These experiments illustrate several important aspects of the relation between oxygen consumption and chloride transport in the rectal gland. Oxygen consumption increases in a linear fashion with increases in the rate of chloride secretion, indicating that chloride secretion depends on aerobic metabolism. The oxygen consumption at low rates of chloride secretion before stimulation by cAMP, is not correlated with changes in chloride secretion implying that at these rates there is no direct coupling. Some portion of oxygen consumption in this basal state appears to be available for transport of chloride when secretion is stimulated. The molar ratio of sodium chloride transported to oxygen consumed in the stimulated gland is greater than 18/1 (a value predicted from the ratio 3 Na/1 ATP found in red blood cells and membrane vesicles), and varies from 20/1 to 30/1. Finally, the chemical gradient against which chloride is secreted does not affect the rate of oxygen consumption.

THE ROLE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) IN THE REGULATION OF ACTIVE CHLORIDE SECRETION IN THE RECTAL GLAND OF *Squalus acanthias*

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The observation that the intracellular level of cyclic AMP modulates active chloride secretion in the rectal gland has stimulated a search for the endogenous factor(s) which evokes this response (Stoff et al. Bull. Mt. Desert Island Biol. Lab. 16:95-98, 1976). A number of humoral and neurohumoral agents were investigated which failed to alter chloride secretory rate in an in vitro isolated perfused gland model; these included: vasopressin and several oxytocin derivatives normally found in the posterior lobe of the pituitary of the dogfish (aspartocin, valitocin, vasotocin), norepinephrine, epinephrine, serotonin, substance P and calcitonin. Since the rectal gland is an appendage of the elasmobranch hind gut, a number of hormones which stimulate intestinal secretion have been studied as well; these include: pentagastrin and a structural related group of three peptide hormones, glucagon, secretin, and vasoactive intestinal peptide (VIP). Vasoactive intestinal peptide (10^{-6}-10^{-8} M) was the only hormone which stimulated chloride secretory rate in this tissue.

All experiments were performed with spiny dogfish of either sex captured by hook and line from Frenchman Bay, Maine. Glands were removed and perfused in vitro as previously described (Stoff et al. J. Exptl. Zool. 199:443-448, 1977). All hormones studied were dissolved in the perfusion media and added to the perfusion reservoir or infused as a single bolus over a one minute time interval into the arterial line. Duct fluid was collected at timed intervals and measured volumetrically. Chloride was measured by amperometric titration. Partial pressure of oxygen in arterial and venous samples obtained under anaerobic conditions was measured by a polarographic oxygen electrode maintained at 15°C.

Two groups of four unanesthetized fish underwent either intravascular volume expansion (50 ml of 1 M NaCl) or intragastro-intestinal volume expansion (100 ml of 1 M NaCl). Blood samples were taken from the dorsal aorta at 0, 5, 15, 30 and 60 minutes. Plasma was assayed for VIP by radioimmunoassay, sodium by flame photometry and osmolality by freezing point depression. A third group of four fish were sacrificed and nine different organs were removed, frozen in liquid nitrogen and stored at -20°C for one month. Tissue was then thawed, homogenized in 0.3 M phosphate buffer (pH 7.4), centrifuged at 15,000 RPM for 30 minutes and the supernatant assayed directly by radioimmunoassay for VIP.

In the nonstimulated state the rectal gland secretory rate gradually declines to 10.3 ± 4.67 µl/min/g wet weight. Under these conditions the calculated chloride secretory rate averaged 4.95 ± 2.64 µEq/min/g wet weight and the oxygen consumption 0.34 ± 0.22 µM/min/g wet weight (n=15) (Table 1). These values are quite similar to previous data reported by our laboratory (Silva et al. Am. J. Physiol. 233:F298-F306, 1977)

TABLE 1

Effect of potential secretagogues on the isolated perfused rectal gland flow rate and secretory rate

Potential Secretagogues	Concentration	N		Chloride Secretory Rate µEq/min/gww
NONE		15	10.3 ± 4.67	4.95 ± 2.64
KPO_4 buffer	3×10^{-3} M	3	10.0 ± 2.17	4.49 ± 1.40
VIP	1×10^{-6} M	6	73.3 ± 18.2*	31.2 ± 9.00*
VIP	1×10^{-8} M	6	43.0 ± 19.3*	19.7 ± 9.60*
VIP	1×10^{-9} M	3	8.00 ± 2.83	3.43 ± 1.60
Secretin	1×10^{-6} M	2	3.00 ± 0.33	1.19 ± 0.30
Glucagon	2.8×10^{-4} M	3	8.44 ± 4.04	3.03 ± 0.98
Pentagastrin	1×10^{-6} M	3	2.33 ± 0.33	1.00 ± 0.11
Theophylline	2.5×10^{-5} M	7	12.3 ± 8.33**	5.03 ± 4.20*
VIP plus theophylline	1×10^{-8} M 2.5×10^{-5} M	3	39.3 ± 13.7	20.8 ± 3.40
VIP plus theophylline	1×10^{-9} M 2.5×10^{-5} M	2	14.0 ± 6.67	6.10 ± 4.40
Indomethacin	1×10^{-5} M	3	25.8 ± 12.0**	9.82 ± 3.50*
VIP plus indomethacin	1×10^{-8} M 1×10^{-5} M	2	63.8 ± 13.5*	25.0 ± 3.30*

Values are means ± SEM. Potential secretagogues were added after two or three 10 minute control periods and the effect measured 10 minutes after addition. P values were calculated by paired t-tests. Each experiment included its own controls which are summarized in the entry marked NONE. Comparisons were made to the basal state (NONE) when a single agent was prevent or when two agents were present to the effect of a single agent alone.

* $p < .005$, ** $p < .01$

A bolus of VIP (10^{-6} M) markedly stimulated rectal gland secretion, producing a greater than 500% rise in the secretory volume and chloride secretory rate while the oxygen consumption rose 265% (Table 1). Stimulation of secretory volume and chloride secretory rate occurred immediately with a peak response at 10 min and a decline to basal within 30 min (Figure 1). An equivalent volume of buffer (300 mM KPO_4, pH 7.4) produced

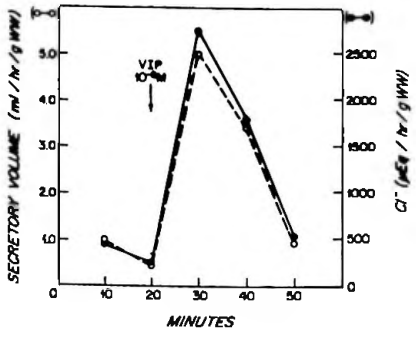

Figure 1. Effect of VIP (10^{-6} M) on secretory volume and chloride secretory rate in isolated perfused rectal gland. (n=6).

no response when given by bolus infusion. The response of the gland to VIP was dose dependent and could be elicited at a concentration as low as 10^{-8} M when addition of the hormone was made directly into the perfusion reservoir. The addition of VIP (10^{-9} M) and a sub-threshold concentration of theophylline (0.025 mM) produced a small synergistic effect on secretory volume and chloride secretory rate.

All other gastrointestinal hormones studied including glucagon, secretin and pentagastrin failed to stimulate secretion when present alone in high concentration or in combination with a sub-threshold concentration of theophylline (0.025 mM).

Additional studies were carried out to determine the influence of endogenous prostaglandins on basal and VIP stimulated secretory rate. Indomethacin (10^{-5} M), an antagonist of prostaglandin synthesis, produced a 150% increase in secretory flow rate and 100% increase in chloride secretory rate (Table 1). There was a small synergistic effect when this concentration of indomethacin was added to a submaximal concentration of VIP (10^{-8} M).

An infusion of hypertonic saline to the unanesthetized fish resulted in a peak increase in plasma osmolality to 1381 mosm/L and plasma sodium to 358 meq/L at 5 min, a rapid decline to a plateau level, plasma osmolality 1290 mosm/L and plasma sodium 300 meq/L at 15 min and then a gradual decline to pre-infusion values by 60 min (Table 2). Simultaneous with this increase in plasma tonicity, VIP plasma levels fell 45% ($p < .001$) and then gradually rose to basal levels by 60 min. Infusion of hypertonic saline by gastrointestinal intubation resulted in a more modest rise in peak plasma osmolality to 1237 mosm/L and

TABLE 2

Effect of intravenous (IV) and intragastrointestinal (IG) hypertonic saline volume expansion on plasma osmolality, sodium, and VIP level

	Osmolality		Sodium		VIP	
	IV	IG	IV	IG	IV	IG
Control	1254 ±20.5	1192 ±38.4	245 ±1.89	238 ±8.78	159 ±38.4	148 ±32.6
5 min	1381 ±37.1	1193 ±23.6	358 ±26.8	242 ±7.64	81.3 ±14.9	192.0 ±63.7
15 min	1290 ±34.2	1237 ±43.7	300 ±20.3	251 ±12.0	92.0 ±13.5	189 ±13.6
30 min	1300 ±21.8	1172 ±42.2	285 ±10.3	236 ±9.38	96.0 ±19.8	253 ±92.8
60 min	1248 ±38.6	1199 ±47.0	268 ±9.98	242 ±8.84	124 ±15.8	137 ±21.6

Values are means ± SEM. Four fish were studied in each group.

plasma sodium to 251 meq/L at 15 min and then a gradual decline to basal conditions. Under these circumstances there was an immediate rise in plasma VIP in all four animals studied to a peak increase of 71% at 30 min and a decline to basal by 60 min. Despite this marked increase in mean plasma VIP concentration, the marked variability in individual response, and a small sample size resulted in a lack of a statistically significant response ($0.05 < p < 0.1$). In order to localize the tissue site which was predominantly contributing to the plasma VIP level a variety of tissues were analyzed for VIP (Table 3). Tissue levels of

TABLE 3

Tissue level of vasoactive intestinal peptide (pg/100 mg wet weight)

Brain	66 ± 7.02	Duodenum	142 ± 18.0 *
Gill	47 ± 2.52	Rectum	135 ± 28.2 *
Pancreas	55 ± 2.52	Kidney	50 ± 1.15
Liver	56 ± 2.83	Rectal gland	88 ± 0
Stomach	63 ± 6.19	Mean all tissue[a]	56.2 ± 2.98

Each value represents Mean ± SEM. N is 4 for all samples except rectal gland where n is 2.

*$p < .001$ when compared to Mean all tissues.

[a]Mean all tissue excluding duodenum and rectum.

VIP were generally higher than those found in mammalian tissue by this laboratory. Especially interesting was the high concentration of hormone localized to the gastrointestinal tract of the fish, in particular the duodenum and rectum.

These studies provide further evidence that vasoactive intestinal peptide is an important humoral regulator of chloride secretion in the rectal gland. It seems likely that during feeding the ingested load of hypertonic sea water stimulates the release of VIP from the intestinal epithelia into the blood. This response must be mediated by an increase in luminal hypertonicity rather than in extracellular space since plasma VIP levels declined following intravenous hypertonic volume expansion. The high circulating level of VIP produced after gastrointestinal hypertonic infusion activates chloride secretion by a cyclic AMP-dependent mechanism. This effect may be modulated in part by endogenous prostaglandins. The increase in rectal gland secretion of sodium chloride produced by the hormone would then restore salt homeostasis.

OPEN-CIRCUIT Na^+ AND Cl^- FLUXES ACROSS ISOLATED OPERCULAR EPITHELIA FROM SEAWATER-ADAPTED *Fundulus heteroclitus* AND THE INFLUENCE OF ADRENERGIC STIMULATORS

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The opercular epithelium of the killifish, *Fundulus heteroclitus*, contains an abundance of chloride cells (Burns and Copeland Biol. Bull. Mar. Biol. Lab. Woods Hole 99:381-385, 1950) identical in ultrastructure to the gill chloride cell (Karnaky, Jr. and Kinter, J. Exptl. Zool. 199:355-364, 1977). When isolated and mounted in a lucite chamber under short-circuited conditions, this epithelium actively secreted Cl^- at a rate equivalent to the short-circuit current (Isc) with no significant net flux of Na^+ (Karnaky, Jr. et al Science 195:203-203, 1977; Degnan et al. J. Physiol. 271:155-191, 1977). More real life conditions can be experimentally studied by monitoring the open-circuit isotope fluxes across these epithelia when bathed on the mucosal side with seawater (SW) and on the serosal side with Ringer.

Opercular epithelia from SW-adapted killifish were isolated, pinned out as a flat sheet on a Sylgard (Dow Corning) disk with a 0.24 cm^2 aperture, and mounted in lucite chambers. Two epithelia were obtained from each fish, mounted in matching chambers, bathed on the mucosa with artificial SW (gassed with air, pH 7.9) and on the serosa with Ringer (gassed with 95% O_2/5% CO_2, pH 7.15), and kept open-circuited. The Na^+ and Cl^- concentrations of the SW and Ringer were determined with a flame photometer and a chloridometer respectively. The SW and Ringer Na^+ concentrations were 480.6 and 151.0 m-equiv/l. respectively and the SW and Ringer Cl^- concentrations were 533.1 and 142.5 m-equiv/l respectively. Isotope fluxes were performed on