ATP SECRETION BY THE RECTAL GLAND OF THE SHARK, *SQUALUS ACANTHIAS*

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Shark rectal gland cells grown in culture express electro-diffussional pathways permeable to ATP (Cantiello, H.F. et al. Am. J. Physiol. 272:C466-C475, 1997). The movement of ATP out of the cells can be stimulated by adenosine 3',5'-cyclic monophosphate (cAMP) and blocked by either nifedipine or glibenclamide. The molecular pathways that mediate the movement of ATP are not currently known but could be associated either with CFTR (Reisin, I.L. et al. J. Biol. Chem. 269:20584-20591, 1994), or P-glycoprotein (Prat, A.G. et al. Am. J. Physiol. 270:C538-C545, 1996) that have been shown to mediate the movement of ATP out of cells. The present experiments were designed to examine whether intact rectal glands release ATP under basal conditions and also when the secretion of chloride is stimulated.

Shark rectal glands were perfused as described in Silva, P. et al. (Methods Enzymol. Vol 192:754-66, 1990). Aliquots for the measurement of the secretion of chloride and ATP were collected for consecutive 10 minute periods. There was an initial thirty minute control period (three collection periods) to allow the secretion of chloride to reach a stable rate. At the end of this control period, vasoactive intestinal peptide (VIP) 5 x 10^{-7}M, final concentration, was added to the perfusate and collections continued for additional thirty minutes. Aliquots for the measurement of ATP in the venous effluent were also collected at ten minute intervals. ATP was measured using the luciferase assay (Lyman, G.E. et al. Anal. Biochem. 21: 435-443, 1967). Chloride was measured by amperometric titration.

Figure 1 summarizes the results of the ATP measurements. The secretion of ATP dropped after the beginning of the perfusion to a stable basal state. After the addition of VIP the secretion of ATP rose six fold and remained elevated at 2.5 times basal for the remainder of the perfusion. In contrast, under basal conditions, release of ATP into the venous effluent was two orders of magnitude greater than in the rectal gland secretion and fairly constant (Figure 2). Stimulation of chloride secretion by VIP caused a progressive decline in the release of VIP into the venous effluent. Figure 3 shows that the secretion of ATP is correlated with the secretion of chloride.

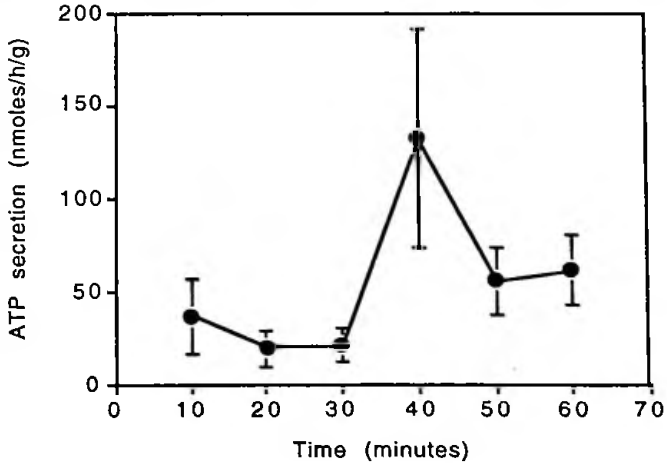

Figure 1. Secretion of ATP by the rectal gland in response to VIP. Stimulation of chloride secretion by VIP caused a six-fold increase, $p < 0.05$, in the secretion of ATP into the rectal gland lumen. The secretion of ATP remained elevated throughout the rest of the perfusion. Values are mean± SEM, n=10.

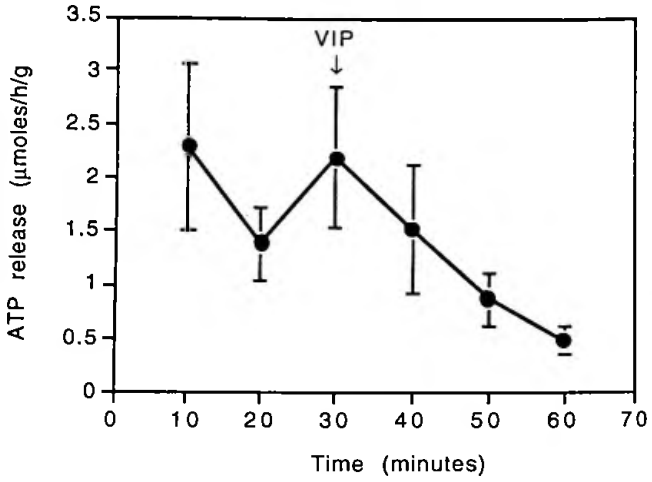

Figure 2. Release of ATP into the venous effluent of perfused rectal glands. ATP was released into the perfusate of rectal glands at a stable rate during the control periods of the perfusion. After stimulation of chloride secretion with VIP the rate of release steadily declined. Values are mean± SEM, n=6.

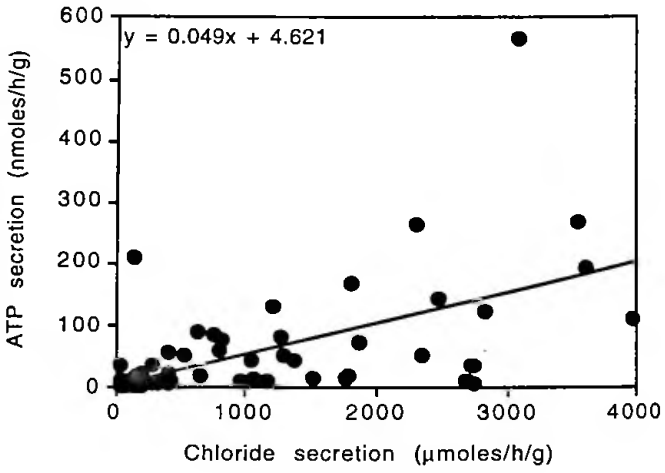

Figure 3. Correlation between the secretion of ATP and that of chloride. The rate of ATP secretion was correlated with that of chloride. The points shown in this figure include collections obtained under both basal and stimulated conditions. The secretion of ATP was 20 times lower than that of chloride on a molar base. R=0.57. $p < 0.01$, n=60.

These studies indicate that ATP is secreted into the rectal gland lumen in a manner that is directly correlated with the secretion of chloride. Furthermore, stimulation by VIP of chloride secretion is associated with an increase in the rate of secretion of ATP. The rate of secretion of ATP is notably substantially lower than that of chloride. It should be noted that hydrolysis of ATP occurs in the venous effluent and probably also in the rectal gland secretion. Therefore, it is possible that the absolute amounts of ATP released into the venous effluent or secreted into the rectal gland fluid are much larger than those measured here. Although these studies do not provide any evidence as to the mechanism(s) that mediate the secretion of ATP they suggest that stimulation of chloride secretion increases the leak of ATP out of the cell into the luminal space. The opposite occurs at the basolateral space where stimulation decreases the rate of release. These observations suggest that the movement of ATP out of the cell responds to changes in the electrochemical potentials that attend the stimulation of rectal gland secretion.

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