EVIDENCE FOR A RIBOSE SPECIFIC ADENOSINE RECEPTOR (R_a) MEDIATING STIMULATION OF CHLORIDE SECRETION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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Secretion of sodium chloride by the rectal gland of Squalus acanthias is stimulated by cyclic adenosine monophosphate (cyclic AMP). Adenosine is known to modify both cyclic AMP concentration and cyclic AMP mediated physiologic function in a variety of cells possibly by interacting with external receptors. Erlij et al., first reported in this bulletin (18: 92, 1978), that adenosine added to the perfusate markedly stimulated chloride secretion in the perfused rectal gland of Squalus acanthias. Recently, Londos et al., (Proc. Nat. Acad. Sci. USA 77: 2551, 1980) have proposed that the effects of adenosine on adenylate cyclase activity and cyclic AMP accumulation in a variety of tissues are mediated by specific subclasses of adenosine receptors. With the use of adenosine analogs, these workers have identified two distinct sites at which adenosine mediates either stimulation or inhibition of adenylate cyclase. One site termed R_a mediates stimulation of adenylate cyclase, requires the integrity of the ribose moiety of adenosine, and accepts as agonists only adenosine analogs modified in the purine moiety. A second site, designated P, requires the integrity of the purine component of adenosine, and accepts as agonists only analogs modified in the ribose moiety; this site mediates inhibition of adenylate cyclase in all systems examined. Methylxanthines, particularly theophylline, competitively inhibit adenosine elicited increases in cyclic AMP and thus are specific antagonists at the R site, but have no effect on P site effectors. In the present experiments performed during 1979 and 1980, we have utilized a series of adenosine analogs and methylxanthines to characterize the response to adenosine in the isolated perfused rectal gland of the elasmobranch. Our results provide evidence consistent with a ribose-specific stimulatory adenosine receptor (R_a) stimulating sodium chloride secretion in this tissue.

Rectal glands were obtained from spiny dogfish (Squalus acanthias) of either sex weighing 2-6 kg. The rectal gland was removed and cannulae were placed in the rectal gland artery, vein, and duct and the rectal gland was perfused with an elasmobranch Ringer's solution under conditions previously described (Forrest, Bull. MDIBL. 18: 10, 1978). Measurements of rectal gland fluid flow rate, chloride secretion, and rectal gland fluid chloride concentrations were made at ten-minute intervals in all experiments.

The effect of theophylline to inhibit reversibly adenosine stimulated chloride secretion is shown in Figure 1. When chloride secretion is stimulated by 10^{-4}M adenosine, increasing concentrations of theophylline (to 10^{-4}M) result in greater than 80% inhibition of adenosine stimulation. This inhibition is rapidly reversed by removing theophylline from the perfusate. The results of 10 experiments demonstrating a dose dependent inhibition by theophylline of adenosine stimulated chloride secretion are summarized in Table 1. During stimulation with adenosine 10^{-5}M, equimolar concentrations of theophylline inhibited chloride secretion by 91%. At 10^{-4}M adenosine, chloride secretion was inhibited by 49% with 10^{-5}M theophylline and by 80% with equimolar (10^{-4}M) theophylline. Under all conditions studied, theophylline antagonism of the adenosine effect was rapidly reversible with return of chloride secretion following perfusion with adenosine without theophylline.

We next studied the effects of adenosine analogs modified in the ribose moiety of adenosine (Table 2). In other systems both analogs used here, 9B-D-arabinofuranosyladenine and 2' deoxyadenosine, have been shown to be P site effectors. In the rectal gland at 10^{-4}M concentrations neither analog stimulated chloride secretion above baseline values. However, the addition of adenosine, 10^{-4}M to perfusate containing these analogs increased chloride secretion approximately 10-fold in each experiment.

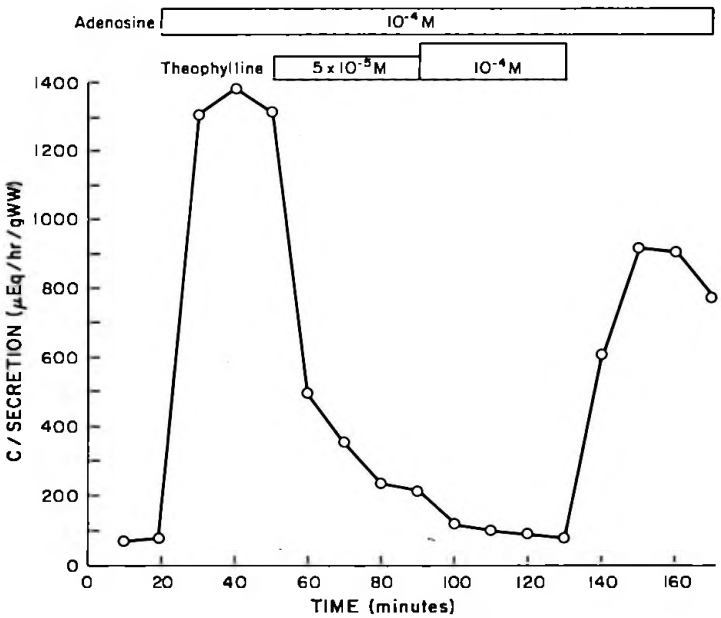

Figure 1.--Inhibition by theophylline of adenosine stimulated chloride secretion in the perfused rectal gland.

Table 1.--Stimulation of Chloride Secretion in the Rectal Gland by Adenosine and Reversible Inhibition by Theophylline

Experimental Conditions	n exp	n total periods	Cl secretion μEq/hr/gww	% Inhibition
adenosine, 5×10^{-6}M	2	8	47.7	
+ theophylline, 10^{-5}M		7	15.2	68%
adenosine, 5×10^{-6}M		8	26.2	
adenosine, 10^{-5}M	3	8	521 ± 69	
+ theophylline, 10^{-5}M		9	48 ± 16†	91%
adenosine, 10^{-5}M		7	246 ± 67	
adenosine, 10^{-4}	5	14	1133 ± 156	
+ theophylline, 10^{-5}M		15	544 ± 132*	49%
+ theophylline, 10^{-4}M		15	222 ± 60*	80%
adenosine, 10^{-4}M		16	748 ± 85**	

†$p < 0.02$ compared to adenosine 10^{-5}M. *$p < 0.01$ compared to adenosine 10^{-4}M.
**$p < 0.025$ compared to theophylline 10^{-4}M.

Table 2.--Effects of Purine Intact Adenosine Analogs (P Site Agonists) on Chloride Secretion in the Perfused Rectal Gland

Purine Intact Analog	Chloride Secretion μEq/hr/gww baseline*	10^{-4} analog	10^{-4} analog 10^{-4} + adenosine
9-B-D- arabinofuranosyladenine	57 ± 36	14.2 ± 3	462 ± 95
	(2,4)**	(2, 6)	(2, 8)
2' deoxyadenosine	103 ± 40	61 ± 24	1197 ± 163
	(2,4)	(2, 9)	(2, 9)

*In each experiment baseline perfusion (20 min.) was followed by addition of 10^{-4} analog (30-50 minutes) followed by 10^{-4} adenosine + 10^{-4} analog (40 minutes). Values are mean ± SEM for all periods under each condition.

**First number in parentheses indicates number of experiments; second number indicates total number of periods under each condition.

In contrast to the previous experiments, each adenosine analog modified in the purine component (R site effectors) stimulated chloride secretion (Table 3). The relative potency of these adenosine analogs on chloride secretion is of

Table 3.--Effects of Ribose Intact Adenosine Analogs (R Site Agonists) On Chloride Secretion in the Perfused Rectal Gland

Ribose Intact Analog	Chloride Secretion μEq/hr/gww baseline*	10^{-5}M	5×10^{-5}M	10^{-4}M
phenylisopropyladenosine (PIA)	35 ± 11	114 ± 25	254 ± 5	
	(6, 6)**	(4, 14)	(2, 6)	
adenosine	54 ± 15	534 ± 76†	780 ± 80†	920 ± 104
	(17, 28)	(8, 28)	(2, 6)	(8, 24)
5'N-ethylcarboxamideadenosine (NECA)	72 ± 10	871 ± 73††		
	(4, 9)	(4, 13)		

*In each experiment baseline perfusion (20 min.) was followed by addition of analog (30-50 minutes). Values are mean ± SEM for all periods under each condition.

**First number in parenthesis indicates number of experiments; second number indicates total number of periods.

† $p < 0.02$ compared to PIA at same concentration. †† $p < 0.025$ compared to adenosine at same concentration.

particular interest. Phenylisopropyladenosine (PIA) was the least potent agonist, stimulating chloride secretion to only 114 ± 25 μEq/hr/gww at 10^{-5}M and 254 ± 5 at 5×10^{-5}M. In contrast to PIA, adenosine was a more potent

agonist, increasing chloride secretion to 534 ± 76 at 10^{-5}M and 786 ± 80 at 5×10^{-5}M. However, the analog 5' N-ethylcarboxamideadenosine (NECA; kindly provided by J. Wolff, NIAMDD, NIH) was the most potent agonist studied. Table 3 compares the relative potency of 10^{-5}M concentrations of PIA, adenosine and NECA in 16 experiments. Mean chloride secretion was 114 ± 25 μEq/hr/gww with PIA, 534 ± 76 with adenosine and 871 ± 73 with NECA, each at 10^{-5}M. These differences in chloride secretion rates were highly significant ($p < 0.025$). It is of considerable interest that the relative potency of these agonists on chloride secretion in the rectal gland, NECA > adenosine > PIA, is identical to the potency order observed by Londos et al, for activation of adenylate cyclase in the turkey erythrocyte and for the steroidogenic response in Leydig tumor cells (Proc. Natl. Acad. Sci. USA, 77: 2551, 1980). This affinity ranking NECA > adenosine > PIA would appear to be characteristic of R_a adenosine receptors that stimulate both adenylate cyclase and physiologic responses in selective tissues.

Finally, we investigated the specificity of theophylline to inhibit adenosine stimulated chloride secretion. Smellie et al., (Life Sci., 24: 2475, 1979), have recently demonstrated that different alkylxanthines have widely varying potencies as antagonists of the adenosine receptor and as inhibitors of phosphodiesterase. For example, theophylline is a potent adenosine antagonist at low concentrations and inhibits phosphodiesterase at higher concentrations. In contrast, 1-isoamyl-3-isobutylxanthine has little, if any, affinity as an adenosine antagonist and appears to be relatively selective for inhibiting both calcium dependent and calcium independent phosphodiesterase. In contrast to the inhibition by theophylline of adenosine stimulated chloride secretion shown in Figure 1 and Table 1, we found that 10^{-5}M 1-isoamyl-3-isobutylxanthine had no effect on chloride secretion stimulated by 10^{-5} NECA or 10^{-4} adenosine (mean chloride excretion 1051 ± 122 following analog stimulation and 933 ± 144 after addition of 1-isoamyl-3-isobutylxanthine, n=4; the latter compound was kindly provided by Dr. J.N. Wells, Vanderbuilt University).

In summary, the present work provides strong support for the presence of a ribose-specific adenosine receptor (R_a) capable of stimulating chloride secretion in cells of the rectal gland of Squalus acanthias. This conclusion is suggested because: (1) adenosine analogs modified in the purine component (with the ribose moiety intact) activate stimulation of chloride secretion, whereas analogs modified in the ribose moiety do not activate secretion and their presence does not inhibit the stimulatory effects of adenosine; (2) the potency ranking of adenosine analogs in stimulating chloride secretion, NECA > adenosine > PIA is identical to the affinity ranking of these analogs for activation of adenylate cyclase and physiologic responses in other tissues containing R_a receptors; (3) Theophylline, an alkylxanthine that alone stimulates chloride secretion in the rectal gland presumably by inhibiting phosphodiesterase activity, actually inhibits (by 80-90%) in a reversible fashion the effects of adenosine. This result is consistent with the known effect of theophylline to inhibit R site but not P site adenosine receptors; (4) finally, 1-isoamyl-3-isobutylxanthine, an alkylxanthine that in other systems is a potent phosphodiesterase inhibitor but has little effect on adenosine receptors, had no effect on adenosine or NECA stimulated chloride secretion in the rectal gland. Taken together, these findings suggest that R_a adenosine receptors contribute to the regulation of sodium chloride secretion in the rectal gland of Squalus acanthias.