3 VOLUME INDUCED SECRETION BY THE RECTAL GLAND IS MEDIATED BY A HUMORAL AGENT(S)

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Studies were undertaken in the intact, pithed dogfish to explore the nature of the stimuli which mediate the secretory response of the rectal gland to a volume load. To differentiate between neural and humoral stimuli, experiments were performed in pithed dogfish which were volume loaded and in explanted glands perfused via a PE catheter with arterial blood originating in the dorsal aorta of a recipient fish.

Sharks were pithed and the rectal gland duct catheterized (Solomon, et al., Bull. MDIBL, 20:138, 1980). The animals were given 1000μ of sodium heparin IV and the rectal gland vein was then exposed by a ventral incision and catheterized with PE 160 tubing. All rectal gland venous blood was collected by gravity drainage into a calibrated centrifuge tube. The collected venous blood was periodically reinfused into the dorsal aorta through PE 50 tubing. Rectal gland blood flow was calculated as the sum of the venous flow and the duct flow and reported as ml/h/kg fish.

In separate experiments, a rectal gland was removed from a fish (donor); the duct and vein were catheterized and the artery was connected to PE 50 tubing, one end of which was inserted into the dorsal aorta of a second, intact fish (recipient). Perfusion pressure was measured by a Statham P25 transducer attached to the tubing connecting the rectal gland artery to the dorsal aorta of the recipient. The implanted gland was placed upon a glass chamber cooled to 15°C and positioned at the level of the recipient's heart. Rectal gland duct and venous flow were collected by gravity. VIP, somatostatin and theophylline were dissolved in shark Ringer's and infused into the dorsal aorta of the recipient or the arterial supply of the explanted gland. All samples of rectal gland duct fluid had chloride concentrations between 480 and 560 mEq/L.

Results

Following infusion of 150 ml of isotonic shark Ringer's solution to the pithed shark, there was an increase in rectal gland duct flow within the first hour that continued for at least two hours (Table 1). The augmented secretion was always accompanied by an increase in blood flow to the rectal gland. Rectal gland blood flow usually increased within 30 minutes of the saline infusion and preceded the increase in rectal gland secretion.

Table 1.--

	Duct Flow (ml/h/kg)			Blood Flow (ml/h/kg)		
Group (N)	Basal	60'	120'	Basal	60'	120'
IN VIVO						
SR (8)	.13 ± .05	.58 ± .16	.97 ± .20	3.7 ± 1.6	11.2 ± 1.8	19.2 ± 8.0
SR/M (3)	.37 ± .16	.75 ± .07	1.78 ± .09	5.5 ± 1.6	32.9 ± 4.6	44.4 ± 12.3
HS (9)	.03 ± .01	.43 ± .15	.91 ± .34	2.6 ± .9	7.5 ± 2.1	7.1 ± 2.8
EXPLANT						
SR (10)	.13 ± .03	.75 ± .20	.83 ± .16	4.9 ± .8	15.5 ± 3.4	14.4 ± 2.5

Values are mean ± SEM.
All values at 60' and 120' minutes are statistically different from basal values ($p>.001$).
SR = isotonic shark Ringer's (150 ml)
SR/M = isotonic mannitol: shark Ringer's::1:1 (150 ml)
HS = hypertonic saline - 1 M NaCl (50 ml)

The same volume of an isotonic mannitol-shark Ringer's solution in which half the concentration of sodium and chloride was replaced by an equi-osmolar concentration of mannitol produced a similar enhancement in rectal gland duct and blood flow (Table 1). Also noted for comparison is the effect of an infusion of 50 ml of 1 M NaCl on duct and blood flow (data collected in 1980).

The response of an explanted gland to volume expansion of the recipient fish was similar to the response of glands in situ (Table 1). Duct flow and blood flow were significantly enhanced within the first hour. Perfusion pressure did not change with a volume load, so that the increase in blood flow was entirely a result of a decrease in vascular resistance within the gland.

To explore the relationship between duct flow and blood flow in the explanted gland, the tubing perfusing the explanted gland was placed in a roller pump so that blood flow could be controlled. In 5 experiments, increasing blood flow for 1 hour from 4.4 ± 1.6 ml/h/kg to 12.3 ± 3.4 ml/h/kg ($p > .01$ by paired "t" test) failed to cause an increase in duct flow. In contrast to the fall in vascular resistance noted above following a volume load, the increase i blood flow following an increase in pump rate was accompanied by a fourfold increase in perfusion pressure. With flow maintained at the elevated level, a volume stimulus to the recipient resulted in the expected enhancement in secretory rate (Figure 1), and a fall in vascular resistance (not shown).

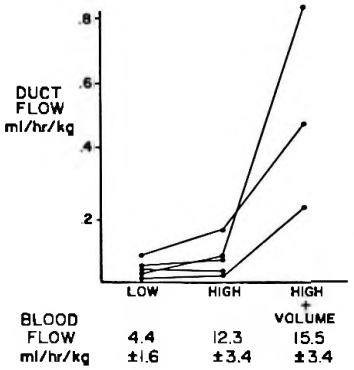


Figure 1.--The effect of increasing blood flow via a perfusion pump on the rectal gland duct flow rate. After one hour at the high flow rate, a volume stimulus (150 ml shark Ringer's infusion) was given to the recipient animal in three of the experiments.

We next studied potential mediators of the blood flow and duct flow responses to a volume load. Vasoactive intestinal peptide (VIP) 10 μg, when infused either directly into the explanted gland (N = 3) or into the recipient (N = 2) produced a small but statistically significant enhancement in duct secretion (Figure 2). Blood flow was not enhanced. A more marked increase in both blood flow and duct flow was seen when a volume load was subsequently given to the recipient animal.

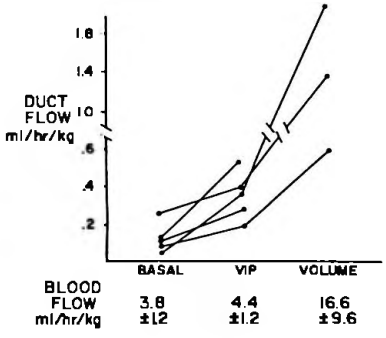
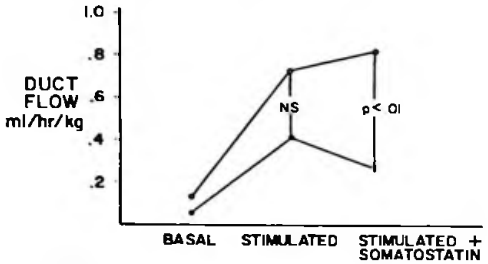


Figure 2.--The effect of VIP on rectal gland duct flow and blood flow in the explanted gland. A volume load (150 ml shark Ringer's) was given one hour after the VIP infusion to three of the recipient animals.

Following stimulation of four explanted glands by volume expansion of the recipient fish with shark Ringer's, somatostatin (1.4×10^{-7}M) was infused directly into the rectal gland artery of the explanted glands. Duct secretion was inhibited in every case by an average of 40%, though there was no change in blood flow to the gland (Figure 3).



BLOOD FLOW ml/hr/kg	BASAL	STIMULATED	STIMULATED + SOMATOSTATIN
	3.8 ±1.1	13.0 ±2.8	10.5 ±2.8

Figure 3.--The effect of a volume load in control explanted glands (0-0) and glands which were infushed with somatostatin (10^{-7}M) during the second hour of stimulation (●-●) (N = 4). Blood flow responses in the latter glands, given somatostatin, are indicated along the abscissa.

Finally, in 4 experiments, theophylline (10^{-6} to 5×10^{-5}M) was infused directly into the explant during basal collections and was continued following a volume stimulus to the recipient. At this concentration, theophylline blocks stimulation of the isolated perfused gland by adenosine (Forrest et al., Bull. MDIBL, 20: 152, 1980). Theophylline did not affect either duct flow or blood flow in the basal state and did not prevent the increase in both, following volume expansion (Table 2). In 2 experiments, adenosine (10^{-4}M) infused directly into the explanted gland failed to stimulate either duct flow rate or blood flow.

Table 2.--Effect of theophylline on volume stimulation of explanted rectal gland

	Basal 0-60 min	Basal 60-120 min	Theophylline Stimulated 120-180 min	Stimulated 180-240 min
Duct Flow ml/hr/k	.37 ± .25	.37 ± .21	1.1 ± .48*	1.35 ± .45*
Blood Flow	5.7 ± 2.2	6.1 ± 2.5	27.0 ± 8.1*	26.7 ± 6.5*

Values are mean ± SEM (6).
*Different from basal plus theophylline.
Theophylline was infused to achieve a final concentration of 10^{-6} to 5×10^{-5}M.

Discussion

The prompt and marked secretory response of intact and explanted rectal glands to an intravascular infusion, regardless of whether the concentration of sodium in the infused fluid is high, isotonic or low, compared with the plasma sodium of the recipient, make it likely that the primary stimulus to rectal gland secretion in the intact fish is a change in volume, rather than tonicity or salt concentration, of some component of the body fluids. Since the explanted gland has no neural connection with the recipient, the fact that it responds to volume loading in the same way as the gland *in situ* clearly establishes that the mediator of this response is blood-borne and not neural.

It should be noted that enhanced secretion was always accompanied (and usually preceded) by an increase in blood flow to the gland, without measurable change in dorsal aortic blood pressure. An increase in blood flow, however, was not the primary stimulus for secretion, since when glandular blood flow was increased by a pump, there was no change in secretory rate (Figure 1). Although an increase in blood flow is not sufficient to cause an increase in duct secretion, it seems to enhance the response (Figure 2), possibly because oxygen supply becomes rate-limiting at high rates of secretion.

In isolated rectal glands perfused with shark Ringer's solution, both vasoactive intestinal peptide and adenosine have been identified as humoral substances capable of stimulating secretion. The present experiments suggest that VIP, but not adenosine, is a factor in mediating the secretory response to volume expansion in vivo. Somatostatin, which inhibits stimulation by VIP (but not by adenosine) of isolated rectal glands, also inhibited secretion by the explanted gland when infused into the gland at a concentration of only 1.4×10^{-7}M. On the other hand, theophylline ($10^{-6} \times 10^{-5}$M) which blocks adenosine stimulation of isolated glands, did not prevent secretion by explanted glands. Furthermore, adenosine (10^{-4}M) produced no change in blood flow or secretory rate when infused directly into the explanted gland. The mechanism of the local vasodilatation induced by volume loading, which permits blood flow to the gland to rise greatly without an increase in perfusion pressure, remainds to be determined.

(4 EFFECT OF GANGLIONIC BLOCKADE ON RESPONSE OF DOGFISH BLOOD PRESSURE, HEART AND RESPIRATORY RATES TO INJECTION OF A GANGLIONIC STIMULATOR AND POTASSIUM ION

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The systemic arterial pressor response of dogfish (Squalus acanthias) to both a ganglionic stimulator, 1,1-dimethyl-4-phenylpiperazinium (DMPP) and potassium ion (K^+) is catecholamine (CA) mediated (Opdyke et al., Comp. Physiol. Biochem., 42A:611-620, 1972; Opdyke et al., Am. J. Physiol., 241:R228-232, 1981). This poses the question: Is the release of CA initiated by each of these treatments caused by neurogenically mediated stimulation of chromaffin cells, or, are the mechanisms of inducing CA release basically different? The following experiments were undertaken to illuminate this question.

Materials and Methods

The dorsal aortae of unanesthetized, healthy dogfish caught by gill-netting in Frenchman Bay, Maine were catheterized via the caudal artery. The fish were placed in a long and narrow restraining box provided with a flow-through of cold sea-water. Phasic dorsal aortic blood pressure (DAP) was monitored by a P23AA Statham gage and transcribed on an E for M CRO recorder. Heart rates were obtained from the blood pressure records. A sea-water filled P260 polyethylene catheter was inserted into the pharyngeal cavity through a spiracle and the changes in pressure associated with gill ventilation were recorded by another P23AA Statham gage. All drug injections and infusions (Harvard infusion pump) were made through the dorsal aortic catheter during a brief interruption of pressure recording. All drugs were prepared in the appropriate dose and delivered in 1 ml elasmobranch saline with allowance for catheter dead space. Control injections of 1 ml saline were made in 10 dogfish. The responses to injections of DMPP (0.2 mg/kg) and K^+ (0.05 mEq/kg) were observed before and after a 10 min continuous infusion of hexamethonium (0.83 mg/kg/min). Twenty min were allowed for recovery between the injections of DMPP and K^+, which were given in alternating order. The significance of the results was assessed by the application of Student's paired t test ($\alpha = 0.05$) with the aid of a programmed calculator.