Discussion

The electron microscopic investigation demonstrates the presence of ionocytes at the afferent side of the gill pouch: in the epithelium of the primary folds and the epithelium covering the water outlet. In Myxine the chloride cells are not accompanied by accessory cells which are typically found in the pseudobranch (Dunel and Laurent, J. Microsc. Biol. Cell 16:53, 1973), the gill (Hootman and P hilpott, Am. J. Phys. 238:199, 1980), and the operculum (Karnaky et al., Am. J. Phys. 238:185, 1980) of marine teleost and euryhaline teleost in salt water. In these hyposmoregulators chloride cells with their accessory cells are considered to form a functional unit for salt secretion (Sardet et al., ibid.). Furthermore the fine structure of the ionocytes of Myxine resembles that of the chloride cells of hyperosmoregulating fresh water teleost and of the dogfish Squalus acanthias (Doyle, this bulletin Vol. 15:27-28, 1975). Fresh water fish are able to accumulate ions via the chloride cells. We would like to conclude that the slight hyperosmolar body fluid of Myxine (body fluid 1140 mOsmol/1, Raguse-Degener et al., Contr. Nephr. 19:1, 1980) could be the result of an ion-accumulating function of the ionocytes. The authors wish to acknowledge the use of the equipment kindly provided by Dr. B. Schmidt-Nielsen and Harold H. Church, and Suzanne Taylor, Jackson Laboratory, for operating the electron microscope. This work was supported by the Deutsche Forschungsgemeinschaft.

CELLULAR MECHANISM OF NaCl SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS. STUDIES ON IN VITRO PERFUSED GLANDULAR TUBULES

Rainer Greger, Eberhard Schlatter, Fong Wang and John N. Forrest Jr., Max-Planck-Institut für Biophysik, Frankfurt/Main, West Germany and Department of Internal Medicine, School of Medicine, Yale University, New Haven, Ct.

Individual glandular tubules (n=200) were dissected from rectal glands (n=60) of specimen of Squalus acanthias of either sex. The glandular tubules were perfused in vitro by the method described recently by Forrest et al. (J. Clin Invest. 1983, 72, 1163-1167). Electrical parameters were measured as described for the thick ascending limb of Henle's loop by Greger and Schlatter (Pflügers Arch: 1983, 396, 315-324). The present study comprises 4 series. A first series was aimed at testing whether the carrier, which mediates secondarily active chloride secretion and which is localized in the basolateral membrane of the rectal gland, has a K^+-binding site. In the second series, we tested the conductivity properties of the lumen and basolateral cell membrane of resting and stimulated glandular tubules. In the third series cellular ion activities for Cl^-, K^+, and Na^+ were measured with double barrelled microelectrodes in resting and in stimulated glandular tubules, and in those treated with 10^{-5} - 10^{-4} mol$\cdot l^{-1}$ furosemide (blood side). In the fourth series we tested the sequence of events occurring during the process of stimulation.

In the first series we measured transepithelial PD (PD_{te}) and the PD across the basolateral membrane (PD_{bl}) as a function of the periglandular K^+ concentration. Pilot experiments revealed that furosemide and ouabain resulted in a reduction of the lumen negative PD_{te}. While furosemide hyperpolarized PD_{bl} rapidly, ouabain lead to a delayed depolarization. The furosemide induced hyperpolarization is caused by a fall in cell Cl^- towards equilibrium. The ouabain induced depolarization is explained by the fall in basolateral K^+-conductance and the increase in cell Cl-. The hypothesis was, that K^+-involvement in the basolateral, furosemide-sensitive carrier, as it has been documented for the thick ascending limb of Henle's loop (Greger and Schlatter, Pflügers Arch. 1981, 392, 92-94), should lead to an initial hyperpolarization followed by a depolarization. This was predicted since K^+ reduction should initially block the carrier, as does furosemide, and only thereafter the ($Na^+ + K^+$)-ATPase. This hypothesis was verified in all 18 experiments.

In the second series K^+-, and Cl^--concentration step experiments (n=108) were performed on the lumen and basolateral cell side. It was shown that a reduction in bath K^+ hyperpolarized PD_{bl} rapidly and completely reversibly

in non-stimulated and stimulated (v.i.) glandular tubules, both in the absence and presence of furosemide. This K^+-conductance could be blocked by Ba^{++} (0.5 mmol · l^{-1}). A Cl^- downward concentration step depolarized the apical membrane only if the cells were stimulated (v.i.) or if the basolateral K^+-conductance was blocked by Ba^{++}. The basolateral membrane exposed no Cl^- conductance, and, conversely, the lumen membrane exposed no K^+ conductance.

The results of series 3 are summarized in Table 1. Stimulation of the glands by 10^{-4} mol·l^{-1} dbcAMP + 10^{-6}

Table 1: Effect of stimulation (dbcAMP 10^{-4}, adenosine 10^{-4}, and forskolin 10^{-6} mol·l^{-1}, bath) and furosemide (10^{-5}–10^{-4} mol·l^{-1}, bath) on isolated perfused rectal glands.

	PD_{te} (mV)	R_{te} (Ωcm^2)	PD_{bl} (mV)	VDR	$a^{cell}_{K^+}$	$a^{cell}_{Cl^-}$	$a^{cell}_{Na^+}$ (mmol·l^{-1})
NON STIMULATED	- 1.1	41	-85	29	122	45	11
	± 0.2	± 7	± 1	±13	±11	± 4	± 3
	(n=35)	(n=13)	(n=157)	(n=3)	(n=8)	(n=29)	(n=4)
STIMULATED	-11.1*	27*	-75*	4.0*	109	38*	30*
	± 1	± 2	±0.4	±0.4	±22	± 4	± 9
	(n=67)	(n=47)	(n=260)	(n=33)	(n=4)	(n=36)	(n=4)
STIMULATED + FUROSEMIDE	- 2.3*	24*	-79*	6.8*	114	19*	17*
	± 0.2	± 1.8	±0.9	±1.5	±11	± 1.9	± 2.8
	(n=63)	(n=26)	(n=99)	(n=21)	(n=6)	(n=14)	(n=14)

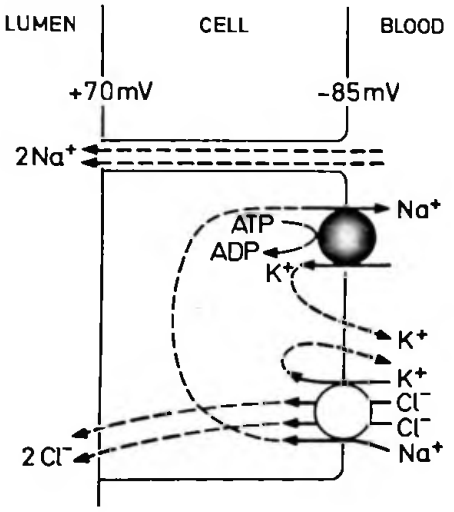
* significantly different from respective control

forskolin + 10^{-4} adenosine lead to a marked increase in PD_{te}, a significant fall in R_{te} (thus to a 15 fold increase in equivalent short circuit current). The cells depolarized and voltage divider ratio (VDR, corresponding to the radio of lumen divided by basolateral membrane resistance = R_l/R_{bl}) fell from 29 to 4. The cell K^+ activity was reasonably constant. Whereas cell Cl^- fell slightly and cell Na^+ increased dramatically.

Furosemide converted the stimulated glandular tubules into "resting" ones, inasmuch as it leads to a fall in PD_{te}, a hyperpolarization of PD_{bl}, and a fall in cell Na^+. In addition, however, cell Cl^- fell markedly.

The data of series 1-3 can be explained by the model proposed in Figure 1. In addition, the results of this series suggest that the primary event in cAMP stimulation of the rectal gland is an increase in the apical Cl^- conductance. In the experiments of series 4 we have further ellucidated this point. In 3 experiments we showed that furosemide lead to a delayed fall in cell Cl^- in unstimulated glandular tubules and a rapid fall in stimulated glandular tubules, but that the increase in cell Cl^- upon removal of furosemide was equally fast in non-stimulated and in stimulated glandular tubules. In another 3 experiments, we observed that the decrease in transepithelial resistance and the fall in voltage divider radio (R_l/R_{bl}) can also be induced by stimulation if the carrier is blocked by furosemide. Thus, we suggest that induction of the apical Cl^--conductance is the primary event induced by cAMP in the rectal gland cell. This study was supported by the Deutsche Forschungsgemeinschaft Gr 480/8-1.

Figure 1.



ATP-DEPENDENT SODIUM UPTAKE BY BASOLATERAL MEMBRANE VESICLES FROM THE GILL OF GREEN CRAB, CARCINUS MAENAS

David W. Towle, Department of Biology, University of Richmond, Richmond, Virginia

The gills of euryhaline decapod crustaceans actively absorb NaCl from the ambient medium, producing hyperosmotic hemolymph in animals which are acclimated to salinities below a species-specific value (typically 24-28 o/oo) (see Mantel and Farmer, Biology of Crustacea, Vol. 5, 1983). External application of amiloride inhibits both Na^+ uptake and NH_4^+ excretion by the gills of these crabs, supporting the idea that Na^+/NH_4^+ exchange occurs at the apical membrane of ion-transporting cells within the gill (Kormanik and Cameron, Mar. Biol. Lett. 2:11-23, 1981; Pressley et al., Am. J. Physiol. 241: R370-R378, 1981). Support for the opposing idea that Na^+/NH_4^+ exchange is a basolateral process is provided by the ability of NH_4^+ to substitute for K^+ in enzymatic assays of the basolaterally-localized $Na^+ + K^+$-dependent ATPase (Towle et al., J. Exp. Zool. 196: 315-321, 1976; Towle et al., Amer. Zool. 23(4): Abst., 1983). To distinguish between these alternatives, it is necessary to study apical processes separately from basolateral processes.

We have prepared highly enriched basolateral membranes from ion-transporting cells of blue crab (Callinectes sapidus) gill, using $Na^+ + K^+$-ATPase as a marker (Towle et al., 1983). These membranes are vesicular, and are capable of taking up Na^+ from the medium when ATP is supplied externally and a counterion (K^+ or NH_4^+) is provided internally (Fuhrman et al., Amer. Zool. 23(4): Abst., 1983). The present work was undertaken to describe the ion-exchange processes occurring at the basolateral membrane of ion-transporting cells of green crab gill, using isolated basolateral membrane vesicles. The green crab is somewhat less able to regulate blood Na^+ levels than the blue crab, and might be expected to demonstrate a less efficient transport mechanism at the membrane level.

Green crabs (Carcinus maenas) were collected from the intertidal zone at Salsbury Cove and were maintained for 6-10 days in aerated sea water of 10 o/oo, a salinity at which the hemolymph contains 305 mM Na^+, compared to an external Na^+ concentration of 97 mM (Siebers et al., Mar. Biol. 69: 37-43, 1982). The three most posterior gills, containing the highest $Na^+ + K^+$-ATPase activity (Siebers et al., 1982), were homogenized in a buffer composed of 250 mM sucrose, 6 mM EDTA, 20 mM imidazole (pH 6.8), and 0.1% sodium deoxycholate (Hendler et al., Am. J. Physiol. 222: 754-760, 1972). Basolateral membranes were separated from apical membranes and other organelles by an adaptation of the method of DePew and Towle (Mar. Biol. Lett. 1:59-67, 1979; Towle et al., 1983). The