formations (species formations) without lineage splitting. This model differs from the punctuational model in that new species are not assumed to evolve through lineage splitting and in that the transformations are not assumed to be geologically instantaneous.

The acceleration of evolution that led to the transition of *G. plesiotumida* to *G. tumida* may have been driven by an increase in selection pressure across the Miocene-Pliocene boundary. Density changes in the upper water column associated with the Mediterranean salinity crisis (*13*) could have caused the morphologic changes. Density changes may induce morphologic adaptations in planktonic foraminifera in order to maintain buoyancy in the water column (*14*).

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26 January 1984; accepted 17 April 1984

An Endogenous Peptide Stimulates Secretory Activity in the Elasmobranch Rectal Gland

Abstract. *Extraction and partial purification of peptide material from the intestine of the elasmobranch* Scyliorhinus canicula *yielded a fraction that shows potent stimulatory activity in the rectal gland. The extracted material appears to contain an endogenous peptide (or peptides) that represents the natural hormone responsible for the control of rectal gland secretion in vivo.*

The elasmobranch rectal gland is an extrarenal salt-secreting organ that is important in maintaining the overall ionic balance of the animal (*1*). The secretion rate in the isolated gland is greatly increased by the cyclic nucleotide analog dibutryl adenosine 3′,5′-monophosphate (cyclic AMP) and the phosphodiesterase inhibitor theophylline (*2*). This stimulation of secretion is associated with marked increases in ouabain binding and ouabain-sensitive oxygen consumption (*3, 4*), which presumably reflect increased sodium pump activity. The cyclic AMP stimulation of sodium pump activity, however, is an indirect effect of a cyclic AMP–induced increase in sodium entry via a furosemide-sensitive chloride-coupled cotransport system (*4, 5*). The participation of cyclic AMP in regulating secretion implies a hormonal control. Stoff *et al.* (*6*) therefore investigated a range of exogenous peptide hormones and neurohormonal factors for stimulatory activity in the isolated perfused gland of *Squalus acanthias*. Of the substances tested, only the polypeptide vasoactive intestinal peptide (VIP) stimulated secretion. However, it has been reported that VIP has no effect on rectal gland secretion in another elasmobranch *Scyliorhinus canicula* (*7*), thereby raising doubts that VIP is the natural hormone responsible for controlling rectal gland secretion in elasmobranchs in vivo. We now report the results of an alternative approach, that of isolating the native endogenous secretagogue from elasmobranch tissues.

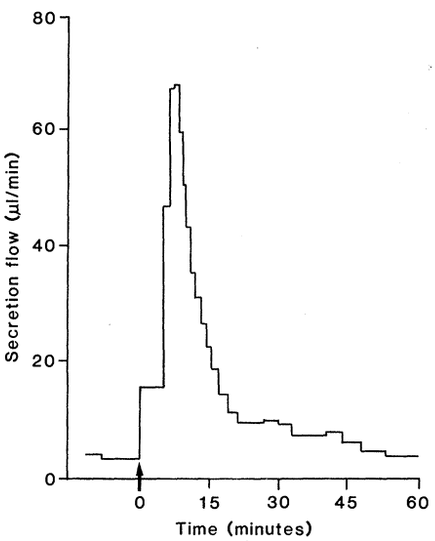

Fig. 1. The effect of fraction 13 on secretion rate in the perfused gland of *Squalus* showing a typical result. The gland was isolated and perfused at a constant pressure (20 mmHg). Secreted fluid was collected by a cannula in the secretory duct, and the flow rate was determined by a microprocessor-controlled flowmeter connected to a drop detector. Output from the flowmeter was proportional to flow rate and was recorded on a chart recorder. Perfusion flow rate was 2.8 ml g^{-1} min^{-1}, and a bolus injection of 100 µl of saline containing 100 µg of fraction 13 was introduced into the afferent perfusion line at time zero.

Extracts of intestines (ileum plus rectum) obtained from freshly killed *Scyliorhinus* were prepared by standard procedures for peptide isolation (*8*). Boiling of the intestines in water for 10 minutes was followed by acidification with acetic acid (final concentration 3 percent) and extraction overnight at 5°C. After filtration, peptides were absorbed to alginic acid and then eluted with dilute hydrochloric acid (0.2 mol/liter). Sequential purification of peptide material was carried out by means of Sephadex gel filtration, ion-exchange chromatography (carboxymethyl cellulose; ammonium bicarbonate stepwise gradient, 0.01 to 0.2 mol/liter) and reversed-phase high-performance liquid chromatography (HPLC). A 0.05 percent trifluoroacetic acid–acetonitrile gradient (20 to 50 percent) was used for HPLC, which was performed with two Waters M6000A pumps and a µBondapak C_{18} column with a model 441 detector operating at 214 nm. Eluted material was freeze-dried after evaporation of acetonitrile under dry nitrogen. This yielded a series of partially purified peptide fractions, which were screened at each stage in the separation procedure for biological activity in the rectal gland by determining their effect on oxygen consumption in slices of the gland of *Scyliorhinus*. Only one of the HPLC fractions (fraction 13) had a potent stimulatory action in the slices from *Scyliorhinus*. This fraction was tested further for activity in *Squalus* and in the ray *Raja clavata*. The peptide content of the tested fractions was determined by biuret analysis of peptide

bonds, and the results were expressed as micrograms of peptide material.

Slices (0.2 to 0.3 mm thick) of the glands from *Scyliorhinus* and *Raja* were prepared and used to determine oxygen consumption and ouabain binding (*3, 4*). Fraction 13 stimulated oxygen consumption in the slices from both species to values very similar to those produced by a combination of cyclic AMP and theophylline (Table 1). Vasoactive intestinal peptide, on the other hand, was completely without effect on the oxygen consumption of the glands from either species. As with cyclic AMP and theophylline, the stimulation in oxygen consumption induced by fraction 13 was entirely ouabain-sensitive. The increases in [^{3}H]ouabain binding produced by fraction 13 in slices from the glands of both *Scyliorhinus* and *Raja* were essentially the same as those produced by cyclic AMP plus theophylline, whereas, again, VIP was completely without effect (Table 2).

The observed increases in ouabain-sensitive oxygen consumption and ouabain binding are indicative of a stimulation of sodium pump activity in the cells. Although it is possible to stimulate the sodium pump in rectal gland cells by processes that do not lead to increases in secretion—for example, by increasing sodium entry with amphotericin B (*5*) or monensin (*9*)—increases in the parameters measured are usually associated with a stimulation of secretory activity in the gland. In such circumstances they result from an increased sodium entry into the cell through a chloride-coupled (and probably potassium-coupled) furosemide-sensitive pathway (*10*) that is stimulated by cyclic AMP. The failure of VIP to affect either oxygen consumption or ouabain binding confirms the earlier report for *Scyliorhinus* and extends it to another species of elasmobranch, *Raja clavata*, and implies that this agent is not able to stimulate secretory activity in the glands of either of these species.

The peptide nature of the active component was confirmed in preliminary experiments showing loss of activity when fraction 13 was digested with protease (Sigma; isolated from *Streptomyces griseus*) for 2 hours at 40°C and then boiled for 5 minutes. Oxygen consumption was only slightly increased (from 147 $\mu l\ g^{-1}\ hour^{-1}$ to 261 $\mu l\ g^{-1}\ hour^{-1}$) in an experiment in which fraction 13 and protease were incubated together. This contrasts with a typical stimulation of oxygen consumption (from 147 $\mu l\ g^{-1}\ hour^{-1}$ to 1090 $\mu l\ g^{-1}\ hour^{-1}$) in an experiment in which fraction 13 and protease were incubated separately, boiled, and then mixed together.

Finally, we investigated the effect of fraction 13 on secretion rate in the isolated perfused gland of *Squalus* (*7*). Fraction 13, injected as a bolus into the perfusion line, produced a pronounced stimulation of secretion flow rate in the isolated gland (Fig. 1). The onset of the response was rapid, reaching a peak within 5 minutes of injection; the response then declined progressively for 30 to 40 minutes. The sodium concentration of the secreted fluid was not affected by the fraction 13 (520.9 ± 13.5 mmol/liter versus 520.6 ± 5.0 mmol/liter). In the 15-minute period after a bolus injection of 100 μg of fraction 13, the sodium secretion rate averaged 16.2 ± 1.04 $\mu mol\ g^{-1}\ min^{-1}$ (mean ± standard error; $n = 6$). This compares with an average rate of 1.3 ± 0.45 $\mu mol\ g^{-1}\ min^{-1}$ (mean ± standard error; $n = 4$) over a similar period after a bolus injection of an identical volume of saline alone.

It is clear from the data presented that VIP does not stimulate secretory activity in the glands of *Scyliorhinus* or *Raja*. This implies that either there is a pronounced species diversity in the natural secretagogue responsible for controlling rectal gland function or the response to VIP in *Squalus* is, in some way, nonspecific or at least nonphysiological. In support of the latter suggestion, fairly high concentrations of VIP ($>10^{-8}$ mol/liter) were required to produce a response in *Squalus* (*6*). In addition, even in *Squalus*, infused VIP did not mimic the stimulation seen in a pithed fish preparation or in an explanted blood-perfused gland after volume loading (*11*). The evidence suggests, therefore, that VIP is not the natural hormonal mediator of the secretory response in the rectal gland in vivo.

Table 1. Oxygen consumption of slices of rectal gland and the effects of cyclic AMP plus theophylline, VIP, and fraction 13. Slices (0.2 to 0.3 mm thick) were prepared on a mechanical tissue chopper and incubated in saline in a differential respirometer at 11°C. Readings of oxygen consumption were taken at approximately half-hourly intervals. After an initial stabilization period, one of the following was added: cyclic AMP and theophylline (final concentrations 0.05 and 0.25 mmol/liter, respectively); VIP (final concentration 0.1 μmol/liter); or fraction 13 (final concentration 50 μg/ml). Oxygen consumption was read until stable values were obtained (usually 30 to 60 minutes in each case). Then, ouabain (final concentration 0.1 mmol/liter) was added to the flasks and readings continued until stable values were again obtained (usually within 60 minutes). Values represent means ± standard error.

Treatment	n	Oxygen consumption ($\mu l\ g^{-1}\ hour^{-1}$)		
		Control	Experimental	
			Without ouabain	With ouabain
		Scyliorhinus		
Cyclic AMP and theophylline	6	222 ± 43	1034 ± 122*	173 ± 15
VIP	6	254 ± 23	220 ± 21	
Fraction 13	4	123 ± 27	1285 ± 62*	112 ± 16
		Raja		
Cyclic AMP and theophylline	5	189 ± 28	888 ± 98*	90 ± 17
VIP	6	139 ± 6	125 ± 6	
Fraction 13	4	122 ± 24	962 ± 63*	79 ± 18

*Significantly different from corresponding control value ($P < 0.001$).

Table 2. Ouabain binding in slices of the rectal gland and the effects of cyclic AMP plus theophylline, VIP, and fraction 13. Slices of the gland, prepared as before, were incubated in the appropriate saline containing [^{3}H]ouabain (0.25 μCi/ml) and "cold" ouabain (final concentration 2.2 μmol/liter), at 11°C for 2 hours. After being washed three times, for 5 minutes each time, in ice-cold ouabain-free saline, the tissue was dried, weighed, and digested with Soluene (Packard). The radioactivity in the tissue was determined by liquid scintillation spectroscopy with channels ratio quench correction, and the results are expressed as picomoles of ouabain bound per milligram of dry tissue. Concentrations of agents used were as given in Table 1. Values are means ± standard error. The number of glands tested is given in parentheses.

Control	Ouabain bound (pmol/mg)		
	Cyclic AMP and theophylline	VIP	Fraction 13
	Scyliorhinus		
9.6 ± 0.5 (8)	26.8 ± 2.2* (4)	10.0 ± 0.5 (6)	18.3 ± 1.8* (4)
	Raja		
7.4 ± 1.9 (4)	17.3 ± 3.1* (4)	8.9 ± 1.9 (4)	20.2 ± 2.6* (4)

*Significantly different from corresponding control values ($P < 0.05$).

In contrast, the partially purified peptide extract obtained from the intestine of the elasmobranch *Scyliorhinus*, which we refer to as fraction 13, contains a component or components that show potent stimulatory activity in the rectal glands of at least three widely differing species of elasmobranch. This component probably has a major role in the control of rectal gland secretion in vivo and is clearly responsible for determining the secretion rate. Preliminary experiments suggest that this component is restricted to the intestine, as similar extracts prepared from the stomach of *Scyliorhinus* were not active in the assays described. Although the constituents of fraction 13 are unknown, preliminary evidence based on methanol solubility and retention times on octadecyl silane reversed-phase HPLC with acetonitrile-trifluoroacetic acid gradients indicates that the peptide (or peptides) has properties distinct from those characteristic of VIP. In addition, radioimmunoassay of fraction 13 showed negligible quantities (<1 fmol/μg) of VIP-like immunoreactivity. Although its precise identity must await further purification and sequencing, we propose the name "rectin" for this putative peptide hormone.

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9 January 1984; accepted 20 April 1984

Antibodies to the Core Protein of Lymphadenopathy-Associated Virus (LAV) in Patients with AIDS

Abstract. *Lymphadenopathy-associated virus (LAV), a human T-lymphotrophic retrovirus isolated from a homosexual man with lymphadenopathy, has been causally associated with acquired immunodeficiency syndrome (AIDS). A sensitive and specific radioimmunoprecipitation test was developed for the detection of antibodies to the major core protein of LAV, p25 (molecular weight 25,000). Antibody to LAV p25 was found in the serum of 51 of 125 AIDS patients, 81 of 113 patients with lymphadenopathy syndrome, 0 of 70 workers at the Centers for Disease Control (some of whom had handled specimens from AIDS patients), and 0 of 189 random blood donors. Of a group of 100 homosexual men from San Francisco whose serum was obtained in 1978, only one had antibody to LAV p25; in contrast, of a group of 50 homosexual men in the same community whose serum was obtained in 1984, 12 had antibodies to LAV p25.*

One of the first indications that a human retrovirus might have a role in the etiology of AIDS was the finding of an increased prevalence, in patients with AIDS and lymphadenopathy syndrome (LAS), of antibodies to membrane antigens of human T-cell leukemia virus type I (HTLV-I) infected lymphocytes (*1*). HTLV-I had been frequently isolated from patients with mature T-cell malignancies and is etiologically associated with adult T-cell malignancy endemic in southern Japan, the Caribbean islands, and Africa (*2*, *3*). However, the rarity of HTLV-I isolates from AIDS and LAS patients and the low titer of antibodies to HTLV membrane antigens, together with the low prevalence of antibodies to virion core protein antigens of HTLV-I (*4*), suggested that cross-reacting antibodies to a related retrovirus was responsible for the initial observations. At the same time, a T-lymphotrophic retrovirus, subsequently termed lymphadenopathy-associated virus (LAV), was described (*5*). This cytopathic retrovirus was isolated from a homosexual man with lymphadenopathy. That this virus is linked to AIDS is suggested by (i) the presence of antibody to the virus in AIDS and LAS patients (*6*), (ii) the repeated isolation of the virus from AIDS and LAS patients (*6*), and (iii) the isolation of the virus from a blood donor–recipient pair of AIDS patients (*7*). Another T-lymphotrophic retrovirus, termed HTLV-III, has been isolated repeatedly in similar circumstances and antibodies to this virus have also been found in a high proportion of AIDS and LAS patients (*8*). Although comparative studies of LAV and HTLV-III have not been reported, it is possible that the two viruses are the same.

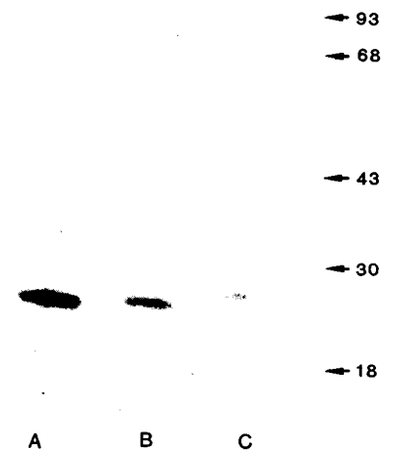

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of ^{125}I-labeled core proteins of (lane A) HTLV-I, (lane B) HTLV-II, and (lane C) LAV. HTLV-I p24 was purified from HTLV-I as described (*9*). By the same techniques, HTLV-II p24 was purified from a cell line (MC) developed in our laboratory. This cell line, which contains the complete integrated genome of HTLV-II, produces large quantities of virus. LAV was purified from supernatant fluids of primary lymphocyte cultures as described (*5*). Virus pellets were prepared from supernatant fluids obtained at the time of peak reverse transcriptase activity. The resuspended pellets were concentrated by centrifugation through a 30 percent sucrose cushion and then subjected to phosphocellulose chromatography. The product of this purification procedure was examined for homogeneity by electrophoresis on polyacrylamide gel.

We have developed a highly sensitive radioimmunoprecipitation assay that is specific for the major core protein, p25, of LAV. We used this assay, together with similar assays for antibodies to the core proteins (p24) of HTLV-I and HTLV-II, to determine antibody prevalence in groups of patients with AIDS on LAS, homosexual men, laboratory workers, and blood donors.

LAV was propagated in primary cultures of blood lymphocytes (*5*). At the time of peak reverse transcriptase (RT) activity, supernatant fluids were harvested from infected lymphocyte cultures by low-speed centrifugation, and virus was pelleted by ultracentrifugation