Therefore, the only common hemodynamic variable in the two experimental situations described above is a fall in perfusion flow. It is clear therefore that this must be the specific parameter responsible for the decline in secretion rate observed and that changes in perfusion pressure and vascular conductance are, themselves, of little effect.

It is known that perfusion flow through the gland is dramatically affected by physiological concentrations of circulating catecholamines (Shuttleworth and Thompson, Bull. MDIBL 21:59-62, 1981; Shuttleworth, J. Exp. Biol. 103:193-204, 1983). For example, perfusion flow through the gland was shown to be reduced to approximately 20% of control levels by 3 10^{-7} mol l^{-1} norepinephrine, a value within the range found in vivo. During stimulation of the gland however, the perfusion pathway is vasodilated via a cAMP-mediated processes that is independent of the secretory process itself (Shuttleworth, loc. cit. and Amer. J. Physiol., in press). The data presented in this study permit a quantitative assessment of the significance of this vasodilatory effect in the overall secretory response. At a perfusion flow equivalent to 20% of control levels the maximum secretion rate is approximately 8.0 μmol g^{-1} min^{-1} (see Figure 2) or only 30-33% of that seen in the fully vasodilated controls. Thus, in the presence of only a moderate circulating concentration of catecholamines, the independent, but simultaneous, vasodilation response described above will increase secretion rate some three times. This work was supported by grants from the U.K. Science and Engineering Research Council (GR/B/67063 and GR/C/41777) and the Marshall and Orr Bequest of the Royal Society, London.

STIMULATION OF RECTAL GLAND SECRETION BY AN ENDOGENOUS PEPTIDE

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The involvement of cAMP in regulating ion secretion in the elasmobranch rectal gland implies a hormonal control and, with this in mind, Stoff et al. (Am. J. Physiol. 237:F138-F144, 1979) investigated a range of exogenous peptide hormones and neurohumoral factors for stimulatory activity in the isolated perfused gland of Squalus. Of those tested, only the polypeptide vasoactive intestinal peptide (VIP) was successful in stimulating secretion. However, it has been shown that VIP is totally without effect on ouabain-sensitive oxygen consumption and ouabain binding in the glands of two other elasmobranch species - Scyliorhinus canicula and Raja clavata (Shuttleworth, in preparation). This failure of VIP to affect oxygen consumption or ouabain binding implies that this agent is not able to stimulate secretory activity in the glands of either of these species and thereby raises doubts as to whether VIP is indeed the natural hormone responsible for controlling rectal gland secretion in elasmobranchs in vivo. This report describes some initial success we have had in pursuing an alternative approach, that of isolating the native endogenous secretagogue from elasmobranch tissues.

Extracts of the intestines obtained from freshly sacrificed Scyliorhinus were prepared according to standard procedures for peptide isolation (Mutt, in "Gut Hormones", ed. S. Bloom, pp. 21-27, 1978), involving boiling in water and subsequent acetic acid extraction. Following absorption of peptides to alginic acid and elution with dilute hydrochloric acid (0.2M), sequential purification of polypeptide material was carried out by means of Sephadex gel filtration, ion exchange chromatography and reversed phase high performance liquid chromatography (HPLC). High performance liquid chromatography was performed utilizing an 0.05% trifluoroacetic acid:acetonotrile gradient (15%-28%) using a detector operating at 214 nm. This procedure yielded a series of partially purified peptide fractions which were screened for activity in the rectal gland by determining their effect on oxygen consumption in slices of the gland of Scyliorhinus. One of these fractions ("Fraction 13") was found to be a potent stimulator of ouabain-sensitive oxygen consumption and ouabain binding in the slices from both Scyliorhinus and Raja

clavata. The peptide content of the tested fractions was determined by Biuret analysis of peptide bonds and the results expressed as μg of peptide material.

The effect of Fraction 13 on secretion rate was investigated in the isolated perfused gland preparation of Squalus. The technique for perfusion was the same as that described previously (Shuttleworth, J. Exp. Biol. 103:193-204, 1983) with the exception that perfusion flow and secretion flow were monitored by means of a microprocessor-controlled flow recorder. Fraction 13, given as a bolus injection (100 μg in 100 μl) into the afferent perfusion line, produced a pronounced stimulation of secretion flow rate in the isolated gland (Figure 1). The

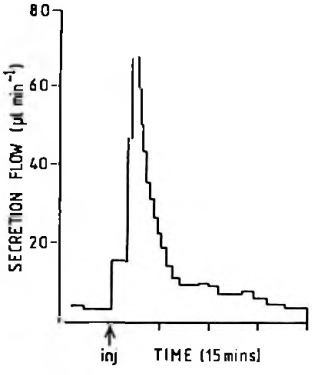

Figure 1.--Effect of a bolus injection (100 μg) of Fraction 13 on secretion flow in an isolated perfused gland. Representative trace.

response was rapid in onset reaching a peak within five minutes of injection and lasted, at a progressively declining level, for some 30-40 minutes. The sodium concentration of the secreted fluid was not affected by the Fraction 13 (520.9 ± 13.5 mmol l^{-1} vs 520.6 ± 5.0 mmol l^{-1}). The mean secretion rate over the fifteen minute period following bolus injection of 100 μg of Fraction 13, is shown in Table 1. Peak secretion flows were more than twice the mean

Table 1.--Effect of Fraction 13 (100 μg) on Rectal Gland Secretion Rate

Perfusion medium	Injection	N	Mean secretion rate (μmol g^{-1} min^{-1})
Saline	Saline	4	1.3 ± 0.5
Saline	Fraction 13	6	16.2 ± 1.0
Saline + furos.	Fraction 13	4	4.2 ± 0.7

Injection volume = 100 μl in all cases. Mean ± S.E. Furosemide concentration = 0.5 mmol l^{-1}.

flows over this time period (peak flow = 67.3 ± 0.4 μl g^{-1} min^{-1}, mean flow over 15 mins = 30.9 ± 1.4 μl g^{-1} min^{-1}). It can be seen from Table 1 that, even with the declining response over the 15 minute measuring period resulting from the utilisation of a bolus injection procedure, Fraction 13 produced a greater than 12-fold stimulation of secretion rate over control levels. Peak stimulations represented secretion rates equivalent to some 25-30 times that seen in control glands.

Also shown in Table 1 is the effect of a similar dose of Fraction 13 given in the presence of 0.5 mmol l^{-1} furosemide. As can be seen, the diuretic produces a clear inhibition (amounting to approximately 80%) of the stimulation of secretion by Fraction 13. Peak values of secretion flow were similarly inhibited in the presence of furosemide.

As mentioned above, VIP does not stimulate secretory activity in the glands of Scyliorhinus or Raja. The

implication of this is that, either there is a pronounced species diversity in the nature of the natural secretagogue responsible for controlling rectal gland function, or that the response to VIP seen in Squalus is, in some way, non-specific or at least non-physiological. In support of the latter suggestion it should be pointed out that fairly high concentrations of VIP ($> 10^{-8}$ mol l^{-1}) were required to produce a response in Squalus. The evidence suggests, therefore, that VIP is not the natural hormonal mediator of the secretory response in the rectal gland in vivo.

In contrast to this, the partially purified peptide extract obtained from the intestine of the elasmobranch Scyliorhinus and here described as Fraction 13, clearly contains a component (or components) that shows potent stimulatory activity in the rectal glands of all three widely differing species of elasmobranch studied to date. This component is therefore likely to play a major role in the control of secretion by the gland in vivo and possibly represents the native hormone responsible for determining secretion rate by the rectal gland. Although the precise constituents of Fraction 13 are, as yet, unknown preliminary evidence based on methanol solubility and retention time on HPLC gradients indicate properties distinct from those characteristic of VIP. Whilst its identity must await further purification and sequencing, we would like to propose the name "rectin" for this putative peptide hormone.

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BASOLATERAL CHLORIDE CONDUCTANCE IN FLOUNDER URINARY BLADDER

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The isolated urinary bladder of the winter flounder actively absorbs sodium and chloride and actively secretes potassium (Dawson and Andrew, Bull.MDIBL 19:46, 1981, and 20:89, 1982). The basolateral sodium transport step probably involves an electrogenic, Na/K ATPase (Dawson and Andrew, Bull. MDIBL 20:89, 1982), but the mechanism of chloride exit from the cell is unknown. The object of the present experiments was to provide a simple test for a basolateral chloride conductance in flounder urinary bladder.

Portions of flounder bladder were mounted as flat sheets in Ussing chambers as previously described (Dawson and Andrew, Bull. MDIBL 19:46, 1981), and were treated with ouabain (0.1 mM, serosal) to abolish the active transport of sodium, chloride and potassium. The ouabain-treated bladders were bathed on both sides by Ringer's solutions which contained (in mM) Na:147.5, K:2.5, Ca:1.5, Mg:1.0, Cl:5.0, gluconate:142.5, HEPES:15.0. Both sides were stirred with air and the pH was approximately 7.5 at room temperature. The serosal solutions also contained Verapamil (10^{-5} M) to inhibit smooth muscle contractions.

In these experiments we exploited the fact that the polyene antibiotic, amphotericin-B, when added to one side of a biological or an artificial membrane, forms pores which although they are moderately cation selective also possess a significant chloride conductance. Thus we added amphotericin-B (10 μM) to the mucosal bathing solution to increase the chloride conductance of the apical membranes and then measured short circuit currents generated by imposing a chloride concentration gradient across the tissue. Rendering the bathing solutions initially low in chloride enabled us to change the chloride concentration by simply adding a small volume of concentrated NaCl to either bathing solution.

Figure 1 shows the results of an experiment in which the amphotericin-B was first added to the mucosal bath in the absence of any transmural chloride gradient. The short circuit current before addition of the polyene was near zero since active transport had been inhibited by ouabain. The addition of amphotericin-B increased the trans-epithelial conductance more than six-fold (from 1.5 to 10 mS/cm^2), but had only a slight effect on the current. This result is expected in the absence of driving forces for transepithelial current flow. The addition of NaCl