The Shark Rectal Gland: A Model for the Active Transport of Chloride

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Received May 8, 1979

The rectal gland of the spiny dogfish, Squalus acanthias, provides an easily studied model of active chloride transport powered indirectly by Na-K-ATPase. Co-transport of sodium with chloride can be demonstrated in membrane vesicles isolated from basolateral membranes of the gland. Chloride secretion is under the hormonal control of vasoactive intestinal peptide, and possibly other agents, via adenyl cyclase and cyclic AMP. A similar mechanism is probably responsible for the active transport of chloride across other biological membranes.

Thirty years ago, when James Gamble summarized the facts of water and electrolytes for an admiring generation of medical students, the transport of chloride was considered to be entirely passive. Excluded from cells, chloride was regarded as a kind of filler, its movement dependent entirely upon the varied whims of sodium, potassium, and bicarbonate. Gamble epitomized this notion of chloride by calling it "a mendicant ion."

It is becoming apparent that far from being a mendicant ion, chloride is in fact actively transported by a number of animal tissues, including the central nervous system, and especially by secretory epithelial organs. It has been the study of the rectal gland of the shark that has provided much of the critical information necessary to establish the mechanism of this active transport.

THE OSMOTIC DILEMMA OF THE SHARK

From an evolutionary standpoint, the shark is said to have developed in fresh water. But for millions of years sharks have lived in the ocean where they are subject to special environmental stresses that menace the composition of their body fluids. These are diagrammed in Fig. 1. The shark swims in a sea containing approximately 500 mEq/L of sodium chloride, or 1,000 mosm/L. The serum sodium of the shark is higher than that of man, approximately 260–290 mEq/L, but sodium and its associated anions amount to only half the external osmotic pressure of the ocean. In order to counterbalance the hypertonicity of the sea, which if unopposed would rapidly dehydrate the shark, elasmobranchs manufacture urea, which circulates in high concentration in the blood and also permeates all cells. The concentration of mineral salts plus urea precisely balances the osmotic concentration of seawater,

517

Supported by Public Health Service Grant AM-18078, and National Science Foundation Grant PCM-77-01146 (to F.H. Epstein) and Grant BG 5781 from the National Science Foundation (to Mount Desert Island Biological Laboratory)

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preventing the depletion of body water. Nevertheless, sharks must combat a tendency to become hypernatremic even beyond their accustomed plasma concentrations of sodium, which to man or other bony vertebrates would appear very high indeed. Sodium chloride tends to diffuse inward through the gills and is also absorbed when the shark swallows his food. The mechanism that elasmobranchs have devised for getting rid of unexpected increments of salt is the rectal gland [1].

RECTAL GLAND OF ELASMOBRANCHS

The rectal gland of the spiny dogfish shark, *Squalus acanthias* (Fig. 2) is a relatively simple structure about as long as your little finger, that looks somewhat like the appendix. It is composed of tubules packed tightly together that look superficially like sections of the renal cortex, except that the movement of fluid and electrolytes is entirely in the secretory direction, from the blood to the lumen. It has a single artery, vein, and central duct. This makes it easy for the rectal gland to be isolated, removed from the shark, and perfused in the laboratory with a shark Ringers solution resembling an ultrafiltrate of shark plasma. The gland is a hardy organ, used to the temperatures of the ocean, so that it does not mind being handled and perfused at the temperature of seawater, approximately 15–17°C [2].

FIG. 2. Photograph of the rectal gland of *Squalus acanthias*. The dorsal aorta is above, and the tail of the shark to the right.
The rectal gland is necessary to the economy of the shark. When it is removed (Fig. 3) there is a progressive rise in serum sodium and chloride [3]. The composition of rectal gland secretion consists almost entirely of hypertonic sodium chloride, at approximately the concentration of seawater, 1½ to 2 times the concentration of salt in the shark plasma. There are miniscule amounts of urea in the rectal gland secretion, which is isotonic (in osmotic terms) with plasma. An important feature of rectal gland secretion as observed in the artificially perfused gland, is that the duct is always electrically negative to the perfusate. Thus, chloride appears to be moving against both a chemical and an electrical gradient into the duct of the gland. Sodium, on the other hand, moves down an electrical gradient although against a chemical gradient [4].

HORMONAL CONTROL OF RECTAL GLAND SECRETION

Rectal gland secretion is under the control of cyclic AMP [5]. The addition of either cAMP or theophylline to solutions perfusing the gland results in an enormous increase in secretory activity. When secretion is stimulated in this way, the electrical potential difference between duct and perfusate invariably becomes more negative and the concentration of sodium chloride in the secretion sometimes increases [4]. Thus, cyclic AMP stimulates the movement of chloride against an even higher electrochemical gradient than existed previously, underlining the active nature of chloride transport in rectal gland secretion. Control of biological activity by adenylcyclase, the "second messenger," usually implies the existence of a hormonal "first messenger." The active polypeptide hormone in this case appears to be vasoactive intestinal peptide or VIP. Of some 20 peptide hormones tested, this has thus far been the only one consistently to elicit rectal gland secretion in the perfused preparation, as illustrated in Fig. 4. Furthermore, the stimulatory action of VIP is inhibited by somatostatin, as it is in mammalian intestine. These two peptide hormones occur naturally in shark tissues and blood, as determined by radioimmunoassay.

The ease of study of the rectal gland and the ability to turn on massive chloride secretion at will have stimulated intensive study of the characteristics of the gland in an attempt to delineate the mechanisms of chloride secretion.
KEY PHYSIOLOGICAL FEATURES OF RECTAL GLAND SECRETION

Some key experimental features of rectal gland secretion that must be incorporated into any general hypothesis of its mechanism are as follows:

1. The gland, as already indicated, is turned on by cyclic AMP and theophylline.
2. Chloride is actively secreted against an electrical and chemical gradient, from blood to duct.
3. Secretion is inhibited by ouabain and by omitting potassium from the perfusing solutions. It therefore is likely to depend on Na-K-ATPase [4].
4. Radioautographic studies indicate clearly that Na-K-ATPase lines the basolateral border of rectal gland cells in extensive basolateral infoldings, positioned in such a way as to pump sodium out of the cell into the blood rather than into the duct [6].
5. Secretion of chloride depends on the presence of sodium in the perfusate [4].
6. Secretion of sodium depends on the presence of chloride in the perfusate [7].
7. Secretion is inhibited by furosemide and thiocyanate, agents which inhibit the active transport of chloride in other tissues like the cornea, the gill, and the mammalian kidney [4].
8. Finally, the intracellular concentration of chloride, as deduced from chemical measurements, as well as the activity of chloride in cellular cytoplasm, as determined directly with the chloride electrode, is 70–80 mEq/L, which is considerably higher than that predicted from the intracellular electrical potential (-60 to -80 mV) [4,8]. Chloride must therefore be transported uphill into the cell across the basolateral cell border. It should be noted that an intracellular chloride concentration exceeding that predicted from the Nernst equilibrium is a feature of other secretory tissues as well. Examples of these include gastric mucosa, kidney, mammary gland, intestinal mucosa, and the goose salt gland.

A GENERAL HYPOTHESIS FOR ACTIVE CHLORIDE TRANSPORT

These features suggest a general hypothesis for the active transport of chloride that is schematized in Fig. 5. Chloride is transported across the basolateral cell borders into the interior of rectal gland cells by a process of co-transport with sodium. The mechanism for co-transport is entirely analogous to that well established for the co-transport of glucose and amino acids with sodium across cell membranes. The energy
FIG. 5. Schematic model for movement of chloride across rectal gland epithelium. Passive ion movements are shown by dotted lines; active transport by solid arrows. A neutral sodium chloride carrier located in basolateral cell membrane effects active movement of chloride into cell, coupled to downhill movement of sodium. Low intracellular sodium concentration and downhill electrochemical gradient for sodium is maintained by activity of Na-K-ATPase. Chloride diffuses passively from cell into tubular lumen down an electrical gradient. Sodium moves down its electrochemical gradient into tubules through paracellular pathways, though an Na-K-ATPase pump on luminal cell border is not excluded. Lower two columns represent the electrochemical potentials (EC) for chloride and sodium across peritubular and luminal membranes, respectively. Calculations are based on Nernst equation where $EC = PD + \text{chemical potential}$, and chemical potential $= \frac{(RT)}{zF} \ln \left(\frac{C}{C_0}\right)$. Values for PD and electrolyte concentrations (mM) in extracellular, intracellular, and ductal fluid are shown in upper columns [4].

for chloride movement derives from the passive inward movement of sodium along its electrochemical gradient. This in turn is maintained by the action of Na-K-ATPase. The Na-K-ATPase pump maintains a low concentration of intracellular sodium and at the same time is responsible for the negative intracellular electrical potential, through the maintenance of a high intracellular concentration of potassium. Chloride passes across the luminal border of the cell in passive fashion, down its electrical gradient, which exceeds the opposing chemical gradient. The most probable route for the outward passage of sodium is paracellularly, through the intercellular space, impelled by an electrical gradient in the secretory direction.

The virtue of this model is that active chloride movement is linked to the action of Na-K-ATPase even though Na-K-ATPase "faces" in a direction opposite to that in which secretion occurs. The model thus resolves a logical dilemma. In several secretory organs (human sweat gland, intestinal mucosa, salivary gland), Na-K-ATPase lines the basolateral side of the cell poised to pump sodium in a direction opposite to that in which secretion actually occurs. In all of these tissues chloride appears to be actively transported.

**DIRECT EVIDENCE FOR SODIUM AND CHLORIDE CO-TRANSPORT IN MEMBRANE VESICLES**

Direct evidence for the co-transport of sodium with chloride in isolated membranes has recently been obtained in membrane vesicles derived from homogenates of shark rectal gland, as illustrated in Fig. 6 [9]. Membrane vesicles were prepared from basolateral membranes (rich in Na-K-ATPase) of rectal glands of the spiny dogfish (*Squalus acanthias*). The uptake of radioactive sodium into these vesicles was greatly enhanced by the presence of chloride and proceeded much more slowly when nitrate or gluconate was the accompanying ion. The enhanced sodium uptake due to chloride was completely inhibited by pre-incubating the vesicles with furosemide.
These experiments provide the first direct evidence of the co-transport of sodium and chloride in isolated membranes and therefore provide strong support for the general hypothesis of active chloride transport outlined in Fig. 5.

CENTRAL ROLE OF NA-K-ATPASE IN TRANSMEMBRANE TRANSPORT

The central role of Na-K-ATPase in providing energy for transmembrane transport of chloride via sodium coupling [10] is emphasized by these experiments. Na-K-ATPase serves as the primary transducer mechanism to transform the chemical energy of ATP into the potential energy represented by the chemical gradient for sodium across plasma membranes. Existence of a number of co-transporter carriers can then provide the means by which this energy can be transformed into the active transport of any variety of substances against their electrochemical gradients into the cell. We may think of Na-K-ATPase as a kind of Sears Roebuck power tool, with which almost anything can be transported depending on the attachments that are available. The direction of transport across an epithelial membrane can be determined simply by the placement of the sodium co-transport attachments and the arranging of suitable permeabilities on either side of the cell. Transport can be regulated by regulating the velocity of the sodium ATPase pump, the number and location of co-transporter carriers, and also by changing the permeabilities of the plasma membrane lining the cell surfaces. In the case of the rectal gland, it seems likely that the site of action of cyclic AMP is on the permeability to chloride of the luminal membrane of the rectal gland cell. This hypothesis can be tested, since it would predict that after stimulation of the rectal gland, the intracellular concentration of chloride would fall and the negative intracellular potential difference would also increase.

REFERENCES
1. Burger JW: Further studies on the function of the rectal gland in the spiny dogfish. Physiol Zool 38:191–196, 1965
2. Hayslett JP, Schon DA, Epstein M, et al: In vitro perfusion of the dogfish rectal gland. Am J Physiol 226:1118–1192, 1974
3. Forrest JN, Silva P, Epstein A, et al: Effect of rectal gland extirpation on plasma sodium in the spiny dogfish. Bull Mount Desert Island Biol Lab 13:41–42, 1973
4. Silva P, Stoff J, Field M, et al: Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. Am J Physiol 233:F298–F306, 1977
5. Stoff JS, Silva P, Field M, et al: Cyclic AMP regulation of active chloride transport in the rectal gland of marine elasmobranchs. J Exp Zool 199:443-448, 1977
6. Eveloff J, Karnaky KJ Jr, Silva P, et al: Elasmobranch rectal gland cell. Autoradiographic localization of [3H] ouabain-sensitive Na,K-ATPase in dogfish, Squalus acanthias, rectal gland. J Membr Biol, in press
7. Solomon RJ, Silva P, Stevens A, et al: Mechanism of chloride transport in the rectal gland of Squalus acanthias: ionic selectivity. Bull Mount Desert Island Biol Lab 17:59-63, 1977
8. Duffey ME, Silva P, Frizzell RA, et al: Intracellular electrical potentials and chloride activities in the perfused rectal gland of Squalus acanthias: a report of preliminary data. Bull Mount Desert Island Biol Lab 18:73-74, 1978, in press
9. Eveloff J, Kinne R, Kinne-Saffran E, et al: Mechanism of active chloride transport: coupled Na/Cl transport by plasma membrane vesicles. Trans Assoc Am Phys, 91:433-443, 1978
10. Frizzell RA, Field M, Schultz SG: Sodium-coupled chloride transport by epithelial tissues. Am J Physiol 236:F1-F8, 1979