From Efendić et al. [35]

glucose-induced insulin release from the perfused rat pancreas (Fig. 6) [35, 32]. In man, the infusion of 210 μg of somatostatin for 90 min was used for the same purpose [34]. Slightly higher doses were required for the inhibition of *glucagon* release. These findings suggested, but did not prove, a physiological action of somatostatin on the endocrine pancreas, and led us to investigate the possibility that somatostatin was produced in the pancreas. As already mentioned, this hypothesis could be proven.

The fact that somatostatin inhibits insulin as well as glucagon secretion led us to look for analogues of somatostatin that might specifically inhibit one but not the other of these hormones. Deletion of Asn^5 from the molecule preferentially inhibited insulin but not glucagon release (Fig. 7) [14, 31]. The opposite was true for D-Cys^{14}-somatostatin [14, 68]. Thus, somatostatin analogues with specific actions can be produced. These studies have also given some information concerning structure-activity relationship for somatostatin in the endocrine pancreas:

1. neither a free carboxyl terminal nor the amino terminal seem to be essential for the activity of the cyclic peptide
2. addition of aminoacids to the amino terminal did not decrease the activity
3. changes in the structure of linear somatostatin, which lead to the loss of ability to form a cyclic peptide impaired the activity.

In analogy to the findings on the endocrine pancreas, other possible extra-hypothalamic actions of somatostatin were looked for. At present, an exciting area is the action of somatostatin on the release of *gastro-intestinal hormones.* It suppresses the release of gastrin [63, 51], glucagon-like immunoreactivity [92], cholecystokinin [59], motilin [7] and secretin [8, 59]. Our main interest in this respect has been focused on gastrin. Somatostatin inhibited vagus-induced gastrin release in the isolated antrum preparation of cats, while atropine was without-effect (Fig. 8) [103]. In addition to gastrin, the inhibition comprised gastric acid secretion [6, 3, 42, 61, 65].

All these studies demonstrate that, most likely, exogenously administered somatostatin exerts effects in those hormone producing tissues where it has been localized, and that these actions are of an inhibitory nature.

On Glucose Metabolism. A factor like somatostatin with such strong inhibitory effect on the secretion of pituitary and pancreatic hormones, could be expected to have a major impact on carbohydrate metabolism. This was soon documented. Thus, short term infusion of somatostatin induced hypoglycemia in man, whereas prolonged infusion led to significant hyperglycemia (Fig. 9) [64].

The initial hypoglycaemia in over-night fasted subjects was due to suppression of glycogenolysis which led to a 70% inhibition of splanchnic glucose output (Fig. 10) whereas, in 60 h fasted subjects, the hypoglycaemic effect was also due to suppression of gluconeogenesis [106]. The hyperglycaemia accompanying prolonged infusion of somatostatin was due partially to increased hepatic glucose output, and partially to decreased peripheral uptake of glucose [97].

The described alterations in the handling of glucose by the liver, most probably, can be attributed to inhibition of insulin and glucagon secretion. One important piece of information to be derived from these data is that hypoinsulinaemia – in spite of simultaneously suppressed glucagon secretion – leads to hyperglycaemia, implying that a basal gluca-

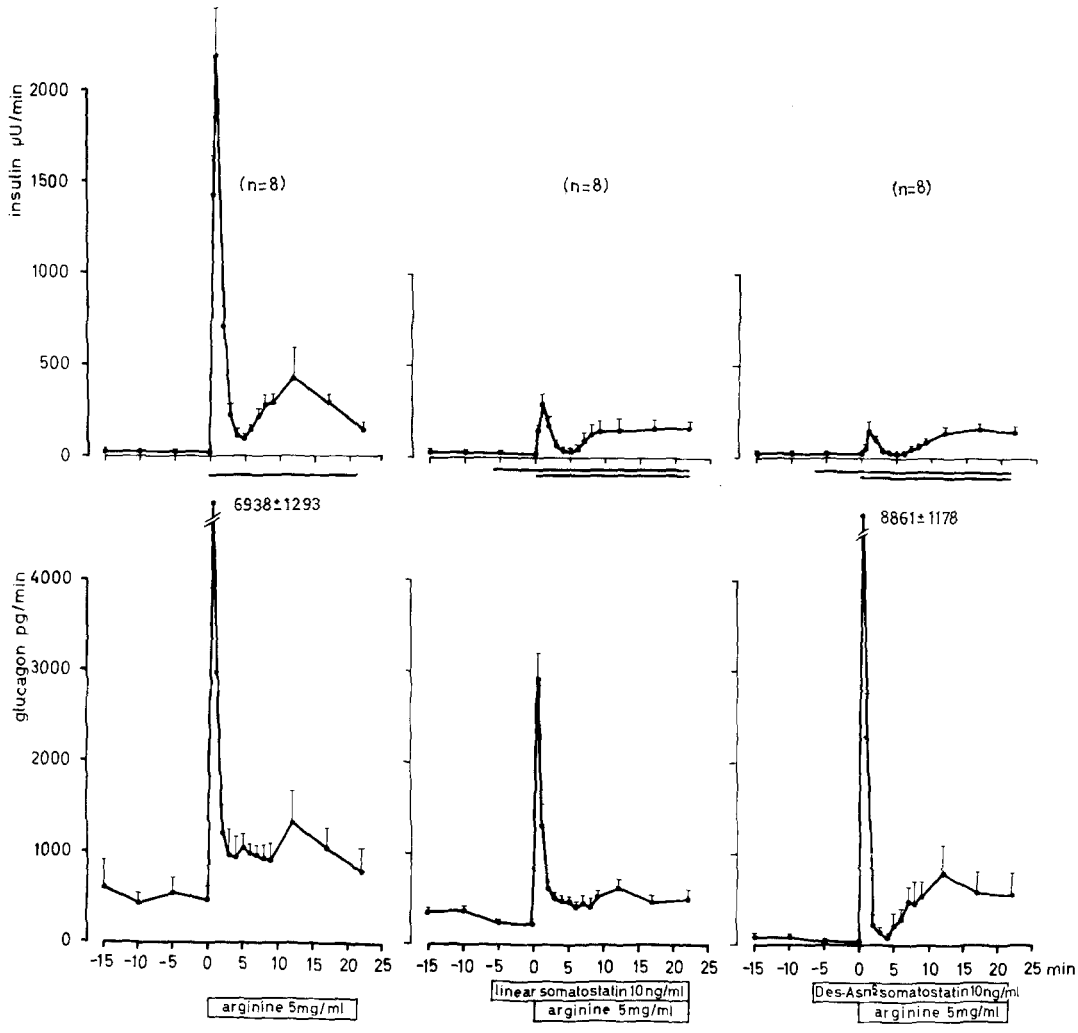

Fig. 7. The effect of linear somatostatin (10 ng/ml) and (Des-Asn^5)-somatostatin (10 ng/ml) or arginine (5 mg/ml) induced insulin and glucagon release from the isolated perfused rat pancreas. Somatostatin and somatostatin analogues were applied 7 min prior and during the arginine stimulation. The results are expressed as mean ± SEM. From Efendić et al. [31]

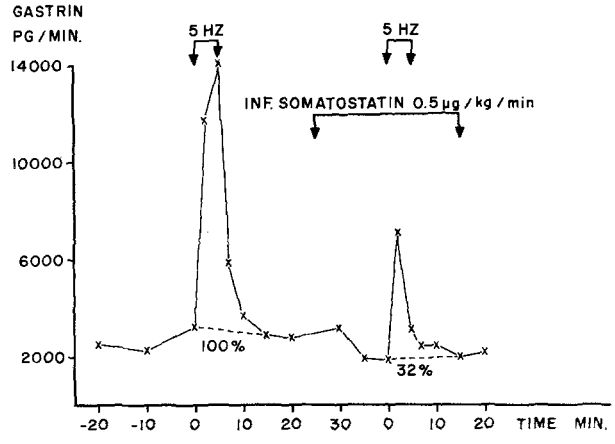

Fig. 8. Experiment demonstrating the effect of somatostatin on basal and vagally induced release of gastrin. Two vagal stimulations (at 5 Hz for 5 min) were performed. Somatostatin (0.5 µg/kg/min) was infused from 15 min before the second stimulation until 10 min after it was ended. From Uvnäs-Wallensten et al. [103]

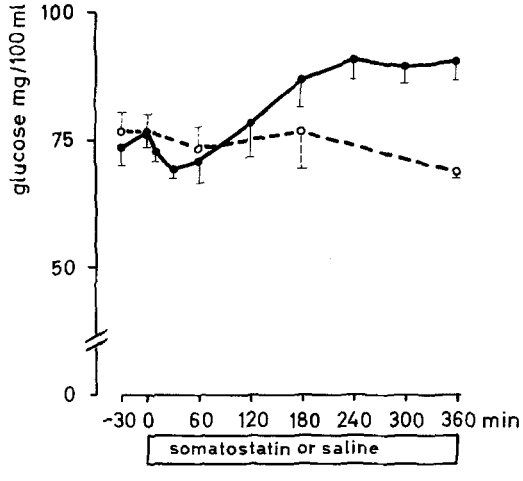

Fig. 9. Effect of prolonged somatostatin infusion (200 µg as a bolus and 50 µg per hour over 6 h) on blood glucose levels in five healthy non-obese men. Solid line denotes experiments with somatostatin, broken line control experiments with saline. Mean ± SEM. From Lins and Efendić [64]

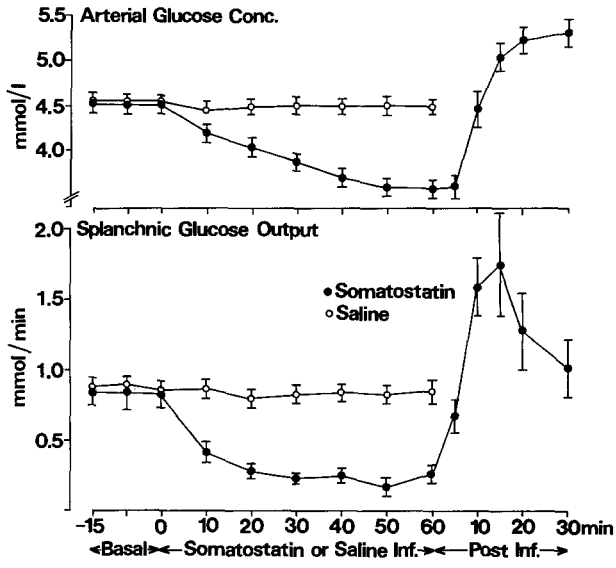

Fig. 10. Arterial glucose concentration and splanchnic glucose output in the basal state and during infusion of somatostatin (filled circles) at 10 μg per min or infusion of saline (open circles) in postabsorptive subjects. Mean ± SEM. From Wahren et al. [106]

gon secretion is not a prerequisite for fasting hyperglycaemia.

2. On Nervous Functions

There are three lines of evidence for the significance of somatostatin in the regulation of functions in the nervous system: *1.* its presence in several neuron systems; *2.* its direct depressant action on the activity of neurons at several levels (cerebral and cerebellar cortex, brain stem and hypothalamus) [86]; *3.* its behavioural effect: it decreased spontaneous motor activity [95], it interfered with strychnine induced effects [13], it potentiated the behavioural effects of L-dopa [81], it decreased locomotor activity to the point of catalepsia and induced unusual rotational behaviour in the rat [20], it prolonged pentobarbital induced narcosis [84]. It also induced a variety of behavioural, motor and electrophysiological changes when injected into the hippocampus [87], the cortex [88] or intraventricularly [44]. In addition, it has been suggested that somatostatin acts as partial agonist-antagonist on opiate receptors in the CNS [102].

In this connection it is of interest that some other peptide hormones exert depressant (LH-RH, TRH) or excitatory actions (substance P, angiotensin II) on the activity of central neurons [70, 28, 58, 60, 80, 86, 100]. The observations that only a certain population of neurons display peptide sensitivity, argues for a degree of selectivity and specificity of effect with respect to the individual peptides.

Less known are the effects of somatostatin in the peripheral nervous system. Some fibres may represent peripheral branches of primary sensory neurons. If so, the question is what modality – if a specific one – these neurons may be related to. Their small sized neurons and fine calibre axons suggest involvement in events perhaps related to temperature or pain.

Mode of Action of Somatostatin

The wide distribution of somatostatin producing cells suggests that the peptide might act locally in the places where it is produced. In favour of such a hypothesis are our studies on the isolated gastric antrum of the cat. Vagal stimulation was accompanied by secretion of both somatostatin and gastrin into the antral lumen [104]. The quantities of the two hormones seemed to vary with the pH of the antral perfusate, more gastrin being released at alkaline and more somatostatin at acid pH (Fig. 11). This observation suggests that the antral cells producing gastrin (G-cells) and somatostatin (D-cells) release their hormones into the surrounding interstitial space from which they diffuse in all directions, thereby acting locally on their target cells or tissues. In the context of such a hypothesis, the close anatomical relationship between the somatostatin and gastrin producing cells would be significant.

At present, there is no information available which would suggest that somatostatin from the D-cells in the pancreatic islets and from the parafollicular cells in the thyroid gland is released in the same fashion. In the perfused isolated dog pancreas glucose, arginine and glucagon stimulated whereas diazoxide inhibited the release of somatostatin [75, 94, 107]. This suggests the possibility that somatostatin exerts paracrine effects on the adjacent β-cells and α-cells in the pancreatic islets. Such a paracrine interaction has been suggested also for insulin and glucagon [93].

Some light has been shed on the mechanism of action of somatostatin during the last two years. Available data indicate that at least two effects may be involved: one would be the level of cyclic AMP in the target cells, the other calcium uptake by these cells. Somatostatin suppressed basal as well as (prostaglandin, theophylline, TRH) stimulated levels of cyclic AMP in the pituitary gland and its cells [52, 9, 10]. Our studies on isolated pancreatic islets from the rat also demonstrated a decrease in the level of

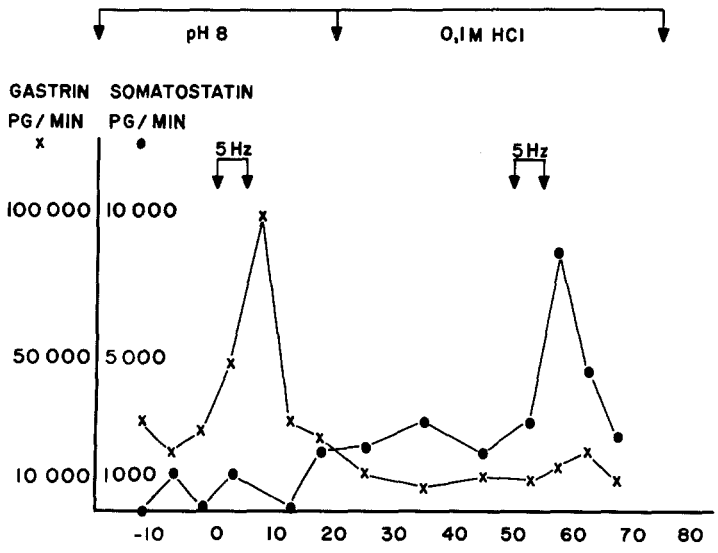

Fig. 11. Release of gastrin (crosses) and somatostatin (filled circles) into the antral lumen of cat. The antrum was perfused with phosphate buffer pH 8 and 0.1 mol/l HCl. Vagal stimulations (5 Hz for 5 minutes) were performed during perfusion with both solutions. From Uvnäs-Wallensten et al. [104]

cyclic AMP [30, 19]. In dose-response studies, the inhibition by somatostatin of the effect of glucose on (^{3}H)-cyclic AMP and insulin release could be overcome by a high concentration of glucose (44.9 mmol/l), suggesting competitive inhibition (Fig. 12). Somatostatin did not affect phosphodiesterase activity. These data indicate that somatostatin induced inhibition of insulin release is, at least partially, mediated by cyclic AMP, probably through inactivation of islet adenyl cyclase.

As for the effect of calcium ions, the inhibitory effect of somatostatin on glucose induced insulin release from the perfused rat pancreas could be overcome by increasing the calcium concentration of the perfusate [21, 22]. Moreover, somatostatin inhibited ^{45}Ca uptake of islets [4, 71].

Few studies have yet been published regarding the mode of action of somatostatin in *the nervous system.* In analogy with the findings in the anterior pituitary and pancreas, indicating that the action of somatostatin involves changes in the levels of cyclic nucleotides, it is conceivable that this mechanism may also contribute to the electrophysiological and behavioural effects of somatostatin in the central nervous system. This hypothesis is supported by some observations which demonstrate that other neurotransmitters can regulate the level of cyclic AMP in the central nervous system. Thus, noradrenaline elevated the cyclic AMP level in the cerebellum [45, 5], and dopamine in the caudate nucleus [54].

Recently Tan et al. [101] have shown that somatostatin can influence the uptake and release of calcium by synaptosomes. In view of the crucial role of this ion in nerve terminal depolarization as well as in the secretion of hormones, it can be speculated that somatostatin exerts its action both as a transmitter and hormone by affecting calcium transport through the nerve cell and endocrine cell membrane, respectively.

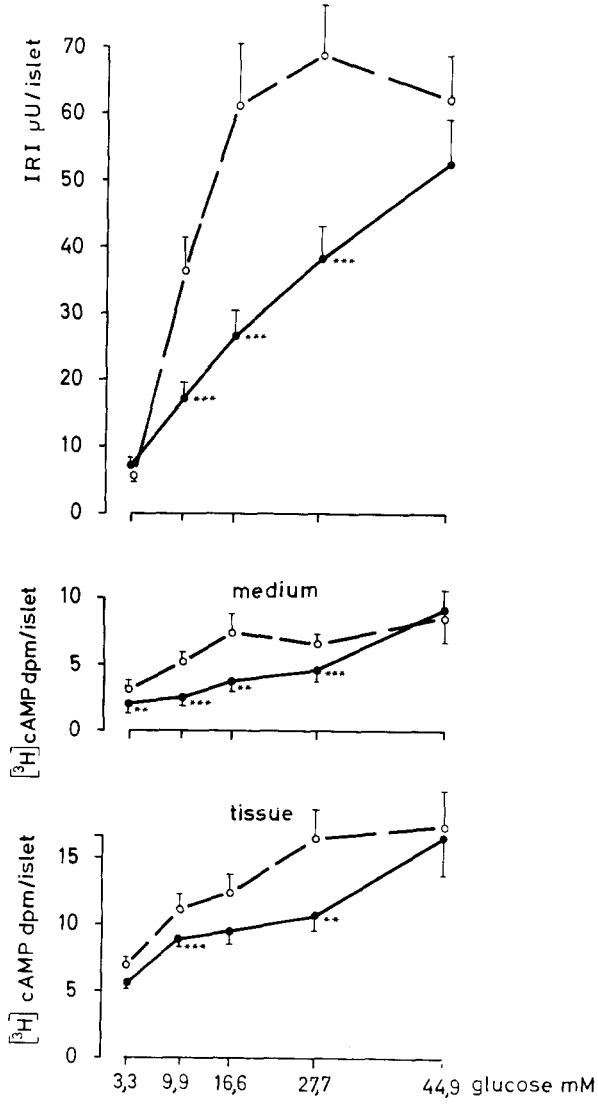

Fig. 12. Effect of somatostatin in the presence of different glucose concentrations (3.3–44.9 mmol/l) on insulin release, and islet and medium (^{3}H)-cyclic AMP. Open circles denote control incubations, filled circles incubations with somatostatin. Incubation time 30 min. Mean ± SEM of 13 complete experiments. *** = < 0.005, ** = p < 0.02, significance of difference from control incubations. From Claro et al. [19]

Conclusions

The tetradecapeptide, somatostatin, is widely distributed in the body, in endocrine-like cells as well as in nervous tissues. Wherever present in endocrine cells, it seems to inhibit the secretion of hormones in the respective glands.

In the central and peripheral nervous systems it probably plays the role of an inhibitory transmitter substance or modulator. This suggestion is supported by the following lines of evidence: (1) the demonstration of somatostatin specific neuron systems with particularly high concentrations in the nerve endings, i.e., at the release sites; (2) the demonstration at the ultrastructural level of somatostatin in storage vesicles in the nerve endings, i.e., at the subcellular compartment which previously has been associated with release of transmitters; (3) the effects of iontophoretically administered somatostatin on the electrical activity of neurons in various brain regions; and finally, (4) the existence of an inactivation mechanism probably based on breakdown of somatostatin at specific places of the amino acid chain by peptidases.

The concomitant presence of noradrenaline and somatostatin in one and the same neuron probably represents the first evidence for two putative transmitters in one and the same mammalian neuron.

As for its mode of action, available data suggest that interference with the cellular levels of cyclic nucleotides as well as Ca^{++} uptake may be of importance.

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