vesicles were able to use NH_4^+ as a counterion for Na^+ uptake. However, actual transport of NH_4^+ itself was not demonstrated in this preparation. Preliminary experiments with vesicles from blue crab gill, using an enzymatic determination for extravesicular NH_4^+, have provided direct evidence for trans-membrane exchange of Na^+ for NH_4^+ (Fuhrman and Towle, unpublished).

These results indicate that Na^+/NH_4^+ exchange by crab gill is an ATP-dependent, basolaterally-localized process, probably mediated by Na^++K^+-ATPase. Manipulations of external media in experiments using intact animals would be expected to affect this basolateral process only indirectly (see Kirschner, Am. J. Physiol. 244: R429-R443, 1983). Isolation of apical membranes and their use in similar kinds of experiments will elucidate whether Na^+/NH_4^+ exchange is exclusively basolateral or may also be achieved by apical membranes as well. This research was supported by the Jeffress Memorial Trust.

RECTAL GLAND FUNCTION IN THE LITTLE SKATE, RAJA ERINACEA

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The physiology of the rectal gland of elasmobranchs has been studied extensively in sharks (Squalus acanthias, Scyliorhinus canicula) but not in rays. Accordingly, rectal gland function was examined in male and female specimens of Raja erinacea, the little skate, found abundantly in the waters of northern Maine. While in the skate the rectal gland is much smaller than in Squalus acanthias (mean ± s.e. of weight of 22 specimens = 184±8.5 mg, range 100-244 mg), its single artery and duct permit isolated perfusion in a way similar to our previous studies with Squalus acanthias.

Oxygen consumption of rectal gland slices was studied by techniques previously described (Bull. M.D.I.B.L., 21:13-14, 1981). Slices of chilled fresh rectal gland tissue, 0.5 mm thick, were prepared using a Stadie-Riggs microtome, and 6 to 20 mg were placed in 2 ml of elasmobranch Ringer's solution, buffered with 40 mM Hepes at pH 7.6, with 5 mM glucose as substrate. Oxygen consumption was measured at 25°C using a polarographic oxygen electrode (YSI) attached to a recorder. The electrode was calibrated using the known solubility of oxygen at 25°C and the barometric pressure. The rate of oxygen consumption was calculated from the tangent of the recorded slope of pO_2 against time. Basal QO_2 was 40.3±4.0 μMoles/hr/g wet weight (mean ± s.e.) in 12 experiments, and increased consistently to 142±8% of that level (n=11, $p<0.01$) on the addition of dibutyryl cAMP and theophylline (final concentration of each = 1 mM). The subsequent addition of ouabain, 10^{-4}M, promptly inhibited QO_2 to a level of 30.4±3.2 μMoles/hr/g wet weight. Oxygen uptake of skate rectal gland slices was not stimulated by 10^{-6}M vasoactive intestinal peptide (VIP, n=5), 10^{-5}M adenosine (n=3), or veratrine, 50 μg/ml (n=2). By contrast, VIP at this concentration regularly increases QO_2 in slices of the rectal gland of Squalus acanthias.

Isolated skate rectal glands cooled to 16°C were perfused at a rate of 0.42 or 1.1 ml/min with elasmobranch Ringer's solution containing 5 mM glucose, gassed with 99% O_2, 1% CO_2 (pH 7.6), from glass syringes, using a Harvard motor-driven infusion pump. The rectal gland artery was cannulated with PE10 polyethylene tubing, and the duct with PE tubing intermediate in size between PE50 and PE10. Duct fluid was collected in glass microliter pipettes for successive periods of 10 min each over 60-180 min. Venous drainage was not collected. The results are shown in Table 1.

In the basal state, the chloride concentration of secreted fluid was usually only slightly higher than that of the perfusate (290 mEq/L), averaging 320±19 mEq/L, but varying from 280 to 476 mEq/L. The rate of chloride secretion was 1.03±.19 μEq/min, or about 5 μEq/min/g wet weight, comparable to the basal secretory rate of isolated perfused rectal glands of Squalus acanthias. After the addition of dibutyryl cAMP, 1 mM, and theophylline, 1 mM, chloride secretion increased by about 7x, to 7.12±.72 μEq/min, the result of an increase in both the volume

TABLE I

Function of isolated perfused skate rectal gland

	Basal Control			Experimental		
	Duct Concentration (Cl-) mEq/L	Duct Flow µl/min	Cl- Secretion µEq/min	Duct Concentration (Cl-) mEq/L	Duct Flow µl/min	Cl- Secretion µEq/min
dbcAMP 10^{-3}M Theophylline 10^{-3}M (n=10)	320±19	3.16±.55	1.03±.19	443*±20	14.6*±2.4	7.12*±.72
dbcAMP + Theophylline + Bumetanide 10^{-4}M (n=6)				319†±17	5.89†±1.6	1.83†±.46
VIP $3x10^{-6}$M (n=7)	347±28	5.42±1.95	1.90±.70	359±32	9.19±3.86	3.08±.66
Forskolin 10^{-6}M	280	2.68	.75	427	9.82	4.19
$5x10^{-7}$M (n=2)	294	3.33	.98	455	15.6	7.10

*Significantly different from basal control, $p < 0.01$.

†Significantly different from glands previously stimulated with dbcAMP and theophylline, $p < 0.01$.

Values are mean ± s.e.

and the chloride concentration of secreted fluid. The stimulated rate of chloride secretion, about 2100 µEq/hr/gm wet weight, thus exceeded the rate previously observed in stimulated perfused glands of Squalus acanthias that had, however, been exposed to lower doses of cAMP (.05 mM) and theophylline (.25 mM) (Am J. Physiol., 233:F298-F306, 1977; J. Membr. Biol., 53:215-221, 1980). Chloride secretion was inhibited by bumetanide, 10^{-4}M.

The effects of VIP were equivocal in the skate, in sharp contrast to the striking stimulation regularly observed in Squalus. VIP, 50 µg/5 ml ($3x10^{-6}$M) was infused for 10 min. after a suitable baseline had been obtained, in 7 experiments. In each of these, the viability of the gland was assured at the conclusion of the experiment by eliciting a clearcut secretory response to cAMP-theophylline, as noted above. In 3 glands, VIP appeared to cause an increase in the volume of secreted fluid without, however, increasing its chloride concentration. In the remaining 4 glands, no secretory response was noted. Neither adenosine, 10^{-4}M (2 experiments) nor 2-chloroadenosine (3 experiments) elicited unequivocal increases in chloride secretion (data not shown). In 2 experiments, however, forskolin, 10^{-6}M and $5x10^{-7}$M, increased chloride secretion by 5.6x and 7x, and increased the chloride concentration of duct fluid as well.

These results indicate that the isolated perfused rectal gland of Raja erinacea, like that of Squalus acanthias, is a suitable model for the study of chloride secretion. Secretion is stimulated by cAMP and theophylline, which increase oxygen uptake by tissue slices. The process is inhibited by bumetanide and ouabain. In contrast to Squalus, VIP in the concentration range of 10^{-6}M produced little or no stimulation of chloride secretion or oxygen uptake.

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