sure produced by the central administration of propranolol is associated with an elevation in the concentration of NE in CSF. Furthermore, the high correlation between the hypotensive effects and the elevation in NE strongly suggests that the hypotensive effect produced by propranolol results from increased release or decreased reuptake of NE into central noradrenergic nerve terminals. That prior treatment with phentolamine prevents the hypotension is consistent with this hypothesis and suggests that the decrease in blood pressure is mediated through stimulation of central alpha-adrenergic receptors.

The possibility that the increased level of CSF NE results from a NE-releasing action of propranolol is supported by recent findings in other studies. Saelens *et al.* (*17*) found that propranolol enhances the spontaneous release of ^{3}H-labeled NE from adrenergic nerve endings in isolated guinea pig vas deferens. Daniell *et al.* (*10*) reported that intracoronary injection of propranolol induces the release of NE from adrenergic nerve endings in the canine heart. In both instances this effect of propranolol was not stereospecific.

In summary, our data support the idea that the hypotensive response to centrally administered propranolol results from an action of the drug to release NE, which stimulates central alpha receptors to decrease peripheral sympathetic nerve activity and lower arterial pressure. Although our study does not exclude a role for central beta receptors in the control of blood pressure, it does suggest that they do not play a major role in the hypotension seen after central administration of propranolol.

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Depolarization- and Ionophore-Induced Release of Octacosa Somatostatin from Stalk Median Eminence Synaptosomes

Abstract. *Species of somatostatin of higher molecular weight were present in the nerve terminals (synaptosomes) of ovine stalk median eminences and were released by depolarizing stimuli. One of these species was identified as the biologically active molecule octacosa somatostatin. Octacosa somatostatin appears therefore to be secreted into the hypothalamic-hypophyseal system, supporting the concept of a role for this peptide in regulating pituitary hormone secretion.*

Somatostatin is a tetradecapeptide originally isolated from ovine hypothalami as a result of its ability to inhibit growth hormone release (*1*). A number of immunoreactive species of somatostatin of higher molecular weight have been found in various tissues (*2*) and have been shown to have biological activity (*3, 4*). Recently a putative prohormonal form of somatostatin containing 28 amino acids (octacosa somatostatin) was isolated from pig intestine (*5*) and hypothalamus (*6*) and from sheep hypothalamus (*7*). This molecule has an extension of 14 amino acids at the NH_2-terminus of the somatostatin sequence. Amino acids 13 and 14 are the basic residues lysine and arginine, a characteristic tryptic cleavage point of many prohormones.

Unlike most prohormones, octacosa somatostatin exhibits substantial biological activity, having growth hormone release-inhibiting activity equal to (*6*) or greater than (*7*) that of somatostatin. Thus, octacosa somatostatin may be an active regulator of growth hormone secretion, and somatostatin may be a biologically active fragment (*7*). The presence of biologically active octacosa somatostatin in tissue does not indicate that the peptide is secreted and thus performs a biological role. We have now demonstrated that octacosa somatostatin is contained in nerve terminals (synaptosomes) isolated from ovine stalk median eminences, and that the peptide is released by a depolarizing concentration of KCl (100 m*M*) and by the calcium ionophore A23187. These findings suggest that the peptide is secreted into the hypothalamic-hypophyseal portal system in vivo and support the concept of a biological role for octacosa somatostatin.

Stalk median eminences were dissected from sheep hypothalami and homogenized. Synaptosomes were purified by differential and discontinuous Ficoll density gradient ultracentrifugation (*8*). Electron microscopy showed that the preparation was composed predominantly of intact synaptosomes. The synaptosomes actively sequestered [^{3}H]noradrenaline, γ-[^{3}H]aminobutyric acid, and [^{14}C]acetylcholine at 37°C, while uptake did not occur at 4°C. Intactness of synaptosomes was demonstrated by the high proportion of occluded lactate dehydrogenase, since 82 percent of the total activity was released on exposure to hypoosmotic medium.

Extraction of synaptosomes with 0.2*N* acetic acid and separation of the components on Sephadex G-25 (fine) revealed a major somatostatin immunoreactive peak, which eluted with synthetic somatostatin-14, and two earlier-eluting immunoreactive peaks (Fig. 1A). The apparent molecular weights of the two peaks were 5000 or larger, and 3000. The 3000-dalton immunoreactive peak eluted with synthetic octacosa somatostatin (*6, 9*).

Stimulation of synaptosomes with 100 m*M* KCl or the ionophore A23187 released immunoreactive somatostatin into the medium. The immunoreactive material was fractionated on Sephadex G-25 (fine) into three immunoreactive peaks that eluted in the positions of somatostatin-14, octacosa somatostatin, and the species of 5000 daltons or larger (Fig. 1B). In contrast, luteinizing hormone–releasing factor was released from these

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Table 1. Somatostatin immunoreactive species released from synaptosomes and in extracts of synaptosomes. In experiment 1, synaptosomes were prepared and treated as described in Fig. 1. Extracted immunoreactive somatostatin and released immunoreactive somatostatin were directly chromatographed on a Sephadex G-25 (fine) column without prior treatment with 8*M* urea and 0.1 percent dithiothreitol. Fractions were assayed with antiserum 774. In experiment 2, synaptosomes were incubated with 100 m*M* KCl for 5 minutes (*7*). Extracted immunoreactive somatostatin and released immunoreactive somatostatin were treated with 8*M* urea and 0.1 percent dithiothreitol at 50°C for 3 hours and chromatographed on a Sephadex G-25 (superfine) column. Antiserum S_1^6 was used to assay the fractions since antiserum 774 did not recognize reduced immunoreactive species of somatostatin. Results are expressed as ratios relative to somatostatin-14 immunoreactivity.

Somatostatin species	Experiment 1		Experiment 2	
	Synaptosome extract	A23187 release	Synaptosome extract	100 m*M* KCl release
Somatostatin-14	1.00	1.00	1.00	1.00
3000-dalton species	0.46	1.18 (4.9)*	0.62	0.24 (0.77)*
≥5000-dalton species	0.40	0.32	0.11	0.02

*The molar ratios of the 3000-dalton peaks, corrected for a cross-reactivity with octacosa somatostatin of 11.9 percent with antiserum 774 and 15.6 percent with antiserum S_1^6, are shown in parentheses. With antiserum S39, which does not bind NH_2 terminally extended forms of somatostatin-14, no immunoreactivity was detected in the 3000-dalton peak.

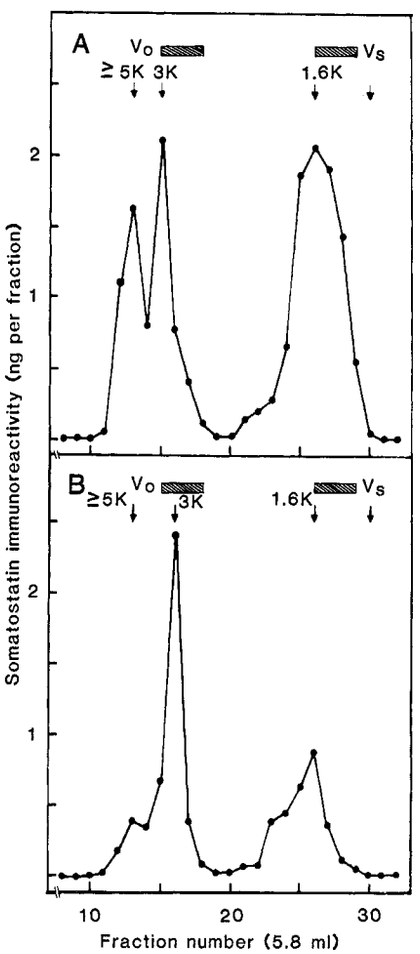

Fig. 1. (A) Separation of immunoreactive species of somatostatin extracted from ovine stalk median eminence synaptosomes on Sephadex G-25 (fine). The stalk median eminences were removed from 50 sheep heads within 20 minutes after death, suspended on 0.32*M* sucrose, 1 m*M* potassium phosphate, 0.1 m*M* EDTA, *p*H 7.4, at 4°C, then homogenized and centrifuged at 1000*g* at 4°C for 15 minutes. The supernatant was centrifuged at 15,000*g* at 4°C for 20 minutes; the mitochondrial pellet was washed twice, suspended in 0.32*M* sucrose, 1 m*M* potassium phosphate, *p*H 7.4 [3 ml per gram (wet weight) of starting tissue], layered on a discontinuous Ficoll density gradient (8.5, 13, and 17 percent), and centrifuged in a Beckman SW 25.1 rotor at 54,000*g* at 4°C for 60 minutes. Synaptosomes, banding between 8.5 and 13 percent and between 13 and 17 percent Ficoll, were suspended in four volumes of 0.32*M* sucrose, 1 m*M* potassium phosphate, *p*H 7.4, and centrifuged at 15,000*g* at 4°C for 20 minutes. The synaptosome pellet was suspended in 3 ml of 0.32*M* sucrose, 1 m*M* potassium phosphate, *p*H 7.4. The synaptosome suspension (1 ml) was extracted with 4 ml of 0.2*N* acetic acid. The extract was centrifuged at 10,000*g* at 4°C for 10 minutes; the supernatant was applied to a Sephadex G25 (fine) column (1.6 by 90 cm) and eluted at 4°C with 0.2*N* acetic acid at a flow rate of 23.2 ml per hour. Fractions were lyophilized and taken up in 1 ml 0.002*M* acetic acid; immunoreactive somatostatin was measured by a specific radioimmunoassay with antiserum 774. Interaction of antiserum 774 with a number of analogs and fragments of somatostatin indicates that it is directed toward the middle of somatostatin-14, from 6-phenylalanine to 8-tryptophan. This antiserum showed 11.9 percent cross-reactivity with octacosa somatostatin and less than 0.06 percent cross-reactivity with reduced somatostatin-14 and reduced octacosa somatostatin. Hatched bars show the elution positions of synthetic octacosa somatostatin and synthetic somatostatin-14. V_o, void volume; V_s, salt elution volume. (B) Sephadex G-25 (fine) fractionation of immunoreactive species of somatostatin released from synaptosomes by incubation with ionophore A23187. Synaptosome suspension (2 ml) was incubated at 25°C in 0.32*M* sucrose, 1 m*M* potassium phosphate, *p*H 7.4, containing 1 m*M* bacitracin for a period of 10 minutes before the addition of 10 μ*M* A23187 (*8*). After incubation for 5 minutes, the synaptosomes were centrifuged, the supernatant was applied to a Sephadex G-25 (fine) column, and somatostatin immunoreactivity was assayed as described in (A). The three somatostatin immunoreactive peaks were also released by stimulation with 100 m*M* KCl. Approximately 10 to 30 percent of the total synaptosomal immunoreactive somatostatin was released on stimulation.

synaptosomes as the single decapeptide species (data not shown). Both the 3000- and the ≥5000-dalton somatostatin immunoreactive peaks have been demonstrated in Sephadex G-25 separations of rat hypothalamic extracts, and both are biologically active (*3*).

The larger species of immunoreactive somatostatin may represent aggregates of somatostatin-14 produced by hydrogen bonding or intermolecular disulfide bridges. To investigate this possibility, we incubated these larger species in 8*M* urea and 0.1 percent dithiothreitol at 50°C for 3 hours and chromatographed them. With antiserum 774, no immunoreactive peaks were detected, since this antiserum does not recognize reduced somatostatin-14 or reduced octacosa somatostatin. With antiserum S_1^6, which recognizes reduced somatostatin (55 percent cross-reactivity) (*10*), the peaks retained their elution volumes.

The possibility of nonspecific interference in the radioimmunoassay due to the presence of somatostatin-binding substances or to peptidases degrading somatostatin in column fractions was ruled out. When ^{125}I-labeled [Tyr^1]somatostatin (Tyr, tyrosine) was incubated for 3 hours at 37°C with material from the three immunoreactive peaks or with radioimmunoassay buffer and then reacted with an excess of somatostatin antiserum, there was no significant difference in the amount of radioactive somatostatin bound. Somatostatin-14 was fully recovered when incubated as above, heated to 100°C for 5 minutes, and then quantified by radioimmunoassay. Binding of ^{125}I-labeled [Tyr^1]somatostatin by material from the three peaks varied between 8.7 and 9.9 percent, an amount similar to that in tubes containing radioimmunoassay buffer alone (10.4 percent). The failure of antiserum 774 to detect any peaks after dithiothreitol reduction, while the peaks were still detected by antiserum S_1^6, further established the authenticity of the somatostatin immunoreactivity.

The somatostatin species were further identified by reverse-phase high-pressure liquid chromatography (Spherisorb column S5-ODS, 4.6 by 25 cm, 5 μm). The smallest immunoreactive somatostatin species again eluted with synthetic somatostatin-14 after 5 minutes, with the use of a linear gradient of 30 to 90 percent acetonitrile in 0.01*M* ammonium acetate, *p*H 5, for 15 minutes. The 3000-dalton peak from the Sephadex G-25 column was resolved into two immunoreactive peaks by isocratic elution with 30 percent acetonitrile for 30 minutes,

then with a gradient to 90 percent for 15 minutes. One of the peaks eluted with synthetic octacosa somatostatin after 35 minutes. The other peak eluted earlier and was tentatively identified, by its similar molecular size, as somatostatin-25 (4-28 octacosa somatostatin), which has been recently isolated from ovine hypothalamic extracts and also exhibits enhanced growth hormone release-inhibiting activity (*7*).

The relative concentrations of somatostatin-14 and the 3000- and ≥ 5000-dalton species in synaptosomes and those released by stimulation with 10 μM ionophore A23187 or 100 mM KCl (Table 1) show that, relative to somatostatin-14, substantial quantities of the 3000-dalton species are released from synaptosomes. Furthermore, since the cross-reactivity of octacosa somatostatin with the antiserums used is low, these values were an underestimate of the mass of the released 3000-dalton species. On a molar basis, approximately 0.8 and 5 times as much 3000-dalton somatostatin as somatostatin-14 is released by stimulation with KCl and ionophore A23187, respectively (Table 1). Since the growth hormone release-inhibiting activity of octacosa somatostatin and somatostatin-25 is 1 to 14 times that of somatostatin-14 on a molar basis (*6, 7, 9*), the total bioactivity released as the 3000-dalton species could be even greater than that secreted as somatostatin-14. Thus, in vivo, the secreted 3000-dalton species potentially exert a greater regulating influence on growth hormone secretion than somatostatin does.

Our findings demonstrate that, in addition to somatostatin-14, immunoreactive species of 3000 daltons (which include octacosa somatostatin) and a species of 5000 daltons or more are present in synaptosomes isolated from ovine stalk median eminences. Moreover, both somatostatin-14 and the octacosa species are released from these synaptosomes by 100 mM KCl or by the calcium ionophore A23187, agents known to stimulate somatostatin secretion from synaptosomes in a physiologically meaningful manner (*8, 11*).

Octacosa somatostatin is thought to be a prohormonal form of somatostatin-14 (*5, 6*). It has also been postulated that somatostatin-14 is a biologically active fragment of a larger molecule of greater specific activity, rather than octacosa somatostatin being a precursor of the tetradecapeptide (*7*). In all probability, octacosa somatostatin has both a hormonal and prohormonal role. Our finding that somatostatin-14 and octacosa somatostatin occur in and are secreted from stalk median eminence nerve endings points to a biological function for both forms. The existence of at least two biologically active secreted forms of somatostatin suggests that there may be subtle differences, yet to be elucidated, in the regulatory function of the two peptides.

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Single Neostriatal Efferent Axons in the Globus Pallidus: A Light and Electron Microscopic Study

Abstract. *Intracellularly labeled rat neostriatal projection neurons were analyzed with both light and electron microscopy. The axons of medium spiny neurons were traced into the globus pallidus and were found to make synaptic contacts with pallidal dendrites. Despite the common somato-dendritic morphology of the neostriatal projection neurons, two different distribution patterns of efferent axons were observed, indicating the presence of functionally different medium spiny neurons in the neostriatum.*

Intracellular labeling techniques combined with intracellular recording allow direct correlative analysis of structure and function of individual neurons (*1*). Traditional classification of neurons, based on morphological characteristics, may now be correlated with physiological properties as determined by intracellular recording. For example, it has been demonstrated that neostriatal projection neurons receive convergent excitatory extrinsic inputs and when labeled with intracellular horseradish peroxidase (HRP), these cells were identified as medium spiny neurons (*2*). The HRP labeling not only revealed the characteristic somato-dendritic morphology of these neurons, which have been described in Golgi studies (*3*), but also enable serial reconstruction of the elaborate arborizations of their intrinsic axon collaterals (*4*). Detailed electron microscopic observations of the somata, dendrites, and intrastriatal connections of these neurons have also been described (*5*). We report that these medium spiny neurons may be further subdivided on the basis of differences in extrinsic axon distribution patterns. We also analyzed the synaptic contacts and postsynaptic targets of individually labeled striopallidal axons by electron microscopy.

Following intracellular recordings, rat neostriatal neurons were labeled by intracellular iontophoretic injections of HRP, fixed, and processed histochemically. Sections containing neostriatum and globus pallidus (GP) were analyzed by both light and electron microscopy (*6*).

All of the neostriatal projection neurons identified in this study were medi-

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