The delay in secretion on stimulation of the rectal gland of the dogfish shark, *Squalus Acanthias*, is largely due to the delay in NKCC1 activation.

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Salt secretion by the rectal gland of the dogfish shark is mediated through the coordinated function of Na pumps, K channels, Cl channels (CFTR), and Na-K-Cl cotransporters[1]. Of these, it is clear that CFTR and NKCC1 are directly involved in the up- and down-regulation of secretion. It is now fairly clear that CFTR is the primary site of transport regulation through PKA-mediated phosphorylation, and that up-regulation of NKCC1 occurs as a consequence of decreases in intracellular [Cl] and cell volume.

We have previously discussed experiments providing evidence that when fluid secretion is stimulated in the rectal gland by perfusion with VIP or forskolin, the 10-minute delay in attaining maximal secretion is largely due to the time taken for activation of NKCC1[2]. We have now completed this series of experiments, one of which is illustrated here. We use a simple and sensitive measurement of the rate of fluid secretion, taking gravimetric measurements at 12 s intervals. At the same time, we monitor activation of NKCC1 by measuring the removal of ^{3}H-benzmetanide (^{3}H-Bz) from the perfusion fluid (shark Ringers), since this compound binds only to activated transporters; this result is expressed as the a ratio of $^{3}H/^{14}C$, with ^{14}C –inulin used as a volume marker.

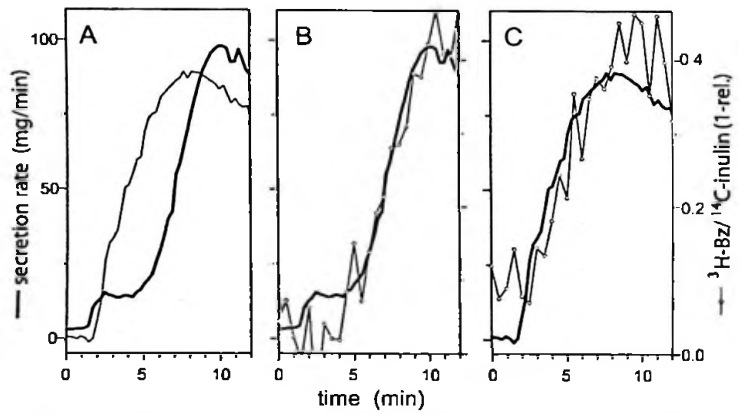

Fig. 1. Secretion and bumetanide binding during perfusion of a rectal gland with VIP.

The results of a typical experiment utilizing these techniques are shown in Figure 1; similar results have been obtained in 5 other experiments. At time zero, the gland was perfused with 50 nM VIP and the rate of secretion is seen to have increased -- however during the interval from 2 min to 5 min, the rate paused at a temporary plateau and then again increased to a maximum at about 10 min (fig. 1A&B, heavy line). We propose that the temporary plateau occurs while the cell loses Cl and NKCC1 is activated. To test this hypothesis, we pre-perfused with 30 mM Cl Ringers to lower $[Cl]_i$ and then repeated the VIP perfusion (light line in fig. 1A, heavy in 1C, offset for the new VIP zero point) – in this case, secretion rose rapidly without a plateau phase. Further support comes from the fact that [^{3}H]bumetanide binding lagged during the initial plateau in the control (fig. 1B, thin line with circles) but not in the low-Cl pre-perfused trial (fig. 1C). Together, these and similar observations strongly argue that activation of NKCC1 is the rate limiting process at the onset of secretion.

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1. **Riordan, J.R., B. Forbush III, and J.W. Hanrahan.** The Molecular Basis of Chloride Secretion in Shark Rectal Gland. *J. Exp. Biol.* 196:405-418, 1994.

2. **Forbush, B., R. Behnke, and J. Forbush, and J.C. Xu.** Coordinate Modulation of the Na-K-Cl Cotransporter and of the Secretory Rate in the Intact Rectal Gland of the Spiny Dogfish, *Squalus Acanthias*. *Bull. Mt Desert Isl. Biol. Lab.*32: 42-44, 1993.