IN VIVO CONTROL OF RECTAL GLAND FUNCTION IN SQUALUS ACANTHIAS. THE ROLE OF HEMODYNAMIC RESPONSES

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INTRODUCTION--The rectal gland of Squalus acanthias responds to systemic volume expansion by secreting a fluid concentrated in sodium and chloride. Many factors which affect epithelial transport have been illuminated by studies in the in vitro perfused gland. However, the in vitro gland does not permit the study of the factors mediating volume expansion induced secretion, the probable physiologic stimulus to rectal gland function.

The present studies support the hypothesis that hormonal factors play a major role in controlling rectal gland function. In addition, local vasodilatory responses to volume expansion appear to be necessary to meet the energy requirements for epithelial transport. Observations during inhibition of volume expansion induced secretion suggest that chloride transport and blood flow are independently regulated.

METHODS--Dogfish of either sex were obtained and prepared for the experiments as previously described (Solomon et al, Bull. MDIBL 21:16-19, 1981). Volume expansion was achieved by an infusion of 150 ml of isotonic shark Ringer's over a 15 minute period into the dorsal aorta of the intact or the perfusing fish.

Infusions directly into the rectal gland artery were performed using an infusion pump (Harvard Apparatus). Solutions of somatostatin, theophylline and dibutyl cyclic AMP, and bumetanide were made fresh prior to each experiment. The concentration of the stock solution was adjusted so that infusion rates of less than 10% of the measured rectal gland blood flow would deliver the desired concentration to the rectal gland.

Rectal gland chloride secretory rate and blood flow were measured for one hour during a control period prior to infusions (C) and for two one hour experimental periods immediately following plasma volume expansion (E_1 and E_2) except as indicated in the text. Dorsal aortic blood was sampled at the mid-point of each collection. Chloride concentration was measured in the samples by amperometric titration in a Buchler-Cotlove chloridometer. Oxygen tension was measured in the arterial and venous blood using a polarographic oxygen electrode calibrated prior to each experiment with calibrated oxygen mixtures and Radiometer pO_2-zero solution.

Results are expressed as mean ± SEM. Statistical analysis was performed using standard t test or paired t test wherever applicable.

RESULTS--Effect of a volume load on blood flow to the rectal gland.--Previous studies (Solomon et al, Bull. MDIBL 21:16-19, 1981) had indicated that blood flow to the rectal gland increases following volume expansion. These studies were confirmed and expanded (Table 1). In both the in situ and the explanted rectal gland, the increase in

TABLE I

THE HEMODYNAMIC RESPONSE TO A VOLUME LOAD IN THE IN VIVO RECTAL GLAND

	BASAL	STIMULATED 1°	STIMULATED 2°
IN SITU (11)			
BLOOD FLOW ML/H/GWW	6.9±.9	20.3**±2.5	28.7**±8.6
DORSAL AORTIC PRESSURE MMHg	20.0+2.0	22+3.1	-
EXPLANT (11)			
BLOOD FLOW ML/H/GWW	8.3±1.0	23.3*±4.4	22.1*±3.4
RECTAL GLAND ARTERY PRESSURE mmHg	15.0±1.8	14.0±1.5	-

* P < .01 BY PAIRED "t" TEST

** P < .001 BY PAIRED "t" TEST

blood flow is mediated by a change in vascular resistance within the gland as perfusion pressure is unaffected by volume expansion.

Oxygen consumption by the in situ gland.--Under basal, non-stimulated conditions, the rectal gland extracts over 95% of the oxygen in the blood (Table 2). This high extraction is unchanged during stimulated conditions. An estimate of the energetics of transport can be derived from the ratio of chloride ions secreted to moles of oxygen consumed (Cl/O_2) corrected for non-secretory oxygen consumption (Silva, et al, J. Memb. Biol. 53:215-221, 1980). Such estimates indicate that the coupling of oxygen consumption and secretory work is not altered during periods of physiologic stimulation (Table 2).

TABLE II

ARTERIAL-VENOUS pO_2 DIFFERENCES ACROSS THE RECTAL GLAND OF SQUALUS ACANTHIAS

	IN SITU	
	BASAL (5)	STIMULATED (15)
ARTERIAL pO_2 (TORR)	146±6	129+12
VENOUS pO_2 (TORR)	15±3	10±1
% EXTRACTION*	95.7%	94.4%
RATIO OF CL/O_2 (Eq/M)	26.4±4.8	24.3±3.9

*BASED UPON THE OXYHEMOGLOBIN DISSOCIATION CURVE FOR SQUALUS ACANTHIAS (Kent, et al. Bull. MDIBL 14:51-55, 1974)

Since the gland extracts such a high percentage of oxygen under all conditions and the energetics of transport are not altered with stimulation, we reasoned that oxygen delivery might be rate limiting for chloride secretion.

Effect of bumetanide on the response to volume loading and cAMP.--In the past, we demonstrated that increasing blood flow to the explanted gland via a roller pump in the absence of a volume stimulus did not enhance chloride secretion (Solomon et al, Bull. MIDBL 21:16-19, 1981). As blood flow always increased pari passu with secretion following a volume load, it was hypothesized that the increase in secretory work itself might provide a signal for vasodilation. The blood flow response of the explanted gland to a volume load was studied while secretion was inhibited by bumetanide (10^{-4}M) (Figure 1). Bumetanide was infused directly into the explanted rectal

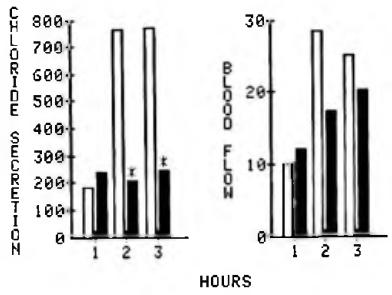

Figure 1.--The effect of volume expansion on the function of the explanted rectal gland (chloride secretion in μEq/h/gww and blood flow in ml/h/gww). The volume load was given during the second hour in control (N=10, open bars) and bumetanide (10^{-4}M) treated (N=5, closed bars) glands. Bumetanide completely inhibits the chloride secretory response (*=$p<.01$) but does not affect the vasodilatory response.

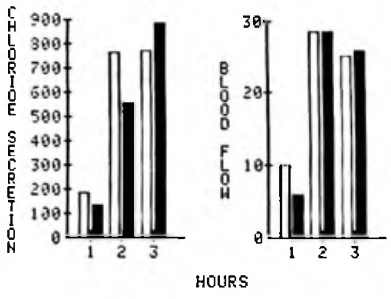

Figure 2.--The effect of volume expansion on the function of the explanted rectal gland (see Figure 1). The volume load was given during the second hour in control (N=10, open bars) and theophylline (10^{-6} to 5×10^{-5}M) treated glands (N=6, closed bars). Theophylline failed to affect either the chloride secretory or the vasodilatory response.

artery commencing 15 minutes before and continuing for two hours following a volume load to the perfusing fish. Bumetanide completely inhibited the secretory response to volume loading but had no affect on the vasodilatory response. A similar inhibition of secretion but not vasodilation was seen when bumetanide and cAMP were infused together (not shown).

Effect of theophylline on the response of explanted rectal glands to saline infusion.--In six experiments, following an initial one hour control period, theophylline was infused into the arterial blood supplying an explanted gland at a rate calculated to reach a concentration of 10^{-6} to 5×10^{-5}M per liter of blood. This concentration of theophylline is an order of magnitude less than that found to produce an increase in intracellular cyclic AMP (Stoff, et al, AM. J. Physiol. F138-F144, 1979) and chloride secretion in the in vitro perfused rectal gland (Stoff, et al, J. Exp. Zool. 199:443-448, 1977). More importantly, this concentration of theophylline has been shown in isolated perfused rectal glands to inhibit the secretory response to adenosine (Forrest, et al, Bull. MDIBL 20:152-155, 1980). The venous blood collected from the explanted gland was not returned to the donor fish during the theophylline infusions. Instead, an equal volume of shark Ringer's was reinfused.

Following an additional collection period, in which there was little change in duct flow or secretory rate flow, isotonic shark Ringer's solution was infused into the perfusing fish and collections were continued for another two hours. Theophylline did not prevent the increase in secretory rate and duct flow following volume expansion with isotonic Ringer's (Figure 2).

The effect of somatostatin on the response of explanted glands to saline infusion.--Somatostatin (4.5×10^{-6}M) was infused directly into the rectal gland artery commencing 15" before and continuing for two hours following the infusion of isotonic shark Ringer's to the perfusing fish. Again the venous blood was not reinfused into the perfusing fish but was replaced by equal volumes of isotonic shark Ringer's. Somatostatin completely inhibited the duct flow and chloride secretory response to volume loading for the entire two hour period. When the somatostatin infusion was stopped and cyclic AMP-theophylline were subsequently infused, the glands responded with an increase in duct flow and chloride secretion (Figure 3).

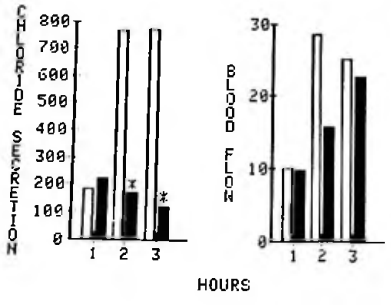

Figure 3.--The effect of volume expansion on the function of the explanted rectal gland (see Figure 1). The volume load was given during the second hour in control (N=10, open bars) and somatostatin (4.5×10^{-6}M) treated (N=6, closed bars) glands. Somatostatin completely inhibits the chloride secretory response (* =$p<.01$) but does not affect the vasodilatory response.

DISCUSSION--The present studies characterize the hemodynamic changes which occur during physiologic stimulation of rectal gland. An increase in blood flow to the rectal gland was found to always accompany a volume induced increase in chloride secretion. Neither dorsal aorta pressure nor rectal gland arterial perfusion pressures were affected by volume loading. Therefore, the increase in rectal gland blood flow occurred as a result of changes in vascular resistance within the rectal gland.

The observation that blood flow increases following volume expansion despite inhibition of chloride transport and presumably transport coupled metabolic changes is consistent with independent stimulation of transport and blood flow. As in other tissues, vasodilation appears to be mediated by cAMP. Infusion of cAMP increases blood flow even when secretion is inhibited with bumetanide. A number of agents stimulating cAMP are capable of producing

vasodilation in the in vitro perfused rectal gland. For example, both VIP and adenosine reverse norepinephrine induced vasoconstriction in this model and the vasodilatory effect is not prevented by furosemide inhibition of secretion (Shuttleworth, et al, Bull. MDIBL 21:59-62, 1981). Thus, a direct vasodilatory effect of VIP and adenosine can be inferred from these observations.

However, on the basis of the experiments with somatostatin and theophylline infusion during volume expansion, only VIP appears to be a candidate as hormonal mediator of the secretory response. If VIP independently stimulates vasodilation and chloride secretion, the membrane receptors on smooth muscle and epithelial cells must respond differently to somatostatin as only the latter are inhibited by this agent. An alternative hypothesis is that another hormonal factor is responsible for the vasodilatory response.

DRINKING IN MARINE STENOHALINE FISH

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Although a considerable amount of research has linked the renin-angiotensin system with drinking behavior in mammals, much less is known of this system in non-mammalian species. Previous work from this laboratory has provided evidence that endogenously produced angiotensin II is a stimulus for drinking in the euryhaline killifish, Fundulus heteroclitus (Malvin, R.L., et al, Am. J. Physiol. 239, 1980), but the role of angiotensin in drinking behavior has not been evaluated in marine stenohaline fish.

METHODS--The current study was performed using two marine stenohaline fish, the flounder, Pseudopleuronectes americanus, and the long-horned sculpin, Myoxocephalus octodecimspinosus. For each model, drinking in response to four interventions was compared to control drinking rates. These four interventions were: 1) injection of antiotensin II (13 ug/100 g body weight in .5 ml); 2) injection of the angiotensin I converting enzyme inhibitor Captopril (250 ug/100 g body weight in .25 ml); 3) hemorrhage (1% body weight by caudal vein puncture); 4) hemorrhage following injection of Captopril. In the flounder, one additional series of experiments examined the effect of hemorrhage in the presence of Saralasin, an angiotensin II competitive antagonist (20 ug/100 g body weight in .2 ml). In all cases the drugs were administered by a single intramuscular injection. Control fish received a similar volume of vehicle (.19 M NaCl) in the same manner.

On the morning of an experiment the fish were placed in tanks containing sea water at 12°-16°C. Temperature was adjusted to this range with ice bags but was to some degree dependant on ambient temperature. After injections were made, all fish were placed in tanks containing 3% polyethylene glycol (PEG) in sea water. After one hour the fish were removed, placed in PEG-free sea water and kept there for another fifteen minutes. Drinking rate during the hour in PEG was determined by killing each fish and flushing the gut contents into a test tube with 12 ml of tap water, combining a portion of this "wash" with an equal volume of 30% TCA, and comparing the absorbance of this mixture at 650 mu with that of PEG standards. Non-specific absorbance was determined by analyzing the gut contents of fish not exposed to PEG, and these values were used to calculate "blank"drinking rates which were averaged and subtracted from all other drinking rates. The fifteen minutes in PEG-free sea water prior to killing allowed ingested PEG to move sufficiently down the gut for adequate recovery in the wash. Separate experiments assured that the transit time through the GI tract for PEG was not exceeded in these studies. Data from experimental groups and control groups were compared using Student's t-test. The control drinking rates in flounder were higher during the last month of this study. For this reason, drinking rates were compared to controls from the same period in the flounder experiments.

RESULTS--Results are summarized in Figures 1 and 2. Control rates of drinking in both groups were less than