HPLC MEASUREMENTS OF ADENOSINE IN THE RECTAL GLAND OF SQUALUS ACANTHIAS: ADENOSINE IS RELEASED AT INHIBITORY CONCENTRATIONS FOLLOWING HORMONAL STIMULATION OF TRANSPORT.

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Adenosine has been proposed as an important inhibitory feedback regulator of metabolic activity. In several tissues, e.g., in cardiac cells, it is formed intracellularly during increased cellular work and is transported out of cells where it interacts with specific receptors that inhibit cellular activity . In the rectal gland, we have previously shown the presence of both stimulatory A_2 (Forrest et al. Bull MDIBL 20:152-155, 1980) and inhibitory A_1 (Kelley et al. Bull MDIBL 26:177-179, 1986) adenosine receptors. Furthermore, we have shown that adenosine deaminase (ADA), an enzyme that deaminates adenosine to inosine, and nitrobenzylthioinosine (NBTI) an adenosine transport inhibitor, both have no effect on basal secretion but enhance forskolin stimulated secretion, (Kelley et al. Bull MDIBL 25:108-110, 1985). From these studies, we proposed that during the increased work of hormonal stimulation, adenosine is released from cells where it inhibits secretion through the A_1 receptor. In the present studies, venous effluent adenosine concentrations were measured by HPLC to demonstrate directly that adenosine is released at inhibitory concentrations during hormonal stimulation.

Chloride secretion and venous effluent adenosine concentrations were measured simultaneously to correlate adenosine release with secretion. Rectal glands were perfused as previously described. Adenosine was measured by a method of combining Sep-pak preparation and high performance liquid chromatography (HPLC) (Dobson et al. Am. J. Physiol. 251:H455, 1986). Venous effluent (approximately 30 ml) was collected for 10 minute periods then immediately passed over equilibrated C_{18} Sep-paks (Waters Associates, #51910) at a flow rate of 4.4 ml/min using a Harvard infusion pump. The Sep-pak was then washed with 1.5 ml of 5 mM KH_2PO_4 and the sample was eluted with 3 ml of 80% methanol, then evaporated to dryness under N_2 at 40 ^{o} C. and resuspended in 500 ul of distilled water. The nucleoside was then assayed by HPLC using an Altex 100 pump, a 7020 Rheodyne Injector, a Rainin Microsorb C18 250 by 4.6 mm column, and a 254 nm Altex 153 UV detector. One hundred ul of sample was loaded onto the column and isocratically eluted with a 5 mM KH_2PO_4 and 10% methanol solution. The retention time for adenosine was routinely 14.5 minutes. The recovery of adenosine was 90.4 $\pm$ 1.2% (n=29).

Table 1 shows the relationship between rates of forskolin stimulated chloride secretion and venous effluent adenosine concentrations. Rectal glands were perfused under basal conditions, then secretion was stimulated with increasing forskolin concentrations from 0.1 uM to 10 uM. This table demonstrates that venous adenosine concentrations increase with increasing chloride transport from a basal value of 3.9$\pm$0.6 nM to 166 fold above basal at 646 $\pm$90 nM when secretion is stimulated maximally with 10 uM forskolin. In other experiments (data not shown) we have demonstrated that 2-chloroadenosine, which has a similar affinity for adenosine receptors as adenosine, inhibits secretion at concentrations <1 uM, with an IC_{50} of approximately 10 nM. Thus, the range of adenosine concentrations released into the venous effluent during hormonal stimulation is sufficient to cause feedback inhibition of secretion. These measurements of adenosine are consistent with our previous hypothesis that ADA and NBTI enhance forskolin stimulated secretion by removing endogenous adenosine from inhibitory A_1 receptors.

TABLE 1 EFFECT OF FORSKOLIN ON SECRETION AND VENOUS EFFLUENT ADENOSINE CONCENTRATION

Forskolin Concentration (uM)	Secretion * (uEqCl/hr/g)	Venous Effluent * Adenosine Concentration (nM)	N
Basal	125±22	3.9±0.6	26
0.1	86±17	3.8±0.7	3
0.3	288±197	38±11	2
1.0	733±132	59±14	10
3.0	1131±394	183±87	2
10.0	1753±206	646±90	5

* Values are from the 20 to 30 minute period after the addition of forskolin except for the basal values which are from the 10 minute period preceding the addition of forskolin.

In an effort to show that adenosine is released secondary to the increased metabolic work of stimulation, the effect of the transport inhibitors bumetanide and ouabain was determined on forskolin stimulated secretion and the venous effluent adenosine concentration. Bumetanide, 10 uM, an inhibitor of the Na, K, Cl cotransporter in the gland, completely inhibited forskolin (10 uM) stimulated secretion and the venous effluent adenosine concentration decreased to basal levels. In three experiments, forskolin (10uM) increased secretion from 97±45 to 1744±218 and increased venous adenosine concentrations from 4.5±2.6 to 571±104 nM. The addition of 10uM bumetamide inhibited the forskolin stimulated secretion to 172±25 ($p<0.05$) and the venous adenosine concentration to 5.7±2.7 ($p<0.05$). Similarly, 10 uM ouabain also completely inhibited forskolin (10 uM) stimulated secretion and reduced the venous effluent adenosine concentration to basal values (data not shown). In addition to showing that venous adenosine concentrations parallel cellular activity, these studies also indicate that adenosine is derived from ATP hydrolysis and not from the metabolism of increased tissue cAMP concentrations.

These studies provide the first measurements of adenosine release in transporting epithelia and demonstrate conclusively that adenosine is released at inhibitory concentrations during hormonal stimulation of chloride transport in the rectal gland. The source of this adenosine is from the metabolic work of chloride transport and not cAMP metabolism because inhibitors of transport that do not inhibit adenylate cyclase, decrease secretion and venous adenosine concentrations in parallel.

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