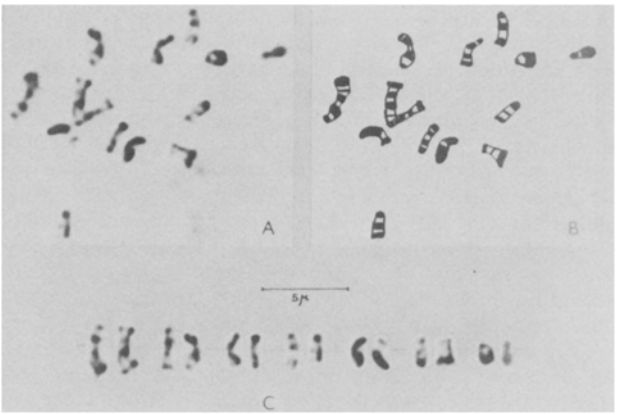
Length, arm ratio, classification and banding patterns of cucumber chromosomes

Chromosome No.	Length	Arm ratio L/S	Classification*	Number of bands Long arm	Short arm
1	2.72	1.58	SM**	3	2
2	2.52	1.39	SM**	2	2
3	2.30	1.00	M	2	2
4	1.69	1.26	SM**	1	2
5	1.48	1.76	ST	1	1
6	1.48	1.10	M	2	1
7	1.25	1.50	SM	1	1

*Giorgi and Bozzini[9]. **Chromosomes with satellite. M, Metacentric; SM, submetacentric; ST, subtelocentric.



A Somatic chromosome complement of cucumber (2n = 14) exhibiting Feulgen banding. *B* Interpretive drawing of figure A. *C* Karyotype based on length, arm ratio and banding patterns.

identification of individual chromosomes. It was possible to study in detail the karyotype following this technique. The banding patterns of chromsosomes are presented in figure A and the interpretive drawing in figure B. The conspectus of the cucumber chromosomes on the basis of length, arm ratio and banding pattern listed in the table are used in karyotyping the complement (fig. C). The specific chromosome identity produced by this technique may pave the way for further cytogenetic and evolutionary studies in cucumbers.

Feulgen banding has already been reported in animal[4-6] and plant systems[7,8]. Several mechanisms have also been postulated by these investigators to explain the chromosome banding following Feulgen staining in conjunction with different pre-treatments. Even though Schlarbaum and Tsuchiya[7] have attributed success of Feulgen banding technique to specific temperature pre-treatments, such pre-treatment is not required in cucumbers.

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Somatostatin-like immunoreactive neurons in a protochordate

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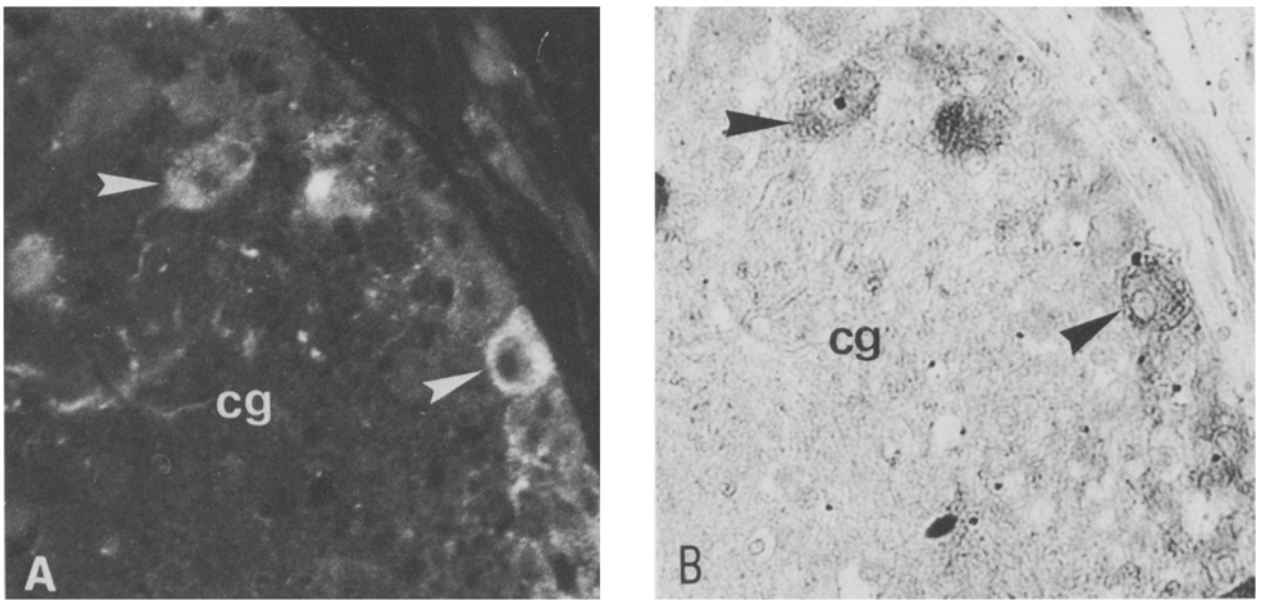
Summary. Peptidergic neurons have been localized in the cerebral ganglion of the ascidian *Styela plicata.* Using an antiserum to mammalian somatostatin, these cells display an intense immunoreactivity. The probable function of somatostatin-like peptides in the nervous system of protochordates is discussed.

Only recently have peptidergic neurons been demonstrated in protochordates. In particular, ACTH-like[1], LH-RH-like[2] and somatostatin-, substance P-, calcitonin-like[3] immunoreactivity has been localized in the nervous system of the phlebobranchiate ascidian *Ciona intestinalis.* Furthermore, CCK/gastrin-like immunoreactive neurons and nerve fibers have been detected in the cerebral ganglion of the ascidians *Styela clava* and *Ascidiella aspersa*[4].

The aim of the present work is the localization, by means of immunocytochemical methods, of somatostatin-like peptides in the cerebral ganglion of the ascidian *Styela plicata,* belonging to the suborder Stolidobranchiata.

Materials and methods. Specimens of the ascidian *Styela plicata* (Protochordata) were collected in the water inside the port of Genova. After dissection, the neural complex was fixed with Bouin's fluid in sea water for 4 h. Then, it was embedded in paraplast and serially sectioned. The sections (4 μm) were placed on albumin-coated glass slides and used for the cytochemical and immunocytochemical assays. In order to verify the presence of neurosecretory cells, the following cytochemical methods were used: Gomori chromohaematoxylin, Alcian B GX 3%-phloxine, Gomori aldehyde fuchsin, Bodian silver impregnation. The indirect immunofluorescence method[5] was applied to dewaxed and rehydrated sections. After incubation for 12–14 h in a moist chamber at 4 °C with rabbit antiserum to cyclic somatostatin conjugated to thyroglobulin (batch No. R-1801; Milab, Sweden) (diluted 1:1600 in 0.04 M



Part of the cerebral ganglion cortex (cg) of *S. plicata* demonstrating (arrows) immunofluorescent (A, × 500) and PAP-immunostained (B, × 500) neurons.

Control tests of the specificity of the antisomatostatin antiserum, performed by immunofluorescence

	Antigen/ml diluted antiserum				
	0.01 µg	0.1 µg	1 µg	10 µg	> 10µg
Somatostatin	+	+	+ +	+ +	+ +
Glucagon	0	0	0	0	0
Insulin	0	0	0	0	0
GIP	0	0	0	0	0

0, Normal fluorescence; +, some reduction; + +, no fluorescence.

phosphate buffered saline-PBS) as the first layer and for 1 h in a moist chamber at room temperature with FITC-conjugated goat anti-rabbit γ-globulin (Behring Institute) (diluted 1:100 in PBS) as the second, the sections were washed in PBS, mounted with glycerol-PBS (9:1, v/v) and examined with a Zeiss epifluorescence microscope. Subsequently, the cover glasses were removed and the sections were rinsed for 30 min in PBS. Then the peroxidase-antiperoxidase (PAP) method of Sternberger[6] was applied by placing one drop of rabbit-PAP-complex (lot 4-1597; Polysciences, USA) on the sections for 1 h at room temperature in a moist chamber. Following rinsing in PBS for 30 min, the peroxidase activity was demonstrated by exposure of the sections to a fresh solution of 3,3'-diaminobenzidine-4 HCl (60 mg/100 ml) and hydrogen peroxide (0.01%) in 0.05 M Tris buffer, pH 7.6, for 1 h. The endogenous peroxidase was previously blocked with 1.2% H_2O_2 in 0.05 M Tris buffer, pH 7.6. Then, the sections were dehydrated, mounted in DPX and examined by an ordinary light microscope.

The specificity of the antiserum was tested by absorptions overnight at 4 °C with different amounts of synthetic somatostatin (Sigma Chem., USA), glucagon (Sigma Chem., USA), insulin (Calbiochem, USA), gastric inhibitory peptide (GIP) (Paesel GmbH, FRG). The results of these tests are shown in the table.

Control staining was carried out with:

- anti-somatostatin preabsorbed overnight at 4 °C with 10 µg/ml diluted antiserum of synthetic somatostatin;
- FITC-conjugated goat anti-rabbit γ-globulin only;
- rabbit-PAP-complex only.

Sections of rabbit pancreas were also used as positive controls.

Results. The neural complex of *S. plicata* consists of a cerebral ganglion and of a neural gland, dorsally located. These anatomical structures are situated between the 2 siphons and they open by means of the neural gland duct in the branchial basket cavity with a ciliated funnel called the dorsal tubercle. In *S. plicata* the cerebral ganglion displays an outer cellular cortex in which appear numerous large neurons, and a fibrous medulla also with some small neurons.

The cytochemical tests show an intensive basophilia and argyrophilia only in some cortically located perikarya. Using an antiserum to mammalian somatostatin, the same cells are immunofluorescent (fig., A) and PAP-immunostained (fig., B). The nerve fibers and the neural gland do not seem to be immunoreactive. After incubation with antiserum preabsorbed with somatostatin, the immunoreactivity disappears in all the neurons.

Discussion. The concept of 'peptidergic neurons'[7] was introduced for the neurosecretory cells of the hypothalamus that release their active factors in the blood stream or at 'neurosecretomotor junctions'. Subsequently, more than 20 peptides have been found in neurons of vertebrates, and Snyder[8] has suggested that at least some of these molecules, including somatostatin, act as neurotransmitters. The presence of neurosecretory cells in protochordates has been a subject for debate. Mazzi[9] did not detect neurosecretory phenomena in the neurons of the cerebral ganglion of *C. intestinalis*. Afterwards, mainly on the basis of ultrastructural investigations, neurosecretory cells were localized in several ascidians[10-13].

In the cerebral ganglion of *S. plicata* the neurosecretory cells contain somatostatin-like peptides with a probable neurotransmitter role, as well as in some species of invertebrates in which somatostatin-like immunoreactive neurons[14-16] have been localized.

After these observations it is necessary to make further investigation on the evolutionary neuroendocrinology of protochordates in order to explain the functions and relationships of the several neuropeptides produced in the neurons of the cerebral ganglion.

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Bombesin-like immunoreactivity in the pituitary gland

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Summary. Bombesin has been shown to stimulate release of anterior pituitary hormones both in vivo and in vitro. The aim of this study was to determine whether bombesin-like immunoreactivity could be detected in the human pituitary. Significant concentrations were found in the human anterior gland (4.6± 1.5 pmoles/g), posterior gland (1.5±0.4 pmoles/g) and stalk (8.1±0.8 pmoles/g). Significant amounts were also observed in the guinea-pig pituitary. Gel permeation chromatography revealed the presence of 2 major molecular forms of bombesin-like immunoreactivity, one co-eluting with porcine gastrin-releasing peptide and the other with amphibian bombesin.

A number of peptides which have been found in the brain and intestinal tract have been shown to be present in the pituitary and have stimulating effects on anterior pituitary hormone production. These include substance P[2-4], vasoactive intestinal polypeptide[5,6], somatostatin[3,7,8] and neurotensin[3,4,9,10].

Pharmacological studies have revealed that bombesin can also alter the release of anterior pituitary hormones, its effect apparently being dependent on the route of administration. For example, in the rat, i.v. injections of bombesin increases plasma levels of prolactin (PRL) and growth hormone (GH), whereas i.c.v. injection of the peptide inhibits stress induced PRL and basal GH secretion[3,10]. Bombesin also stimulates luteinising hormone (LH) release from incubated rat pituitary tissue[11], but as to whether PRL and GH release can be altered in a similar way is unclear[3,12]. A study using cultured bovine pituitary cells has shown that bombesin stimulates GH release, but fails to alter PRL secretion[13].

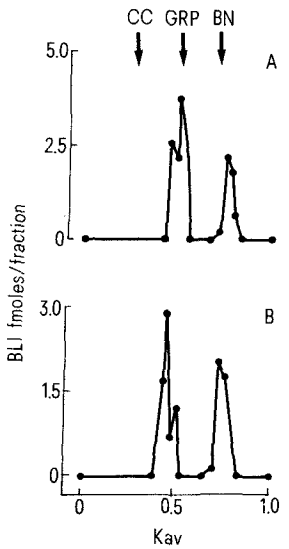
The aim of this study therefore, was to determine whether bombesin-like immunoreactivity (BLI) could be detected locally within the human and guinea-pig pituitary and to characterize this immunoreactivity chromatographically.

Materials and methods. Source of tissue. Human. Human pituitary tissue was collected post-mortem (mean age 80 years, range 34-95 years; mean autopsy delay 39 h, range 15-62 h) from patients who had died without neurological or psychiatric disease. The tissue was divided into the stalk, anterior and posterior lobes (n=6) and stored at −70 °C.

Animals. Adult male Duncan Hartley guinea-pigs (n=10) were killed by decaptitation and the pituitary glands removed.

Extraction. Each tissue was weighed and rapidly placed in boiling 0.5 M acetic acid (10 ml acid/g wet tissue) for 10 min[14]. They were then allowed to cool and stored at −20 °C until assay.

Radioimmunoassay. The tissue extracts were thawed and



Gel permeation chromatography profiles of guinea-pig (A) and human (B) pituitary extracts. CC, cytochrome C; GRP, gastrin-releasing peptide; BN, bombesin.

BLI in the human and guinea-pig pituitary (number of samples in parenthesis)

Species	Region	pmoles/g ± SEM
Human	Stalk (6)	8.1 ± 0.8
	Anterior (6)	4.6 ± 1.5
	Posterior (6)	1.5 ± 0.4
Guinea-pig	Whole (10)	18.09 ± 2.13