gland and are not simply the result of an increase in the extracellular volume of the animal. It is not surprising that stimulation of secretion should result in the reduction in high energy compounds since the stimulated glands were secreting the equivalent of 9 times their own volume every hour. This work was supported by Grants from the New York Heart Association and the NIH (AM 24064 and HLB 10384).

EFFECTS OF ADENOSINE ANALOGUES ON SECRETION BY THE ISOLATED RECTAL GLAND OF THE DOGFISH, SQUALUS ACANTHIAS

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This communication describes the effects of several adenosine analogues on the rate of secretion of the isolated and perfused rectal gland of the dogfish Squalus acanthias. The interest in studying the effects of these analogues stems from our discovery (Erlij, Silva and Reinach, Bull. MDIBL. 18: 92-93, 1979) that very low concentrations of adenosine stimulate secretion by the perfused rectal gland. The study of the effects of analogues can provide evidence concerning several aspects of the mechanism of action of adenosine.

For example, the use of impermeable analogues can provide evidence useful to judge whether their action is either intracellular or extracellular. There is also evidence indicating the presence of different kinds of adenosine receptors in cell membranes; activation of one of them, Ra, leads to stimulation of adenylate cyclase while activation of the other, Ri, results in its inhibition. Comparison of relative potencies of different analogues is the usual way to determine the type of receptor involved in a given response (Londos, Cooper and Wolff, Proc. Natl. Acad. Sci. USA, 77: 2551-2554, 1980).

We perfused the isolated glands following the methods used previously (Erlij, Silva and Reinach, 1978). Figure 1

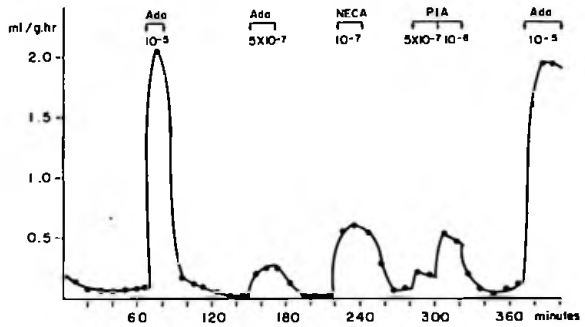

Figure 1.--Comparison of the effects of adenosine analogues on the rate of fluid secretion by the perfused rectal gland of the dogfish. Drugs were infused in periods indicated by horizontal bars. Figures are concentration in moles. "Ado" stands for adenosine; "NECA" for 5'-N-Ethylcarboxamide-adenosine and PIA for N^6-phenylisopropyladenosine.

illustrates the type of procedure used to compare the effects of adenosine and its analogues on the secretory rate of the rectal gland. First, adenosine (10^{-5}M) was perfused. This concentration produced a rapid and large increase in the rate of secretion. The increase was observed within the first 5 minutes after addition of the compound; it disappeared rapidly after perfusion with adenosine-free Ringers. The response to 5×10^{-7} adenosine was then tested, this concentration produced a smaller but still clear-cut effect on secretion. Then the effects of 5' N-ethylcarboxamide-adenosine (NECA) and N^6-phenylisopropyl-adenosine (PIA) were determined. These compounds were selected because their sequence of potency offers a sensitive indication of the receptor type involved in the response (Londos, Cooper

and Wolff, 1980). NECA in a concentration 10^{-7} M produced a larger response than $5x10^{-7}$ adenosine. This response was also reversible. PIA was the least effective of the three compounds tested. A final infusion of 10^{-5} M of adenosine showed that the sensitivity of the preparation had not substantially changed during the course of the experiment. Two other experiments produced similar results. As in our previous observations (Erlij, Silva and Reinach, 1978) chloride concentration in the resting and stimulated samples were not different.

This sequence of activity, NECA > adenosine > PIA, is characteristic of adenosine responses associated with activation of adenylate-cyclase (Ra receptor). While inhibition of adenylate cyclase is associated with the sequence PIA > adenosine > NECA; (Ri receptor; Londos, Cooper and Wolff, 1980). Another purine substituted analogue, 2 methyl-adenosine was tested in four perfused glands. Three of them were clearly stimulated to secrete by 10^{-5} M methyladenosine and all four responded markedly when 10^{-4} M was used; the increases in rate ranged between 2 and 8 times the resting rate ($\bar{x} \pm$ S.E. = 4.84 $\pm$ 1.47).

We also tested the ribose modified analogues 2' Deoxyadenosine and 2'3' isopropylidene-adenosine. Concentrations of 2' Deoxyadenosine ranging between 10^{-5} M and 10^{-4} M were without an effect in 8 glands. Of three experiments with 2'3' isopropylidene-adenosine, only one was stimulated to secrete with 10^{-4} M.

We tested two compounds that penetrate the cell membrane very slowly: adenosine 5'-0- thiomonophosphate and adenyl $(3'\ 5')_9$ - adenosine. Adenosine 5'-0-thiomonophosphate (10^{-5} M) produced about a three-fold stimulation of secretion in four preparations ($\bar{x} \pm$ S.E. = 3.49 $\pm$ 1.05). Adenyl $(3'5')_9$ adenosine, (10^{-5} M) was without effect in 3 preparations. The present experiments suggest a number of points concerning the adenosine effects. First, based on the sequence NECA > Adenosine > PIA it seems that a site analogous to the Ra site of other cell types is involved in the response (Londos, Cooper and Wolff, 1980). This site is usually associated with activation of adenylate cylase. In agreement with this pattern, we have found in a single experiment in which we measured cyclic AMP content in slices of rectal gland that 10^{-4} M adenosine increased the content of cyclic AMP from 5 to 12.9 pmoles/mg protein. Clearly this finding merits additional experiments. A second point, is that the P site, a nucleoside receptor site different from the Ra and Ri sites, and associated with a catalytic subunit of adenylate cyclase is not involved in the regulation of secretion in the rectal gland, since compounds that are effective activators of it, such as 2' deoxyadenosine and 2'3' isopropylidene-adenosine, had no effects or were poor stimulators of secretion.

Finally the effects of 5'-0-Thiomono phosphate, a compound that very likely does not penetrate the cell membrane, are in further agreement with the notion that the action of adenosine is the result of an interaction with a receptor on the outer surface of the cell membrane. This work was supported by Grants from the New York Heart Association and the NIH (AM 24064 and HLB 10384). We thank Dr. C. Londos for a generous gift of PIA and NECA and useful discussion of our results.

PROTEIN PHOSPHORYLATION IN THE DOGFISH RECTAL GLAND

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Cyclic AMP stimulates active Cl secretion in the rectal gland of Squalus acanthias (Stoff et al., J. Exp. Zool., 199: 443, 1977). The major mechanism whereby cyclic AMP is thought to accomplish its second messenger role is by activation of cyclic AMP-dependent protein kinases leading to phosphorylation of tissue-specific substrate proteins (Greengard, Science, 199: 146, 1978). This hypothesis has received support from the finding that the regulatory subunits of cyclic AMP-dependent protein kinases are the sole intracellular receptors for this