ENDOGENOUS ADENOSINE INHIBITS CHLORIDE SECRETION VIA A_1 ADENOSINE RECEPTORS IN THE RECTAL GLAND OF THE SHARK, *Squalus acanthias*

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Adenosine is an important local regulator of many cellular processes including coronary artery vasodilation, inhibition of platelet aggregation and renal vasoconstriction. In many instances, extracellular adenosine concentrations have been linked to cellular metabolic activity. For example, adenosine is a potent coronary artery vasodilator and has been proposed as the vasoactive substance released from the myocardium during hypoxia from increased work (Imai et al. Circ. Res. 15, 1964). Similarly, renal vascular tone has been related to adenosine concentrations and oxygen consumption (Osswald, Kid. Intern. 22, 1982) We have previously identified stimulatory (A_2) and inhibitory (A_1) adenosine receptors in shark (*Squalus acanthias*) rectal gland, a model epithelium for cyclic AMP mediated Cl transport (Poeschla et al. Bull. MDIBL 24, 1984). We now provide evidence that adenosine released from cells during increased metabolic activity is an important physiologic inhibitor of chloride transport.

Rectal glands were isolated and perfused as previously described except when vasoactive intestinal peptide (VIP) was used. In these experiments 0.1 mg/ml BSA was added to the Ringer's solution which was bubbled before the addition of drugs but not during the experiment. Each drug was infused for three 10-minute periods. Results are expressed as µEqCl/h/g and are the mean±SEM of 3 or more experiments.

Adenosine deaminase (ADA) was used in several experiments to examine the role of endogenous adenosine. ADA deaminates adenosine to inosine (which is not an adenosine receptor agonist) thereby removing adenosine from extracellular adenosine receptors. In 10 experiments ADA had no effect on basal secretion. When the gland was stimulated with forskolin, a direct activator of adenylate cyclase, the response to forskolin was two fold greater in the presence of ADA. In the absence of ADA, 1 µM forskolin increased Cl secretion from basal values of 126±67 to 479±50 µEqCl/h/g (n=15); whereas, in the presence of 0.1 U/ml ADA the response to forskolin was significantly ($p<0.01$) greater at 842±149 (n=6). In 12 of these experiments shown in Figure 1, 1 µM 2-Chloroadenosine (2ClADO), an ADA resistant adenosine analogue, completely reversed the stimulation due to ADA and inhibited Cl secretion to basal values. Other adenosine analogues including R-PIA and NECA had similar effects. In the absence of ADA in the perfusate the maximum response to forskolin declined with time ($p<0.01$, 20 min compared to 30 min time period); this decline was prevented entirely by ADA.

The response to the secretagogue VIP was also increased by ADA (Figure 2). From a basal value of 126±30, VIP (3nM) stimulated Cl secretion to 1113±65. In the same experiment, when 0.1 U/ml ADA was added to the perfusate, Cl secretion increased to 1498±85 ($p<0.01$, n=4). Again, this increased secretion rate due to ADA was completely reversed (to 912±140) with 2ClADO ($p<0.05$). We observed that BSA was required in the perfusion medium to optimize the VIP response. In the presence of BSA, the VIP response was more than 10 fold greater than in the absence of BSA. This effect may be caused by binding of VIP to the perfusion resevoir glass.

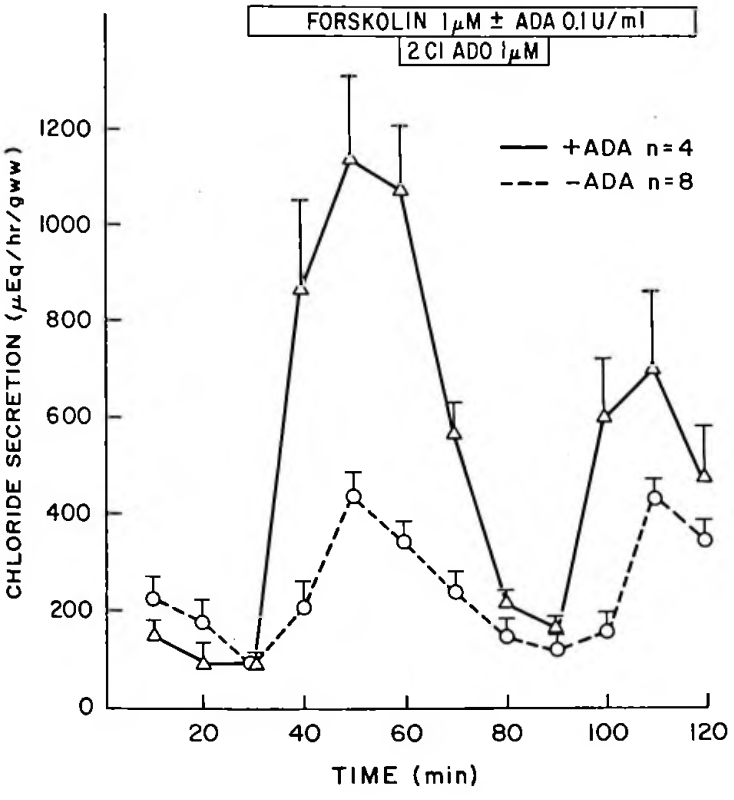

Figure 1: Effects of Forskolin (1 μM) and 2 ClAdo (1 μM) on chloride secretion in the perfused rectal gland in the presence and absence of adenosine deaminase (ADA) in the perfusate (n=12 experiments).

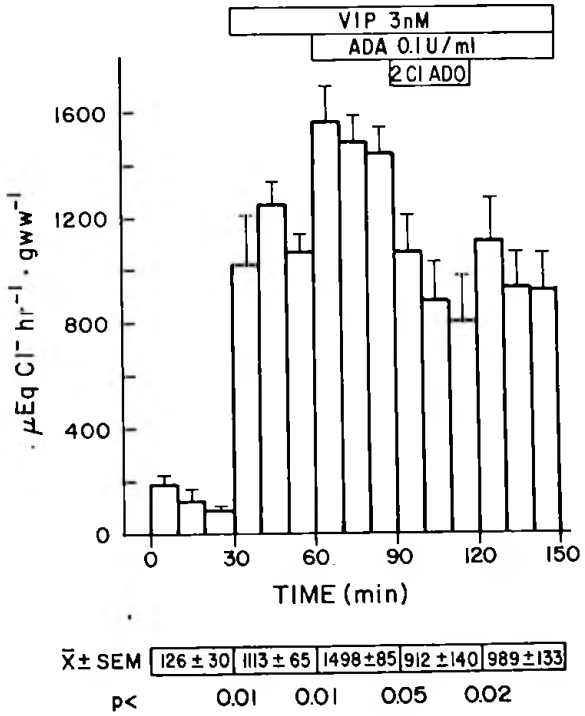

Figure 2: Effects of VIP (3 nM) on chloride secretion in the perfused rectal gland before and after the addition of adenosine deaminase (ADA) to the perfusate (n=4 experiments).

In two experiments, nitrobenzylthioinosine (NBTI) was used as an adenosine transport inhibitor. At 1 μM, NBTI had no effect on basal Cl secretion, but increased forskolin stimulated secretion by 45%. Other investigators have shown that NBTI increases intracellular adenosine concentrations and decreases extracellular concentrations (Dobson J.G. and Schrader J., J. Mol. Cell Cardiol. 16, 1984) . Thus, similar to ADA, NBTI enhances forskolin stimulated secretion by reducing the extracellular adenosine concentrations and preventing interaction with inhibitory adenosine receptors.

These data support the hypothesis that endogenous adenosine is an important physiologic regulator of chloride secretion. Note that neither ADA nor NBTI had effects on basal activity but that both enhanced Cl secretion when the gland was stimulated by secretagogues. These findings indicate that extracellular adenosine reaches significant inhibitory concentrations only during the increased metabolic work of hormonal stimulation. During stimulation of secretion when oxygen consumption, Na-K-ATPase activity and ATP hydrolysis are maximal, the increased release of adenosine may serve as a feedback inhibitor to prevent further tissue hypoxia and possible cellular injury. Since adenosine is linked intimately to ATP hydrolysis and metabolic activity, it is an excellent candidate for this role.

It is now apparent that the rectal gland has both inhibitory and stimulatory adenosine receptors and that a transition from inhibition to stimulation of secretion occurs between 1 μM and 5 μM adenosine. The present studies establish that the inhibitory receptor has an important physiologic role in the perfused gland. Further work is required to clarify the role of the stimulatory (A_2) receptor.