MECHANISM OF SIGNAL TRANSDUCTION OF INHIBITORY A_1 ADENOSINE RECEPTORS IN THE RECTAL GLAND OF THE SHARK, Squalus Acanthias

Grant G. Kelley, Harold Barron, and John N. Forrest, Jr.
Department of Medicine, Yale University School of Medicine, New Haven, CT.

In non-epithelial tissues, inhibitory (A_1) adenosine receptors are coupled to adenylate cyclase, to the calcium messenger system and to specific ion channels (Spielman and Forrest. Proceedings of the 3rd International Congress on Adenosine, Munich, 1986, in press). In the rectal gland, adenosine stimulation of chloride secretion has been shown to be cAMP dependent (Kelley et al. Bull MDIBL 24:102, 1984). It therefore seems reasonable that inhibition of chloride secretion may be mediated by inhibition of cAMP. In this report we present data indicating that inhibition occurs by two mechanisms, one involving cAMP and one independent of cAMP.

Rectal glands were isolated and perfused as previously described. Each drug was infused for three 10-minute periods. Results are expressed as μEqCl/hr/gww and are the mean ± SEM of 3 or more experiments. At specific time points, portions of the tip of the gland proximal to artery were removed and a ligature was placed to prevent leakage of fluid. Tissue cyclic AMP content was measured in these portions of the gland as previously described. Cyclic AMP content is expressed as pmoles cAMP/mg protein and all values are the mean ± SEM of 3 or more experiments.

To determine if the effect of adenosine to inhibit forskolin stimulated chloride secretion is mediated by inhibition of cAMP accumulation, rectal glands were perfused with various drug combinations and chloride secretion and tissue cAMP content were measured simultaneously. These experiments are shown in Figure 1: the top panel depicts rates of chloride secretion and bottom panel shows the corresponding tissue content of cAMP. In the first set of experiments, four rectal glands were perfused to basal values of chloride secretion (64±11 μEq Cl/hr/gww), and cAMP content (11.6±2.8 pmoles cAMP/mg protein). 2-Chloroadenosine (2Clado) (0.1 μM) and forskolin (1 μM) were then added to the perfusate. 2Clado completely blocked forskolin stimulated chloride secretion to 56±7 but cAMP content increased 5-fold to 60.7±28 ($p<0.01$). When the 2Clado was removed, secretion increased promptly to 665±162 and the cAMP content increased to 452±82. These experiments reveal that while the cAMP content did not remain at basal values in the presence of 2Clado, it was significantly less than in the absence of the agonist, suggesting that the inhibition was at least partially cAMP dependent.

A second set of four experiments was performed to determine the cAMP content in glands perfused with forskolin alone. In the absence of 2Clado, forskolin stimulated chloride secretion and increased cAMP content from basal values of 90±9 to 775±119 and 15.5 to 329±45 respectively. Thus, compared to the glands perfused with 2Clado and forskolin in the previous experiment, cAMP content was 5 fold greater in the absence of 2Clado at 50 min. This supports the finding that a component of the 2Clado inhibition is cAMP dependent. Further perfusion with forskolin resulted in no further increase in secretion but cAMP content continued to increase to 639±157.

A third set of three experiments was performed to determine the effect of continued perfusion with 2Clado. Continued perfusion with 2Clado completely inhibited secretion; however, cAMP content steadily increased to 347±96 at 80 minutes, which is not significantly different from forskolin control values noted

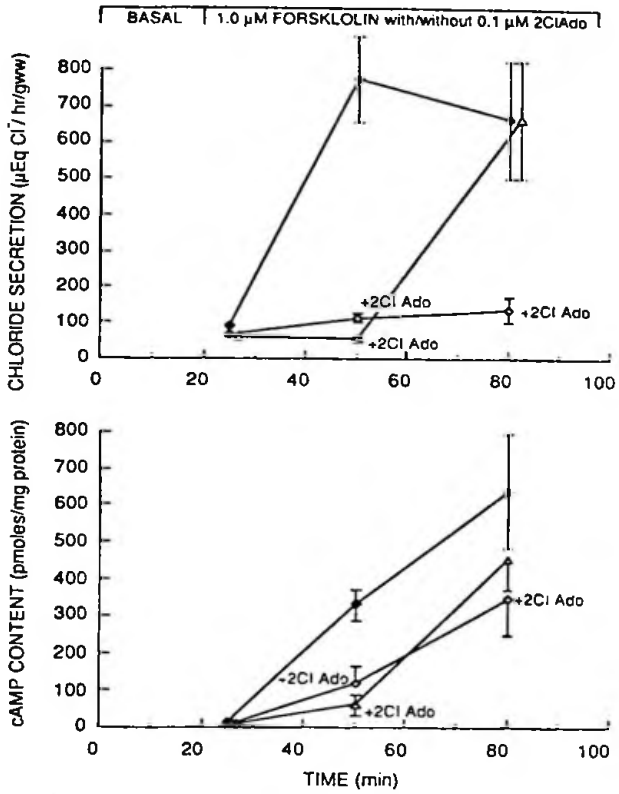

Figure 1. Top panel. Rates of chloride secretion in perfused rectal glands. All glands were perfused for 30 min under basal conditions and then forskolin (1 μM) was added to the perfusate with or without 2Clado as indicated. Bottom panel. Corresponding tissue cyclic AMP content. Values are mean ± SEM of 3 or more experiments in each group.

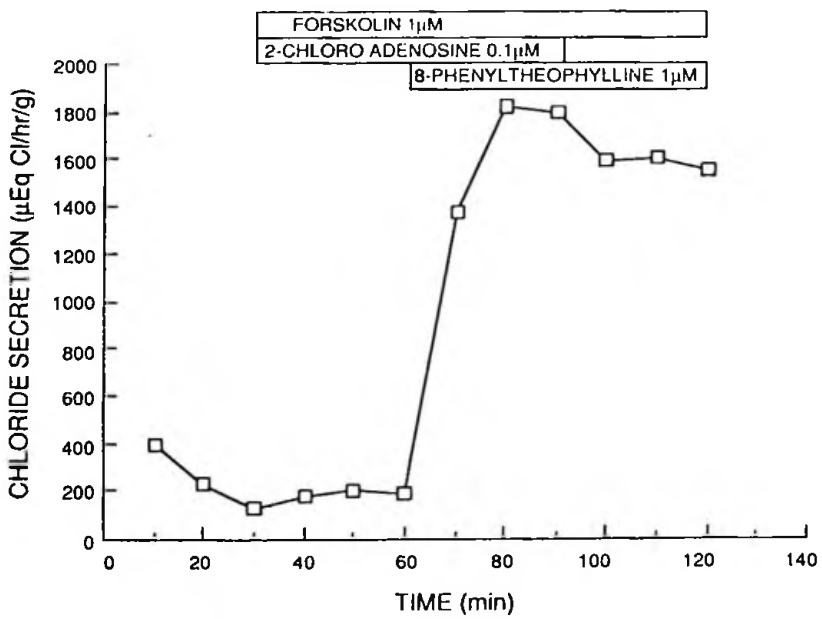

Figure 2. Effect of 8 phenyltheophylline to reverse 2-chloroadenosine inhibition of forskolin stimulated chloride secretion. Values are from one representative experiment of 10 similar experiments.

in the previous experiments. Thus, despite total inhibition of secretion, cAMP content increased to forskolin alone values indicating that under these conditions the inhibition of chloride secretion appears to be independent of the cyclic AMP content.

The effects of 2 Clado to inhibit chloride secretion was entirely reversed by the addition of 8 phenyltheophylline, an antagonist of A_1 adenosine receptors (Figure 2) demonstrating that inhibition of secretion is receptor mediated.

Our results strongly suggest that A_1 adenosine receptor mediated inhibition of chloride secretion occurs by mechanisms that are both cyclic AMP dependent and independent. Inhibition of adenylate cyclase or stimulation of cAMP dependent phosphodiesterase may account for the cAMP dependent inhibition. Receptor coupled effects on specific ion channels (K^+, Cl^- or Ca^{+2}) and/or the calcium messenger system (C-kinase and inositol 1,4,5-triphosphate) are candidates for the cyclic AMP independent mechanisms suggested by there experiments.