INVITED REVIEW

Mechanisms of Rectal Gland Secretion

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Introduction

Swimming in an ocean in which the concentration of sodium chloride approximates 500 mEq/L, all marine vertebrates must continually excrete salt to preserve the constancy of their internal environment. Teleosts, with a serum sodium and chloride that varies from 150 to 180 mEq/L do this via the specialized chloride-secreting cells located in their gills. Elasmobranchs, with serum sodium averaging 280 mEq/L, utilize the rectal gland, a specialized chloride-secreting structure emptying into the terminal large intestine [1].

This review summarizes information about the mechanism of this process, developed at MDIBL over the past 30 years through work on the rectal gland of *Squalus acanthias*, using intact sharks, whole isolated perfused rectal glands [17], rectal gland slices, rectal gland tubules, cultured cells and isolated membranes. Many scientists have worked on the shark rectal gland at MDIBL. We have not attempted a complete or exhaustive review of their contributions in this short paper, but have relied heavily on our own work, to describe the evolution of our own thoughts.

Hormonal Stimulation of Secretion

Chloride secretion by the rectal gland is stimulated by two endogenous polypeptide hormones: vasoactive intestinal peptide,(VIP) [29] and C-type cardiac natriuretic peptide (CNP) [14,28]. In the shark, VIP serves chiefly as a neurotransmitter, rather than as a circulating hormone [21,30]. It is located in rectal gland nerves and released in response to neurostimulation [30]. CNP is released from secretory granules within cardiocytes in response to an increase in the volume of circulating blood (sensed as cardiac dilatation) [21,24-27]. This humoral factor in the blood perfusing the rectal gland causes the gland to secrete chloride. If an isolated rectal gland is perfused by blood from a shark whose blood volume has been expanded, the perfused gland begins to secrete chloride profusely in a way that is blocked by an inhibitor of cellular receptors for cardiac peptides [9].

Shark CNP has two actions on the rectal gland. The first is to release vasoactive intestinal peptide from rectal gland nerves [21]. The second is detected in isolated perfused rectal glands when neurotransmission is blocked by the local anesthetic procaine, or in isolated rectal gland cells and tubules, where no neuronal elements are present [18]. It constitutes a direct action of CNP on rectal gland cells themselves to stimulate chloride secretion.

Secondary Active Transport of Chloride

Chloride secretion is accomplished in rectal gland cells by the process known as "secondary active transport", diagrammed in Figure 1 [5,19,20]. The motive power for active transport is derived from the hydrolysis of ATP to ADP by the enzyme Na-K-ATPase, located on the basolateral border of rectal gland cells [4]. The furosemide-sensitive, Na-K-2Cl cotransporter, also located on the basolateral border, permits sodium to move down its electrochemical gradient into the cell, accompanied by potassium and chloride ions. The concentration of chloride in the cell thus exceeds the concentration predicted at electrochemical equilibrium. Chloride exits the cell into the duct through a chloride channel controlled by a shark version of the cystic fibrosis trasnmembrane regulator (CFTR). Activators

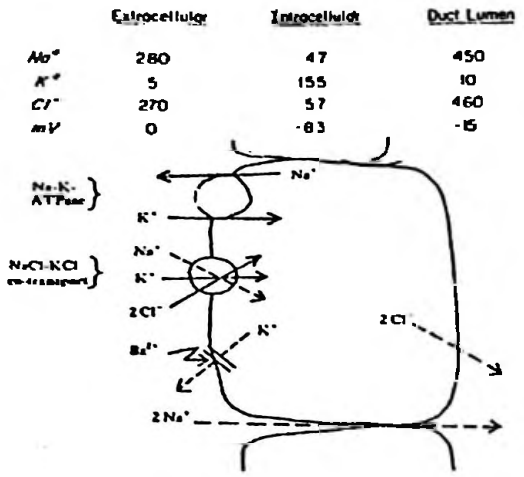

Figure 1. Model of secondary active chloride secretion in shark rectal gland. The motive power for the transcellular movement of Cl^- across the rectal gland epithelium is supplied by the Na-K-ATPase pump, which pumps Na^+ out of the cell into the blood. Na^+ moves into the cell across the basolateral cell membrane down its electrochemical gradient, through the $Na^+K^+2Cl^-$ co-transporter, dragging Cl^- and K^+ with it. Intracellular Cl^- concentration therefore exceeds that predicted by the Nernst equilibrium equation. When the gland is stimulated to secrete, Cl^- channels open in the luminal membrane (controlled by the cystic fibrosis transmembrane regulator - CFTR protein) and chloride exits the cell into the duct lumen. Na^+ moves passively down its electrochemical gradient through paracellular pathways into the duct lumen.

of secretion open this channel, permitting chloride to flow down its electrochemical gradient into the duct. Stimulation of chloride secretion also directly activates the ouobain inhibitable, basolateral Na-K-ATPase [11,13].

Intracellular cascades

The intracellular signals by which VIP and CNP stimulate chloride secretion in the rectal gland appear to follow different pathways. It is clear that, as in other vertebrate tissues that secrete chloride, the chloride exit channel of rectal gland cells is activated by cAMP. Isolated rectal glands are stimulated to secrete by activators of adenylate cyclase, including VIP, forskolin and high concentrations of adenosine. Infusion of cAMP itself into the isolated perfused gland stimulates secretion, as do inhibitors of phosphodiesterase that slow the hydrolysis of cAMP. Inhibiting adenylate cyclase (e.g., by infusing somatostatin) blocks stimulation by VIP or by forskolin [22]. The action of cAMP to stimulate chloride secretion is thought to occur via stimulation of protein kinase A (PKA) which then utilizes ATP to phosphorylate CFTR and open its chloride exit channel.

Different cellular pathways are entrained when the rectal gland is stimulated by CNP [18]. Unlike VIP, CNP does not stimulate the adenylate cyclase of rectal gland membranes. Instead, its action is exerted via protein kinase C (PKC) and guanylate cyclase. Inhibitors of PKC, such as staurosporine, chelerythrine, and bisindolylmaleimide [2,18] block the direct stimulatory action of C-type cardiac natriuretic peptide in isolated perfused rectal glands. These compounds do not inhibit stimulation by VIP. Stimulation of isolated perfused glands with CNP also increases the intracellular accumulation of cyclic guanosine monophosphate (cGMP). If the hydrolysis of cyclic GMP is inhibited: stimulation of chloride secretion by a small dose of CNP is enhanced[16]. Simultaneous activation of PKC and guanylate cyclase appears to be necessary to stimulate rectal gland secretion, if cAMP levels are not raised [18].

Contractile elements of the cytoskeleton may play a role in chloride secretion stimulated by CNP but not by VIP [15]. Direct CNP stimulation of the isolated perfused gland is completely prevented by cytochalasin D, which disrupts actin filaments, and by ML-7, an inhibitor of myosin light chain kinase. These agents have little effect on stimulation of the rectal gland by VIP or by forskolin.

Interacting Pathways

An important question raised by the foregoing studies is whether the intracellular cascades initiated by VIP and by CNP are entirely separate from beginning to end or whether they interact. The patch-clamp studies of rectal gland tubules carried out by Greger and his associates at MDIBL suggested that there is more than one chloride exit channel on

the luminal border of rectal gland cells [6,8]. It seemed possible that chloride secretion stimulated by the direct action of CNP involved a different chloride channel than that opened by VIP or other stimulators of cAMP production. The Forrest laboratory showed that when Xenopus oocytes were transfected with shark CFTR and shark receptor for CNP, exposure to CNP could open the CFTR-associated chloride channel [7]. The discovery of a specific inhibitor of CFTR-associated chloride channels by Dr. Alan Verkman and his associates [12] permitted this question to be investigated in isolated perfused rectal glands. Preliminary exposure of these glands to Verkman's compound markedly inhibited chloride secretion stimulated by CNP as well as VIP [3].

It seems likely, therefore, that the final exit step for chloride secretion, its downhill movement through a CFTR- modulated chloride channel, is the same for CNP and for VIP. Another point of similarity between VIP-induced stimulation and CNP-induced stimulation is that in isolated rectal gland tubules, both are said to increase the fluorescent signal generated by the reaction of intracellular Ca^{++} with fura-2 [7].

Additional interactions between the CNP pathway and the VIP pathway have been uncovered by recent studies of isolated perfused rectal glands, summarized in this edition of the Bulletin. Perfusion of glands with small amounts of CNP greatly enhanced their response to VIP and dibutyryl cAMP, as if the CNP pathway, once activated, amplified the secretory response to cAMP. This synergistic action is consistent with studies by others in mammalian cells, suggesting that CFTR may be phosphorylated by other protein kinases than PKA, and that such phosphorylation renders CFTR more sensitive to activation by PKA [10]. Inhibition of PKA (by the compound H-89) effectively blocks stimulation of intact perfused glands or of rectal gland slices [23] by both VIP and CNP. CNP stimulation thus appears to require PKA activity. It seems possible that a major component of CNP activated chloride

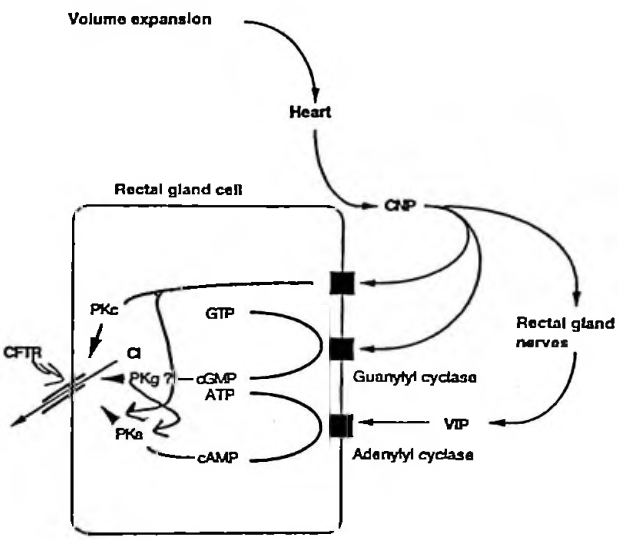

Figure 2. Schema of effect of CNP on secretion of chloride in dogfish rectal gland. Volume expansion causes release of CNP from the heart. CNP binds to a guanylate cyclase-linked B-type receptor and activates guanylyl cyclase producing cGMP. The increase in cGMP is not itself sufficient to produce an increase in chloride transport. A parallel stimulation of protein kinase C (PKC) is probably mediated by phospholipase C and the phosphoinositide pathway producing a synergistic effect on chloride transport. CNP also has an indirect effect to stimulate chloride secretion by eliciting release of VIP from nerves within the rectal gland. VIP stimulates adenylate cyclase, thus increasing cAMP, which activates an apical chloride channel homologous to the human cystic fibrosis transmembrane conductance regulator (CFTR).

secretion involves the sensitization of shark CFTR to the small amounts of cAMP that might be produced within the cell constitutively, that is, by the "idling" of the adenylate cyclase of rectal gland cells at basal levels.

In this perspective, the process of rectal gland stimulation used by elasmobranchs for salt homeostasis appears ingenious and complex. Salt retention and volume expansion lead to release of CNP from a dilated heart. CNP not only stimulates the local release of VIP from nerves within the rectal gland but also greatly sensitizes the gland to stimulation by the cAMP produced by VIP. The result is an immediate burst of salt secretion, which rids

the body of salt and water, and returns the volume of blood and extracellular fluid to normal. These experiments strengthen the view that several parallel and interacting intracellular pathways are involved in the stimulation of rectal gland secretion by endogenous hormones. It seems possible that an analogous pattern of interacting influences may control active transport in other highly developed secreting epithelial organs in man and other vertebrates.

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