FORSKOLIN INCREASES TISSUE ADENOSINE AND INOSINE FORMATION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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We have previously demonstrated that adenosine is released into the venous effluent during hormonal stimulation of chloride secretion in the perfused rectal gland (Kelley et al. Bull MDIBL 27:132-133, 1987). This adenosine then serves as an autocoid inhibitor of transport via extracellular A_1 receptors. We propose that adenosine is formed intracellularly from ATP metabolism during cellular work. The present studies were designed to examine tissue concentrations of adenosine and inosine under basal and stimulated conditions.

Rectal glands were perfused and rates of chloride secretion measured as previously described. Following basal perfusion or 20 minutes of stimulation with 1 or 10 μM forskolin, glands were removed, rapidly weighed and freeze clamped (without interruption of perfusion) with aluminum tongs at the temperature of liquid N_2 in order to avoid hypoxia. Tissue samples were lyophilized at -70^{o} and extracted with ice cold 0.5 N perchloric acid, homogenized with an Ultra Turrax and centrifuged at 10,000g. The supernatant was neutralized to pH 8.0 with 2M potassium hydroxide and then centrifuged to remove precipitated salts. The supernatant was then directly assayed by HPLC using the method we have previously described. The recovery of adenosine was 79.05 $\pm$ 1.92 % (n=10).

Table 1 shows the relationship between forskolin stimulated chloride secretion and tissue concentrations of adenosine and inosine. Tissue concentrations of adenosine increased with increasing chloride secretion from a basal value of 8.62 $\pm$ 1.97 (ng/g wet tissue) to 45.1 $\pm$ 9.2 with 1μM forskolin and to 74.8 $\pm$ 12.4 with maximal secretion at 10 μM forskolin. Inosine concentrations increased in parallel from a basal value of 18.3$\pm$4.52 to 59.1 $\pm$7.83 and 151$\pm$25.6 with 1 and 10 μM forskolin respectively.

Our measurements of basal tissue adenosine concentrations are consistent with those measured previously in brain, liver, and myocardium. In myocardium, it is accepted that the signal for adenosine formation is a decrease in the oxygen supply to consumption ratio. Sparks et al. (Am.J.Physiol.256:H772, 1989) have measured tissue adenosine concentrations in control versus hypoperfused myocardium and found a 2 fold increase in adenosine concentration in the hypoperfused state. Our studies provide the first measurements of tissue adenosine in a transporting epithelial organ and demonstrate a 9 fold increase in tissue adenosine concentration during maximal hormonal stimulation of chloride transport.

TABLE 1

EFFECT OF FORSKOLIN ON SECRETION
AND TISSUE ADENOSINE AND INOSINE CONCENTRATIONS *

Forskolin Conc. (μM)	Secretion (μEqCl/h /g)	Tissue Concentrations (ng/g tissue) Adenosine	Inosine	n
basal	123±16	8.62±1.97	18.3±4.52	8
1	1013±136	45.1±9.2	59.1±7.83	12
10	1887±133	74.8±12.4	151±25.6	8

*Results are expressed as ± standard error of the mean.

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