CYCLIC AMP ACTIVATION OF SODIUM PUMP AND POTASSIUM EXIT IN CULTURED CELLS OF THE RECTAL GLAND OF SQUALUS ACANTHIAS.

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Stimulated chloride (Cl) secretion in the rectal gland of the Squalus acanthias has been extensively examined. Cl secretion can be increased as much as forty-fold by cAMP and various hormones which act via adenylate cyclase. The site and precise mechanism of cAMP action have not been unequivocally defined. In theory, cAMP stimulation might act on any of four different sites in the rectal gland cell: the apical Cl exit channel, the co-transporter-mediated entry of sodium (Na) and Cl, the potassium (K) exit channel, and the Na pump (Na,K-ATPase). Whether the increase in Na,K-ATPase activity is primary or driven by a rise in intracellular Na content is controversial. Since the pump is exquisitely sensitive to small changes in intracellular Na concentration, the rate of pump activity could be altered by passive entry pathways. A second mechanism might involve direct stimulation of the pump, produced, for example, by a change in enzyme phosphorylation or in the number of pump units. Whether intracellular Na rises or falls in response to stimulation is clearly of critical importance in deciding if the Na pump is stimulated directly by cAMP. In the present experiments, we utilized the electron probe to make serial measurements of the electrolyte content of cultured rectal gland cells, before and after stimulation, in an effort to determine the effect of stimulation on two sites: the Na pump and the K exit step.

Using electron probe analysis, we studied the intracellular ion contents of rectal gland cells, cultured from tubules isolated from Squalus acanthias, as previously described (J. Valentich, Bull. MDIBL 26:91-94, 1986). The tubules were suspended in medium (DMEM:Ham's F-12=1:1, supplemented with 5% Nu-Serum, insulin, transferrin and selenium (ITS 1%), NaCl, TMAO (70mM) and urea) and then grown, at 25°, on fibronectin-coated silicon chips to which they attach, until confluent. Each chip was then transported in individual, sterile, refrigerated vials containing medium to the National Electron Probe Resource for the Analysis of Cells (NEPRAC) in Boston. Rectal gland tubules were studied within 10-14 days of plating. Cells were stimulated by the simultaneous addition of the cAMP analog, dibutyryl cyclic AMP (1mM), theophylline (0.5 mM), VIP (5×10^{-8} M), and, in some experiments, forskolin (1 uM). At various timepoints, individual chips were washed and immediately frozen in liquid nitrogen, then freeze-dried, prior to electron probe measurement of ion contents. Intracellular Na, Cl and K contents are normalized to phosphorous content which is an index of cell mass.

The passive rate of Na entry and K efflux were measured under basal and stimulated conditions, in the presence of ouabain (1 mM). Stimulation nearly doubled the rate of passive K exit from 14.5±1.7 to 27.6±3.8 umole/mmole P/min (±SEM, n=6, $p<0.01$). This is consistent with an increase in the basolateral K conductance or a change in the driving force because of the primary increase in the Cl conductance with stimulation. Passive Na entry tended to increase with stimulation, but did not attain statistical significance, possibly because of surface contamination by the high extracellular sodium concentration (280 mM) as compared to low intracellular values.

The intracellular Na and Cl contents of stimulated cultured rectal gland cells, and the unstimulated time controls, are shown in Table I. There is a highly significant fall in cell Na content with stimulation. The Cl content also decreases significantly with stimulation. There was no significant change in K content with time in stimulated cells, (data not shown). The marked decrease in Na content suggests that, by at least 8 minutes after

addition of the stimulatory agents, the increase in pump activity is not driven by high levels of intracellular Na.

An early increase in passive entry of Na, Cl and K at the co-transporter is probable. Our Na entry data and Greger's measurements in isolated tubules at 2 minutes (Pfluegers Arch 402:376-84, 1984) support this possibility. However, our studies of intracellular Na content support a direct effect on Na,K- ATPase with sustained stimulation, independent of intracellular Na content which falls below its resting level. An increase in the luminal Cl conductance is felt by many to be the primary event in stimulation. This results in a net lowering of cell Cl activity, found at an early timepoint by Greger and at later timepoints in this study, in spite of increased Cl entry. Cell depolarization, facilitating an increase in K exit, then occurs. There is no significant change in cell K as a result of stimulation, according to these experiments and Greger's study, most likely because of the increase in the K exit which occurs concomitantly with the increase in passive and active entry of K. Studies of the elasmobranch rectal gland may provide insight into the mechanisms of pump activation in other Cl-transporting epithelia.

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TABLE I. Ion Contents of Cultured Rectal Gland Cells

Time (min)	Sodium Control	Sodium Stim	Chloride Control	Chloride Stim
0	0.35 ± .02 (159)		0.98 ± .03 (159)	
8	0.30 ± .01 (155)	0.21 ± .01* (145)	0.91 ± .03 (155)	0.83 ± .03* (145)
25	0.31 ± .02 (151)	0.23 ± .02* (153)	0.92 ± .03 (151)	0.87 ± .02* (153)
33	—	0.20 ± .01* (276)	—	0.82 ± .02* (276)

Values are expressed in umole/mmole P, as mean± SE, (n).
*p <0.005 as compared to time 0.