during five seasons (1972 through 1976), we never observed three gulls defending a territory together or attending the same nest. The presence of two females sharing the same territory cannot be construed as polygamy, regardless of whether or not the eggs produced by those females were fertilized by promiscuous mating with a male. The finding of one male-female pair incubating four fertile eggs does not invalidate the conclusion that the majority of supernormal clutches in the colony are the result of female-female pairing.

Homosexual pairing has not, to our knowledge, been reported for any group of wild birds (*16*). With the exception of our study it is unknown in gulls, a taxon that has been exposed to widespread and thorough scrutiny. Its existence in western gulls is apparently recent. Before 1968, only two clutches greater than the usual maximum of three eggs were reported for western gulls nesting in the Channel Islands off California and Mexico (*17*). The first records of substantial percentages of supernormal clutches in this species are from 1968 [11.3 percent of 150 clutches examined, San Nicolas Island (*3*)] and 1972 [11.0 percent of 63 clutches examined, Santa Barbara Island (*1*)].

Supernormal clutches have been reported in colonies of ring-billed gulls (*L. delewarensis*) as early as 1942 (*7*). Vermeer (*8*) reported that in one supernormal clutch, two eggs were laid on the same day, but no data were presented in any of these studies on either the fertility or the origin of the large clutches in this species.

At present we do not know whether female-female pairing in western gulls is pathological or if it has adaptive value. Promiscuous matings allow some homosexually mated females to produce fertile eggs. Without being paired, they would not be able to incubate these eggs or raise chicks (*18*). If there were an excess of females in the population (yet to be determined), then homosexual pairing would raise from zero the probability that the excess females would raise offspring.

Although female-female pairs may produce fertile eggs from promiscuous matings, these pairs may still lack other contributions to chick production provided by the male. One difference between pair types is the lack in female-female pairs of net energy input to females by males through courtship-feeding. Eggs laid by the stable homosexual pairs are smaller than those laid by heterosexually paired females (*19*). Chicks from small eggs have lower posthatching survival (*20*). This study may provide a unique opportunity to test the adaptive value of energy provided by the males to the females for egg production.

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5. Clutch size in large *Larus* gulls usually ranges between two and three eggs per clutch, and clutches of four eggs are very rare [K. Paludan, *Vidensk. Medd. Dan. Naturhist Foren. Khobenhavn* **114**, 1 (1951); R. A. Fordham, *Notornis* **11**, 3 (1964); J. Keith, *J. Appl. Ecol.* **3** (Suppl.), (1966); H. Klomp, *Ardea* **58**, 1 (1970); G. L. Hunt and M. W. Hunt, unpublished observations; (*1*, *6*)].
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9. Incubating adult gulls were trapped with either walk-in traps placed over their nests [D. K. Weaver and J. A. Kadlec, *Bird-Banding* **41**, 28 (1970)] or monofilament snares placed around the edge of the nest, which caught the birds around the feet when the line was pulled.
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12. We determined laying patterns by marking nests with numbered wooden stakes and visiting them daily (1973 and 1975) or every other day (1975) at approximately the same hour in order to record nest contents and individually mark newly laid eggs.
13. Intervals range from 48.7 to 54.8 hours [J. Burger, *Anim. Behav.* **22**, 521 (1974)]; R. Drent, *Behav. Suppl.* **7**, 1 (1967).
14. In 1973 and 1974, we examined incubated eggs for evidence of development. In 1975, we distinguished fertile from nonfertile eggs on the day they were laid by examining the blastodisc [B. Marquez and K. Ogasawara, *Poult. Sci.* **53**, 1607 (1974)].
15. M. MacRoberts [*Z. Tierpsychol.* **32**, 62 (1973)] reviewed the literature on "extramarital" courting in gulls and presented detailed information on this behavior for lesser black-backed (*Larus fuscus*) and herring gulls (*Larus argentatus*). In all instances, MacRoberts reported that male gulls attempted to rape females and that the females rejected these advances. No successful copulations were seen. Vermeer (*6*) reported instances of rape in glaucous-winged gulls (*Larus glaucescens*), which apparently resulted in successful copulations. J. Burger and C. G. Beer [*Behaviour* **55**, 301 (1975)] reported rape of incubating female laughing gulls (*Larus atricilla*) by intruding males.
16. Male-male courtship but not pairing has been reported in wild ostriches (*Struthio camelus australis*) [G. Sauer, *Auk* **89**, 717 (1972)]. Homosexual pairings of birds in captivity have been reported by N. Collias and L. John [*Auk* **76**, 478 (1959)], W. Dilger [*Z. Tierpsychol.* **17**, 649 (1960)], and D. Jefferies [*Ibis* **109**, 266 (1967)].
17. L. Kiff, unpublished data on museum collections of eggs taken from the Channel Islands of California and Mexico (337 clutches, 1891 to 1969).
18. In 1975, we compared chick survival and growth rates of 37 young produced by 20 normal pairs with 41 foster young given as pipping eggs to 22 pairs that had laid supernormal clutches. Since growth rates and fledging success in the two groups were nearly identical (*10*), female-female pairs can be considered capable parents under the conditions extant at Santa Barbara Island.
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16 November 1976; revised 4 February 1977

## Somatostatin: Analogs with Selected Biological Activities

Abstract. *[D-Cys$^{14}$]-Somatostatin is the first analog of somatostatin found to be more potent in inhibiting glucagon and growth hormone secretion than it is in inhibiting insulin secretion. [D-Trp$^{8}$]-Somatostatin is eight to ten times more potent than somatostatin in inhibiting insulin, glucagon, and growth hormone secretion. [D-Trp$^{8}$, D-Cys$^{14}$]-Somatostatin is more potent than [D-Cys$^{14}$]-somatostatin, but retains its relative selectivity in being a more potent inhibitor of the secretion of glucagon and growth hormone than of insulin.*

Somatostatin is now recognized as a suppressor of the secretion of various pituitary, pancreatic, and gastrointestinal hormones (*1*). In addition, somatostatin has been shown to influence an array of neurochemical, neurophysical, pharmacological, and behavioral parameters (*1*, *2*).

The plethora of actions of somatostatin and the demonstrations of its anatomic distribution throughout the central nervous system (*3*, *4*), gastrointestinal tract (*4*, *5*), and pancreas (*4*, *6*, *7*) have suggested that somatostatin might play several physiological roles as a local extracellular messenger (*1*).

Somatostatin when given continuously intravenously (*8*) or subcutaneously (*9*) decreases the glucose intolerance of human juvenile diabetes mellitus. This action is at least partially secondary to the lowering of the abnormal elevation of

Table 1. Biological activities of somatostatin (SS) and analogs of SS. The numbers in parentheses are 95 percent confidence limits.

| Peptide | Percent potency based on inhibition of | | |
|---|---|---|---|
| | GH | Insulin | Glucagon |
| SS | 100 | 100 | 100 |
| [D-Cys$^{14}$]-SS | 271 (144 to 522) | 20 (3 to 75) | 310 (151 to 1050) |
| [D-Trp$^{8}$-D-Cys$^{14}$]-SS | 647 (350 to 1168) | 130 (40 to 300) | 950 (420 to 2000) |

plasma glucagon that occurs in diabetes mellitus (*8*).

The multiple pharmacological actions of somatostatin and, therefore, its relative lack of specificity when administered parenterally and the increasing clinical interest in the possible use of this peptide in the treatment of diabetes mellitus have prompted our search for analogs of somatostatin that might have a selected action on inhibition of glucagon secretion without altering insulin or growth hormone (GH) secretion.

Analyses of the biological activities of a variety of somatostatin analogs have led to an appreciation of the strict structural requirements for biological activity. Recently a series of Asn$^{5}$ (asparagine in position 5) deleted somatostatin analogs were reported to preferentially inhibit insulin rather than glucagon or GH secretion (*10, 11*). These studies suggested the feasibility of developing analogs with selective activities.

Analogs of somatostatin have been synthesized by means of solid-phase methodology, purified, and characterized as described (*12*). To assess the biological activity of these analogs relative to somatostatin, multiple doses of somatostatin and analogs were tested for their ability to inhibit the secretion of GH from enzymatically dispersed anterior pituitary cells in vitro (*13*) and to lower the concentrations of insulin and glucagon in vivo in the portal vein in the etherized rat (*11, 14*). Potency values relative to somatostatin standard are thus obtained from four- or six-point bioassays with the use of the computer programs EXBIOL and HUBA.

[D-Cys$^{14}$]-Somatostatin is the first analog of somatostatin to be more potent in inhibition of glucagon and GH secretion (310 and 270 percent) than on inhibition of insulin secretion (20 percent) (somatostatin = 100 percent; Table 1 and Fig. 1). These results, however, may actually underestimate the selectivity of action of this peptide since, in normal animals, pancreatic insulin secretion may decrease as a regulatory mechanism to prevent hypoglycemia secondary to a specific inhibition of glucagon secretion. An analog such as [D-Cys$^{14}$]-somatostatin may be best evaluated for the therapeutic consequences of its selectivity in animals or humans with adult-type diabetes mellitus who have limited insulin reserve. In such animals or adult-type human diabetics, administration of somatostatin may actually increase glucose intolerance in contrast to its beneficial effects in juvenile diabetes (*9*). If this deterioration of glucose tolerance is indeed secondary to inhibition of the physiologically important limited insulin reserve, such an analog of somatostatin as [D-Cys$^{14}$]-somatostatin may be useful. In the best situations, the desired pharmacotherapeutic effect would be preservation of insulin secretion with a concomitant lowering of plasma glucagon.

We have previously demonstrated that [D-Trp$^{8}$]-somatostatin is eight to ten times more potent than somatostatin on inhibition of growth hormone, insulin, and glucagon (*14, 15*). We had observed that the potency of des-Asn$^{5}$-somatostatin, an analog that selectively inhibits insulin secretion, could be enhanced by incorporation of the D-Trp$^{8}$ modification (*11*). Des-Asn$^{5}$-[D-Trp$^{8}$]-Somatostatin retains the selectivity of des-Asn$^{5}$-somatostatin while exhibiting high potency (*13, 16*). We therefore considered it of interest to synthesize and test [D-Trp$^{8}$-D-Cys$^{14}$]-somatostatin. This analog has a higher potency than [D-Cys$^{14}$]-somatostatin for inhibition of the secretion of GH, insulin, and glucagon with the maintenance of a similar selectivity of glucagon to insulin ratio of 10 : 1 (Table 1). Since [D-Trp$^{8}$]-somatostatin has been reported to be less potent on inhibition of pentagastrin-induced gastric acid secretion than on inhibition of GH secretion (*17*), it could be that the D-Trp$^{8}$ modification might result in [D-Trp$^{8}$-D-Cys$^{14}$]-somatostatin possessing lower potency to inhibit gastric acid secretion than glucagon secretion.

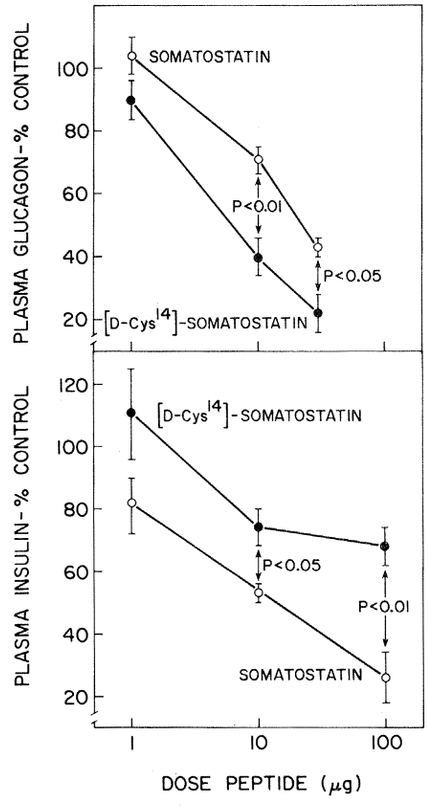


Fig. 1. Effects of graded doses of somatostatin and [D-Cys$^{14}$]-somatostatin on plasma levels of insulin and glucagon. Each point represents data on six animals.

These results suggest that there are exploitable differences between the somatostatin receptors of the various responsive cell types involved regarding their structural requirements for ligand recognition. The existence of potent somatostatin analogs that are relatively selective for glucagon inhibition should accelerate those programs assessing the potential of this approach to the therapy of diabetes mellitus.

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28 September 1976; revised 15 November 1976

# Somatostatin: Electron Microscope Immunohistochemical Localization in Secretory Neurons of Rat Hypothalamus

Abstract. *Somatostatin, the hypothalamic hormone which inhibits the secretion of growth hormone by the pituitary, has been localized in the rat hypothalamus by using electron microscope immunohistochemistry. Somatostatin occurs in secretory granules (80 to 110 nm in diameter) of a few neurons located in the hypothalamic periventricular nucleus. These observations establish the identity of neurosecretory neurons involved in the regulation of adenohypophyseal secretion.*

Since the isolation and characterization of a hypothalamic tetradecapeptide named somatostatin, which inhibits the release of immunoreactive growth hormone (*1*), many reports have been published on the distribution in the brain of this inhibiting hormone. Immunohistochemical techniques at the light microscopic (*2*) and ultrastructural levels (*3*), have shown that somatostatin is mainly concentrated in nerve endings of the median eminence.

Somatostatin-containing cell bodies have recently been described as occurring mainly in the rat preoptic and anterior hypothalamus (*4*). To establish that these somatostatin-containing cells are really secretory neurons, we have conducted an immunohistochemical study of somatostatin in the rat hypothalamus using the electron microscope. We report here that somatostatin is contained within secretory granules present in the perikarya of a few secretory neurons located in the hypothalamic periventricular nucleus.

Brains of seven adult male Sprague-Dawley rats were fixed by perfusion with 4 percent paraformaldehyde or 1 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer (*p*H 7.3). For light microscopic studies, the whole brain was fixed by perfusion for 1 to 3 days and embedded in paraffin. For immunoelectron microscopy, the hypothalami were dissected immediately after the perfusion and embedded in Araldite. Two hypothalami were subsequently fixed in 1 percent osmium tetroxide for conventional electron microscopy.

Immunohistochemical staining of somatostatin for both light and electron microscopy was performed with the peroxidase-antiperoxidase technique of Sternberger (*5*). Rabbits were injected either with synthetic somatostatin conjugated to human $\alpha$-globulin by means of glutaraldehyde, or with synthetic somatostatin conjugated to bovine thyroglobulin by the glutaraldehyde method (*6*). The antiserums thus obtained were used at a dilution of 1:1000 to 1:10,000. Specificity of the staining was verified on adjacent paraffin sections by using antiserums which had been previously absorbed with an excess of synthetic somatostatin ($10^{-4}M$).

The studies with the light microscope showed that positively stained cell bodies occurred in the preoptic and anterior hypothalamus, mostly in areas adjacent to the ependyma of the third ventricle. Both antiserums produced the same results. Adjacent control sections stained with previously adsorbed antiserums confirmed the localization of somatostatin (Fig. 1).

Ultrathin sections from the periventricular nucleus were also stained for electron microscopy. A positive reaction, as indicated by the accumulation of molecules of the peroxidase-antiperoxidase complex, was observed in the secretory granules within the perikarya of a few neurons located in the proximity of ependymal cells (Fig. 2a). Most of the secretory granules, but none of the other

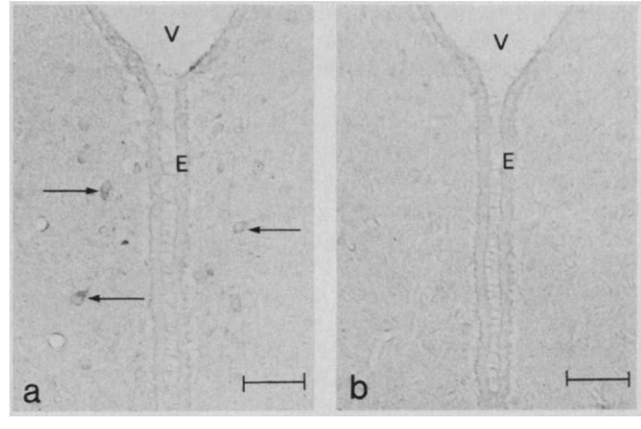


Fig. 1. (a) Frontal section from the periventricular nucleus of a male rat, stained with antiserum to somatostatin serum (diluted 1/1000). Immunostained cell bodies (→) are located adjacent to the ependyma (*E*); *V* indicates the third ventricle. (b) Control section adjacent to that shown in (a). Absorption with excess somatostatin has completely prevented the staining. Scale bars, 50 $\mu$m.

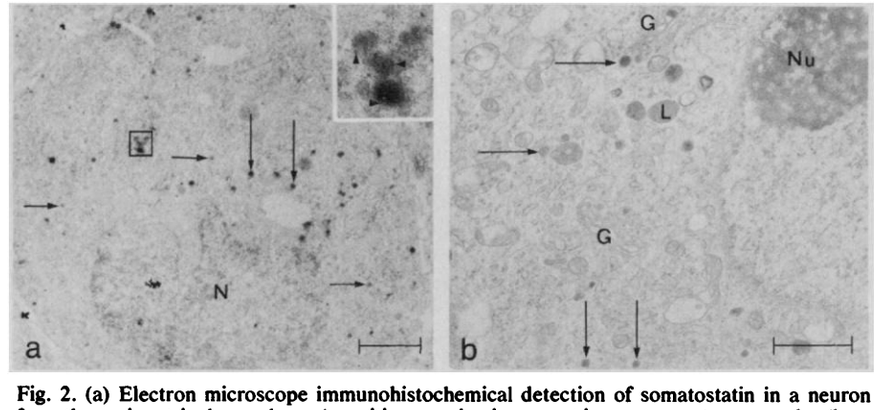


Fig. 2. (a) Electron microscope immunohistochemical detection of somatostatin in a neuron from the periventricular nucleus. A positive reaction is present in most secretory granules (long arrows) whereas a few granules are unstained (short arrows). In the inset, the higher magnification (× 44,600) indicates that the reaction is due to the accumulation of the molecules of the peroxidase-antiperoxidase complex (arrowheads). (b) Section of a neuron treated for conventional ultrastructural studies. This neuron, presumably containing somatostatin, is characterized by the presence of a well-developed Golgi apparatus (*G*), secretory granules (arrows), and lysosomes (*L*). The nucleolus (*Nu*) is prominent. Scale bars, 1 $\mu$m.