Endogenous adenosine is an autacoid feedback inhibitor of chloride transport in the shark rectal gland.

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Research Article

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Endogenous Adenosine is an Autacoid Feedback Inhibitor of Chloride Transport in the Shark Rectal Gland

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Abstract

The present studies define the physiologic role of endogenous adenosine in the perfused shark rectal gland, a model epithelia for hormone-stimulated chloride transport. Chloride ion secretion, and venous adenosine and inosine concentrations increased in parallel in response to hormone stimulation. From a basal rate of 157±26 μeq/h per g, chloride secretion increased to 836±96 and 2170±358 with 1 and 10 μM forskolin, venous adenosine increased from 5.0±1 to 126±29 and 896±181 nM, and inosine increased from 30±9 to 349±77 and 1719±454 nM (all $P < 0.01$). Nitrobenzylthioinosine (NBTI), a nucleoside transport inhibitor, completely blocked the release of adenosine and inosine. Inhibition of chloride transport with bumetanide, an inhibitor of the $Na^+/K^+/2Cl^-$ cotransporter, or ouabain, an inhibitor of Na^+/K^+ ATPase activity, reduced venous adenosine and inosine to basal values. When the interaction of endogenous adenosine with extracellular receptors was prevented by adenosine deaminase, NBTI, or 8-phenyltheophylline, the chloride transport response to secretagogues increased by 1.7–2.3-fold. These studies demonstrate that endogenous adenosine is released in response to hormone-stimulated cellular work and acts at A_1 adenosine receptors as a feedback inhibitor of chloride transport. (*J. Clin. Invest.* 1991. 88:1933–1939.) Key words: adenosine • adenosine receptors • chloride transport • forskolin • 8-phenyltheophylline • shark rectal gland • inosine • hitrobenzylthioinosine

Introduction

A unifying theme for the diverse effects of adenosine is the function of this nucleoside as a regulatory link between cellular energy demand and availability (1–5). Adenosine is uniquely suited for this role because of its relationship to ATP, the energy currency of the cell. According to the adenosine hypothesis, an increase in cellular work or a decrease in oxygen delivery results in an increase in the intracellular production of adenosine (6–8). Adenosine is then released into the extracellular space via facilitated diffusion (9) where it interacts with extracellular adenosine receptors (10) to restore the ratio of energy demand to supply. Newby has termed adenosine a "retaliatory metabolite" because of this regulatory feedback function (2).

Drury and Szent-Gyorgyi (11) first observed the unique renal vasoconstrictive effects of adenosine. Osswald et al. (12, 13) and Spielman et al. (14) later proposed that adenosine acts as a local metabolic regulator of renal hemodynamics, glomerular filtration rate, and renin secretion (for reviews see 14–16). Because adenosine receptors have been localized recently to several distal renal tubular segments, including rabbit cortical collecting tubule cells and medullary thick ascending limb cells in primary culture (17–19), and rat papillary collecting ducts and medullary thick ascending limb (mTAL)[1] tubules (20, 21), it has been proposed that adenosine may directly regulate tubular function (22). However, the metabolic regulation of ion transport in epithelial cells by endogenous adenosine has not been demonstrated.

The rectal gland of the dogfish shark, *Squalus acanthias,* is an epithelial organ composed of homogenous tubules and is an important model for secondary active chloride transport in the thick ascending limb of Henle in the mammalian kidney (23–26). We recently demonstrated the presence of a high affinity inhibitory A_1 adenosine receptor that potently inhibits chloride transport in the rectal gland (27). The present studies define the physiologic significance of this receptor and its relationship to endogenous adenosine. We demonstrate that during hormone stimulation, endogenous adenosine is released from tubular cells and acts as an autacoid at tubular A_1 receptors to inhibit chloride transport.

Methods

Materials. Forskolin and 8-phenyltheophylline were obtained from Calbiochem-Behring Corp., (La Jolla, CA). C_{18} Sep-Paks (#51910) were purchased from Waters Chromatography Div. (Milford, MA). All other reagents were from Sigma Chemical Co. (St. Louis, MO).

In vitro perfusion of the shark rectal gland. Rectal glands were obtained from male spiny dogfish sharks, *Squalus acanthias,* weighing 2–4 kg. Glands were removed and cannulae were placed in the single artery, vein, and duct as described previously (27). Glands were placed in a glass perfusion chamber equilibrated to 15°C with running sea water and perfused with an elasmobranch Ringer's solution containing 270 mM NaCl, 4 mM KCl, 3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1 mM KH_2PO_4, 8 mM $NaHCO_3$, 350 mM urea, 0.5 mM Na_2SO_4, and equilibrated to pH 7.5 by bubbling with 99% O_2 and 1% CO_2. When vasoactive intestinal peptide (VIP) was used, 0.1 mg/ml BSA was added to the Ringer's solution to prevent the binding of VIP to the perfusion bottle. All glands were first perfused for 30 min in the absence of hormones to achieve basal (unstimulated) rates of chloride secretion. Basal values given in the text and figures are the last basal measurement (20–30-min interval). Measurements of duct flow were made at 10-min intervals in all experiments. Venous flow rates and gland weights did not vary significantly between experiments. The mean venous flow rate was 24±1 ml/10 min and the mean gland weight was 1.45±0.06 g (n = 22). Re-

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1. *Abbreviations used in this paper:* ADA, adenosine deaminase; DCF, deoxycoformycin; mTAL, medullary thick ascending limb; NBTI, nitrobenzylthioinosine; 8PT, 8-phenyltheophylline; VIP, vasoactive intestinal peptide.

sults are expressed as microequivalents of chloride secreted per hour per gram wet weight (μeq/h per g)±SEM.

Measurements of adenosine and inosine concentrations in the venous effluent of the in vitro perfused rectal gland. Chloride secretion and venous effluent adenosine and inosine concentrations were measured simultaneously to examine the relationship between adenosine release and hormone-stimulated chloride transport. Rectal glands were perfused as described above and the venous effluent was collected on ice at 10-min intervals for determination of adenosine and inosine concentrations. The venous effluent samples were first desalted and concentrated by passing the fluid over equilibrated C_{18} Sep-Paks at a flow rate of 4.4 ml/min using a Harvard infusion pump (Harvard Apparatus Co., S. Natick, MA). The Sep-Pak cartridge was washed with 1.5 ml of 5 mM KH_2PO_4, pH 7.0, and the nucleosides were then eluted with 5 ml of 80% methanol (28). This solution was evaporated to dryness with a SpeedVac Concentrator (Savant Instruments, Inc., Hicksville, NY) and then resuspended in distilled water.

Adenosine and inosine were assayed by HPLC using HPX pumps, a Gilson 231 automatic sample injector, a Gilson 112 UV detector, and a Microsorb C_{18} 250 mm × 4.6 mm column (Rainin Instrument Co., Inc., Woburn, MA). Samples (100 μl) were loaded onto the column and isocratically eluted with 5 mM KPO_4 and 14% methanol at a flow of 1 ml/min. Data were collected and peak areas were calculated by Rainin HPLC integrator software. The retention times for inosine and adenosine were routinely 5.5 and 12.0 min, respectively. Addition of adenosine deaminase (ADA) completely abolished the adenosine peak. Recoveries for adenosine and inosine were 94±1% (n = 20) and 57±2% (n = 10), respectively, and did not vary over the concentration range of the resuspended samples (0.1–10 μM). Split samples (n = 8) collected in the presence or absence of deoxycoformycin (DCF) had identical concentrations of adenosine, indicating that there was no endogenous ADA activity in the collected samples (data not shown). Results are expressed as nanomolar concentration±SEM.

Statistics. All results are mean±SEM. Data were analyzed for significance with Student's t test for paired or unpaired data.

Results

Correlation of adenosine and inosine release with chloride transport rates

To correlate adenosine and inosine release with rates of chloride transport, rectal glands were perfused in vitro under varying work loads, and chloride secretion, venous effluent adenosine, and inosine concentrations were determined simultaneously.

Effect of stimulation of chloride transport on adenosine and inosine release. In rectal glands perfused with forskolin, venous adenosine, and inosine concentrations increased in parallel with chloride secretion rates. Fig. 1 illustrates chloride secretion rates (*left*), and the corresponding venous adenosine (*middle*) and inosine concentrations (*right*) during basal and forskolin (1 and 10 μM) stimulated conditions. At 50 min of basal perfusion, chloride secretion was 157±26 μeq/h per g (n = 6) and the venous adenosine and inosine concentrations were 5±1 and 30±9 nM, respectively. In glands stimulated with 1 μM forskolin (n = 10), chloride secretion increased to 836±96 ($P < 0.0001$) at 50 min, and adenosine and inosine concentrations increased in parallel to 126±29 ($P < 0.01$) and 349±77 nM ($P < 0.01$). When glands were stimulated with 10 μM forskolin (n = 6), chloride secretion increased to 2170±358 ($P < 0.0002$), and venous adenosine and inosine levels correspondingly increased to 896±181 ($P < 0.001$) and 1719±454 nM ($P < 0.01$). When expressed as release per tissue weight, the release of adenosine increased from basal values of 7.2±1 pmol/min per g under basal conditions to 223±62 and 1611±314 pmol/min per g at 1 and 10 μM forskolin, respectively. The corresponding values for inosine release were 46±12, 584±158, and 3102±804 pmol/min per g. The direct relationship between chloride secretion and venous adenosine and inosine concentrations in these experiments is illustrated in Fig. 2.

In additional experiments, chloride transport and venous effluent adenosine were determined during stimulation with forskolin (0.3–10 μM) and VIP (1–10 nM). Fig. 3 illustrates the direct correlation (r = 0.98) between increasing rates of chloride secretion and venous adenosine concentrations following stimulation with both forskolin and VIP.

Effects of inhibition of chloride transport on adenosine and inosine release. To demonstrate further the parallel relationship between chloride secretion and adenosine release, and to correlate the work of ion transport with this release, two specific inhibitors of transport were used. Table I demonstrates that ouabain, which inhibits membrane Na^+/K^+ ATPase activity in the rectal gland (23), completely inhibited both chloride secretion and adenosine and inosine release. Similarly, Fig. 4 illustrates that addition of bumetanide, an inhibitor of the $Na^+/K^+/2Cl^-$ cotransporter in the rectal gland (29, 30), inhibits adenosine release in parallel with a decrease in chloride secretion. Forskolin (10 μM) stimulated chloride secretion to a maximum value of 1744±217 μeq/h per g. The addition of bumetanide (10 μM) inhibited this stimulatory response to basal values of 172±23. Correspondingly, adenosine values decreased in parallel from 571±104 to 6±3 nM. In forskolin control

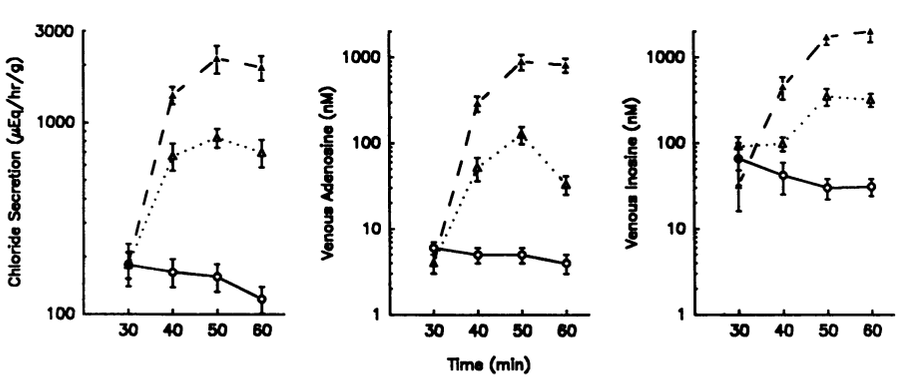

Figure 1. Simultaneous measurements of chloride secretion, and venous adenosine and inosine concentrations. Rectal glands were perfused for 30 min to basal values and then perfused for 30 min under either basal conditions (— ○ —; n = 6) or in the presence of 1 μM forskolin, (— △ —; n = 10), or 10 μM forskolin (— ▲ —; n = 6). Chloride secretion rates, and venous adenosine and inosine concentrations were measured at 10 min intervals and values are mean±SEM. (P values at least $P < 0.05$ for chloride secretion, venous adenosine and venous inosine for basal compared with 1 μM forskolin; P values at least $P < 0.01$ for 10 μM forskolin compared with basal and 1 μM forskolin).

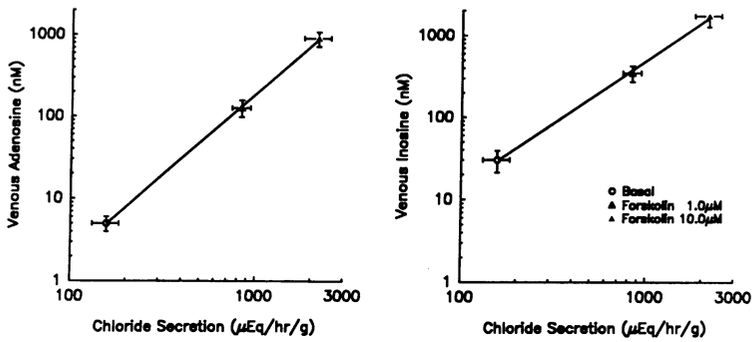

Figure 2. Relationship between chloride secretion and venous effluent adenosine (*left*) and inosine (*right*) concentrations under basal conditions and during forskolin stimulated chloride transport. Values are from the 50 min period of experiments described in Fig. 1 and are mean±SEM. (r = 0.99 for both panels).

experiments (Fig. 4, *closed circles*) chloride secretion and adenosine release did not change over the same time period. Bumetanide also decreased inosine release from 803±118 to 133±16 nM.

Perfusion with NBTI during forskolin-stimulated chloride secretion to determine the source of venous effluent adenosine and inosine. To determine if adenosine and inosine are formed intracellularly and transported to the extracellular space, or formed extracellularly from the metabolism of nucleotides via an ecto 5'-nucleotidase that is present in this tissue (Kelley, G. G., and J. N. Forrest, unpublished data), experiments were performed with the nucleoside transport inhibitor nitrobenzylthioinosine (NBTI). NBTI is a potent inhibitor of the transport of adenosine and other nucleosides in various cell types and binds to plasma membrane sites that are functionally associated with the facilitated diffusion of adenosine (31–33). Table II demonstrates that 1 μM NBTI markedly reduced forskolin-stimulated (1 and 10 μM) increases in extracellular adenosine and inosine, indicating that the source of these nucleosides is transported from tubular cells and not from extracellular degradation of nucleotides.

Physiologic significance of adenosine release

To assess the physiologic significance of the increase in extracellular adenosine in the perfused rectal gland, agents that alter the degradation, transport, and binding of adenosine were employed to inhibit the interaction of this nucleoside with external adenosine receptors.

Effect of adenosine deaminase on forskolin-stimulated chloride transport. ADA deaminates adenosine to inosine, which is inactive at extracellular adenosine receptors. Fig. 5 illustrates the effects of adding adenosine deaminase to the perfusate during forskolin-stimulated chloride secretion in the perfused shark rectal gland. Addition of ADA (0.1 U/ml) to the perfusate increased the response to 1 μM forskolin from 645±56 μeq/h per g (n = 37) to 1,068±100 (n = 27) ($P < 0.0002$).

ADA also increased the stimulatory response to VIP. In these experiments, rectal glands were stimulated with VIP and then ADA was added to perfusate. After 30 min of perfusion with VIP 3 μM, the secretion rate was 1064±69. The addition of ADA 0.1 m/ml increased this rate 1.5-fold to 1,568±131 (n = 4) ($P < .01$).

Effect of a nucleoside transport inhibitor on forskolin-stimulated chloride transport. Similar to the effects of ADA, NBTI also increased the transport response to forskolin. As shown in Fig. 6, addition of 1 μM NBTI to the perfusate increased the chloride transport response to forskolin (1 μM). In the absence of NBTI, the maximum rate of forskolin-stimulated chloride secretion was 836 + 96 μeq/h per g (n = 10). NBTI (1 μM) increased the response to 1 μM forskolin to 1574 + 101 (n = 8; $P < 0.0001$).

Effect of an A_1 adenosine receptor antagonist on forskolin-stimulated chloride transport. In a separate series of experiments, we examined the effects of an A_1 receptor antagonist on forskolin-stimulated chloride transport. 8-Phenyltheophylline (8PT) is a potent adenosine receptor antagonist that would be expected to inhibit the effects of endogenous adenosine by com-

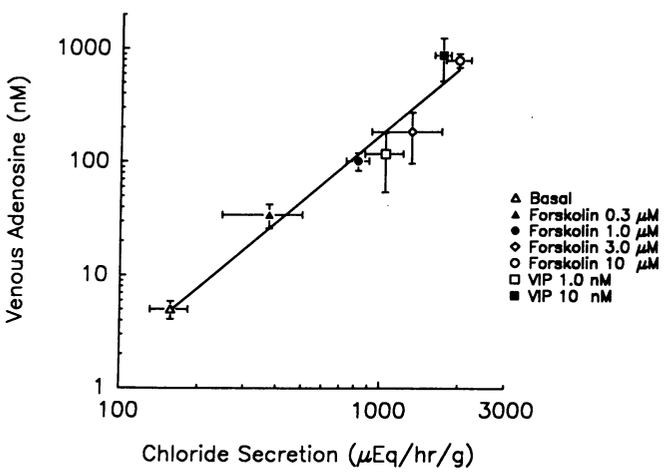

Figure 3. Relationship between venous effluent adenosine concentrations and chloride secretion rates during stimulation with increasing concentrations of forskolin and VIP. Rectal glands were perfused for 30 min to basal values and secretagogues were then added to the perfusate for an additional 30 min. Values are from the 50 min period and are mean±SEM (n = 4–11/group). (r = 0.98).

Table I. Effect of Ouabain on Forskolin-stimulated Increases in Venous Effluent Adenosine and Inosine Concentrations

Condition	Chloride secretion	Venous adenosine	Venous inosine	n
	μeq/h per g	*nM*	*nM*	
Basal	157±26	5±1	30±9	6
Forskolin 10 μM	2006±238*	822±112*	1337±307‡	11
Forskolin 10 μM + Ouabain 100 μM	187±28§¶	5±0.4§¶	60±42‖¶	3

* $P < 0.0001$ compared to basal. ‡ $P < 0.01$ compared to basal. § $P < 0.003$ compared to forskolin 10 μM. ‖ $P < 0.05$ compared to forskolin 10 μM. ¶ P = NS compared to basal.

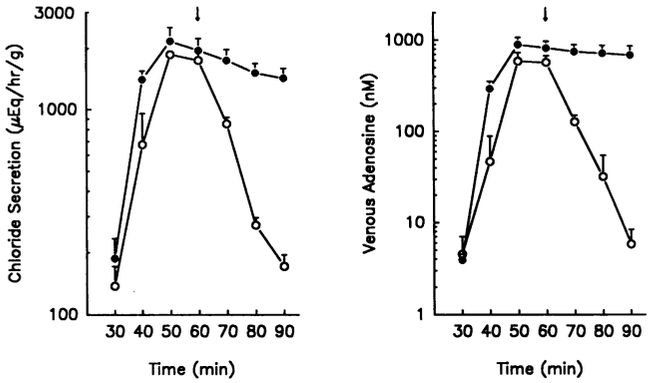

Figure 4. Effects of bumetanide, an inhibitor of the $Na^+/K^+/2Cl^-$ cotransporter, on chloride secretion and venous effluent adenosine concentrations. Rectal glands were perfused for 30 min to basal values and then 10 μM forskolin was added to the perfusate. After 30 min of perfusion with forskolin, glands were perfused with either forskolin alone (— ● —; $n = 6$), or forskolin and bumetanide (— ○ —; $n = 3$). Chloride secretion and venous adenosine concentrations were measured at 10 min intervals and values are mean±SEM. Chloride secretion and venous adenosine values for forskolin alone (compared with forskolin and bumetanide) were significantly different ($P < 0.01$ or less) at 70, 80, and 90 min.

petitively binding to extracellular receptors. In experiments illustrated in Fig. 7, glands were first perfused with forskolin (1 μM) for 30 min and then 8PT (1 μM) was added in the presence of forskolin for an additional 30 min. The addition of 1 μM 8PT increased the chloride secretory response to forskolin 2.5-fold from 380±75 to 966±110 μeq/h per g ($n = 3$; $P < 0.01$). The addition of NBTI to forskolin and 8PT did not further increase the secretory response (data not shown).

Effects of removal of endogenous adenosine on basal chloride transport. ADA, NBTI, and 8PT had no effect on unstimulated (absence of forskolin) rates of chloride secretion. Glands were perfused for 50 min in the absence or presence of these agents. Chloride secretion rates at 50 min were 156±26 μeq/h per g in control glands ($n = 6$), 127±28 in the presence of ADA ($n = 4$), 126±4 with NBTI ($n = 4$), and 122±26 with 8PT ($n = 5$). These results indicate that inhibitory concentrations of extracellular adenosine are present only when the rectal gland is stimulated with secretagogues.

Table II. Effects of NBTI on Forskolin-stimulated Increases in Venous Effluent Adenosine and Inosine Concentrations

Condition	Venous adenosine	Venous inosine	n
	nM	*nM*	
Basal	5±1	30±9	6
Forskolin 1 μM	126±29*	349±77*	10
Forskolin 1 μM + NBTI 1 μM	11±4‡§	68±25‡§	8
Forskolin 10 μM	896±181*	1719±453*	6
Forskolin 10 μM + NBTI 1 μM	37±5*‖	35±18‡‖	5

* $P < .01$ compared to basal. ‡ P = NS compared to basal. § $P < .01$ compared to forskolin 1 μM. ‖ $P < .01$ compared to forskolin 10 μM.

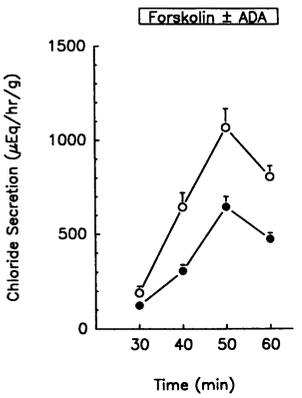

Figure 5. Effect of ADA on forskolin-stimulated chloride secretion. Rectal glands were perfused under basal conditions for 30 min and then 1 μM forskolin was added to the perfusate. Experiments were performed in the absence (— ● —; $n = 27$) or presence (— ○ —; $n = 37$) of ADA, 0.1 U/ml. Chloride secretion was measured at 10-min intervals and values are mean±SEM. (P values < 0.001 at 40, 50, and 60 min).

Discussion

The present studies provide the first direct evidence that endogenous adenosine, produced by the metabolic work of ion transport, is an important autacoid regulator of transport in epithelia. Simultaneous measurements demonstrate a direct relationship between chloride secretion, and adenosine and inosine release from tubular cells of the rectal gland (Figs. 4–6). Over a wide range of chloride transport stimulated by the secretagogues forskolin and VIP, venous adenosine increases in parallel with chloride secretion. When hormone-stimulated chloride transport is inhibited with bumetanide, an inhibitor of the $Na^+/K^+/2Cl^-$ cotransporter (29, 30), or ouabain, an inhibitor of membrane Na^+/K^+ ATPase activity (23), adenosine and inosine release decrease in parallel with chloride secretion. These findings demonstrate a remarkably tight coupling between cell work and adenosine release in a tubular epithelium.

The marked inhibition of hormone-stimulated adenosine and inosine release observed with NBTI indicates that an NBTI-sensitive nucleoside transporter is present on tubular cells of the rectal gland. Furthermore, these data establish that the source of the effluent adenosine is facilitated diffusion from the intracellular to extracellular space and not metabolism of nucleotides by an ecto 5'-nucleotidase. Our findings in this epithelial model are in agreement with studies in the heart indicating an intracellular source for released adenosine (34).

Venous effluent inosine also increases in response to forskolin-stimulated ion transport. The likely source of inosine is intracellular release via the NBTI-sensitive transporter, but it is also possible that inosine is formed from the extracellular metabolism of adenosine. Studies in our laboratory indicate that

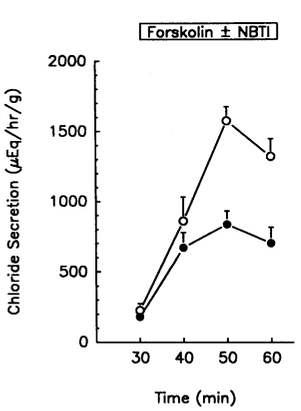

Figure 6. Effect of NBTI, an adenosine transport inhibitor, on forskolin-stimulated chloride secretion. Rectal glands were perfused under basal conditions for 30 min and then 1 μM forskolin was added to the perfusate. Experiments were performed in the absence (— ● —; $n = 10$) or presence (— ○ —; $n = 8$) of 1 μM NBTI. Chloride secretion was measured at 10-min intervals and values are mean±SEM. (P values < 0.0001 at 50 min and < 0.01 at 60 min).

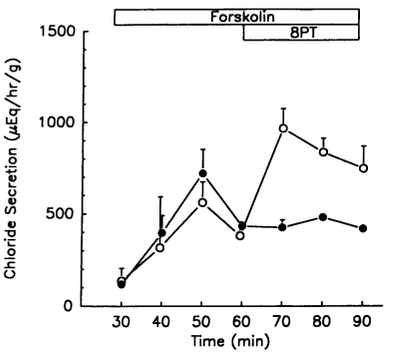

Figure 7. Effect of 8PT, an A_1 adenosine receptor antagonist, on forskolin-stimulated chloride secretion. Rectal glands were perfused for 30 min to basal values and then 1 μM forskolin was added to the perfusate for 30 min. Glands were then perfused either with forskolin alone (— ● —; n = 3), or forskolin and 8PT (— ○ —, n = 3). Chloride secretion was measured at 10-min intervals and values are mean±SEM (P values < 0.02 at 70 and 80 min compared to forskolin controls, and compared with the 60-min time point before 8PT was added).

the intracellular concentration of inosine is more than twofold the adenosine concentration (Kelley, G. G., O. S. Aassar, and J. N. Forrest, unpublished observations) suggesting that a major source of the inosine is efflux from intracellular pools.

The physiologic significance of the released adenosine was demonstrated with agents (ADA, NBTI, 8-PT) that employ diverse mechanisms to prevent the interaction of endogenous adenosine with extracellular receptors. These agents had no effect on basal secretion but markedly increased the secretory response to forskolin. Previous studies defined a high affinity inhibitory A_1 adenosine receptor in the rectal gland and demonstrated that exogenous adenosine agonists potently inhibit hormone-stimulated chloride secretion via this receptor (27). The present studies demonstrate that the endogenous adenosine released in response to forskolin-stimulated chloride transport interacts with this A_1 adenosine receptor to inhibit secretion.

From these studies we propose the following model of adenosine as an inhibitory feedback regulator of chloride transport in the shark rectal gland (Fig. 8). Chloride secretion is stimulated by hormones acting on receptors that activate the catalytic subunit of adenylate cyclase via nucleotide binding proteins (N_s). Cyclic AMP–dependent processes activate chloride channels in the apical plasma membrane (35). Secondary active chloride transport in this tissue has been well characterized adding to the model described by Silva et al. (23). At the basolateral membrane chloride and potassium ions enter the cell tightly coupled to sodium via a bumetanide sensitive $Na^+/K^+/2Cl^-$ cotransporter (29, 30). Potassium ions recycle across the basolateral membrane via a barium-sensitive channel (26, 35). The driving force for ion movement is an inwardly directed gradient for sodium maintained by a high specific activity of membrane-associated Na^+/K^+ ATPase. This enzyme is the primary site of ATP hydrolysis and is responsible for the high rates of oxygen consumption observed in this tissue (36, 37). Stimulation of secretion results in a marked increase in Na^+/K^+ ATPase pump activity (37–39) and ATP hydrolysis resulting in an increased tissue content of adenosine. Adenosine then exits the cell down a concentration gradient via an NBTI-sensitive nucleoside transporter into the extracellular space. At this site it interacts with a high affinity A_1 receptor coupled to an inhibitory nucleotide regulatory binding protein (N_i) to inhibit chloride transport by both cyclic AMP–dependent and –independent processes (27, 40). ADA, 8PT, and NBTI block this feedback inhibition by preventing endogenous adenosine from interacting with the A_1 receptor. Thus, in the rectal gland, adenosine functions as an inhibitory autacoid to regulate the metabolic work of ion transport and link energy demand and availability.

The relationship between metabolic work and the subsequent release of adenosine has been examined previously in

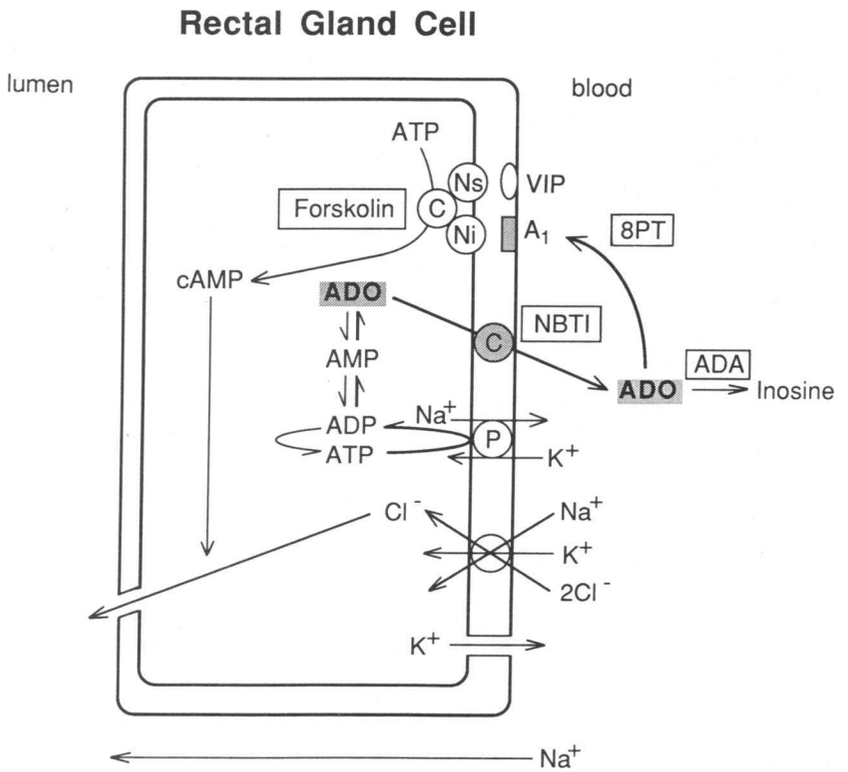

Figure 8. Model of adenosine as an inhibitory autacoid regulator of hormone-stimulated chloride transport in the rectal gland cell. Adenosine production and efflux from the cell is increased following stimulation of chloride secretion by secretagogues. Extracellular adenosine interacts with inhibitory A_1 adenosine receptors to inhibit chloride transport via cyclic AMP–dependent and –independent processes. Only well-established cyclic AMP–dependent elements are shown here. The cyclic AMP–independent pathway(s) for A_1 receptors in the rectal gland are not defined (for discussion see 27). ADO, adenosine; ATP, adenosine triphosphate; VIP, vasoactive intestinal peptide; A_1, inhibitory adenosine receptor; N_s, stimulatory GTP binding protein; N_i, inhibitory GTP binding protein; C, catalytic unit of adenylate cyclase; C, cellular nucleoside transporter; P, Na-K ATPase; ADA, adenosine deaminase; NBTI, nitrobenzylthioinosine; 8PT, 8-phenyltheophylline.

cardiac tissue (41–45), and also in skeletal muscle (46–48) and brain (49–51). In two studies in the isolated perfused guinea pig heart, the release of adenosine into the effluent perfusate increased from basal values of 11–81 to 250–1,479 pmol/min per g following norepinephrine infusion (44, 45). These values are similar to our findings in the perfused rectal gland where adenosine release increases from basal values of 7±1 pmol/min per g, to 223±62 and 1,611±314 pmol/min per g at 1 and 10 μM forskolin, respectively. It is important to note that the metabolic work of cardiac tissue is muscle contraction, whereas in epithelia it is transcellular ion movement. Thus, in cardiac tissue ouabain increases the production of adenosine because of a direct inotropic effect (44), but as demonstrated in the present studies, in epithelia ouabain inhibits the production of adenosine by inhibiting secondary active ion transport.

In the mammalian kidney endogenous adenosine production is increased by ischemia (53), hypoxia (54), and sodium loading (55). Osswald et al. (55) postulated that tubular cells are the primary source of this adenosine and proposed a feedback role for adenosine via receptors on renal vascular and juxtaglomerular cells. Recent studies have defined further the function of adenosine in regulating renal hemodynamics (56–58), renin release (15), and tubuloglomerular balance (59, 60). In contrast, effects of endogenous adenosine on ion transport in tubular segments of the nephron have not been demonstrated. The present studies establish that in secretory tubules of the rectal gland, epithelial cells per se are both the source of adenosine and the target site for feedback inhibition of ion transport by this nucleoside.

Inhibitory A_1 receptors recently have been identified on several tubular segments of the mammalian kidney that are characterized by high rates of ion transport and Na^+/K^+ ATPase activity, including the mTAL (19–21), and cortical and papillary collecting ducts (17, 18). In the isolated perfused rat kidney, A_1 receptor agonists prevent cellular injury to mTAL cells caused by ion transport in an hypoxic environment (61). Given the striking similarities between rectal gland tubules and the mTAL (23–26), it is reasonable to propose a similar autacoid feedback mechanism for adenosine in tubules of the mammalian kidney.

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