Effect of H89, an inhibitor of protein kinase A, on chloride secretion by shark rectal gland stimulated by VIP and by CNP

Franklin H. Epstein[1], Chris Sighinolfi[2], Katherine Hessler[3], and Patricio Silva[4]

[1]Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA 02215
[2]University of Pennsylvania College of Arts and Sciences, Philadelphia, PA 19155
[3]Bowdoin College, Brunswick, ME 04011
[4]Department of Medicine, Temple University Hospital, Philadelphia, PA 19140

Secretion of chloride by the shark rectal gland is stimulated by vasoactive intestinal peptide (VIP), a neurotransmitter released by rectal gland nerves, and by C-type natriuretic peptide (CNP) a circulating hormone secreted by the heart in response to an increase in blood volume. Different intracellular cascades are entrained by these two agonists. VIP activates adenylate cyclase, producing cAMP, which activates protein kinase A (PKA), which is thought to phosphorylate the shark version of the cystic fibrosis transmembrane regulator (CFTR) at the apical membrane of rectal gland cells, thereby opening an apical chloride channel to permit chloride to pass into the duct. CNP, in addition to stimulating the release of VIP from rectal gland nerves, has a direct action on rectal gland cells, which is blocked by inhibitors of protein kinase C (PKC) and is mediated in part by the stimulation of guanylate cyclase. This direct action of CNP, apparent even when neurotransmission and VIP release are blocked (e.g., by perfusing with procaine), does not involve the stimulation of adenylate cyclase,[2] but is prevented by a specific inhibitor of the CFTR chloride channel.[1]

The present experiments were designed to see if inhibition of PKA (a maneuver expected to inhibit secretion stimulated by VIP) would affect as well chloride secretion stimulated by the direct cellular action of CNP. The compound used to reduce PKA activity was H-89, an isoquinoline said to exert a strong selective inhibitory action on PKA.[3]

Shark rectal glands were perfused as described by Silva et al.[4] Duct fluid was collected at 10 minute intervals in small tared plastic centrifuge tubes and the volume was estimated by weighing. The concentration of chloride was measured by amperometric titration using a Buchler-Cotlove chloridometer. An initial 30-40 minutes of control perfusion allowed the gland to reach a stable basal state. At the end of the control period, a bolus of 1 ml of shark Ringer's solution was infused directly into the rectal gland artery over one minute, containing an amount of VIP (Sigma) calculated to deliver a final concentration of 10^{-7}M to the gland, or of recombinant human CNP (Sigma) in an amount calculated to deliver a final concentration of 5 x 10^{-7}M. In the experiments with CNP, procaine 10^{-2}M, was added to the perfusate in order to present the release of VIP from rectal gland nerves.[6] In the experiments with H-89, this compound was added to the perfusate from the start of the experiment.

Preliminary perfusion with 25 micromolar H-89 inhibited the secretion of chloride induced during the first 10 minutes after a one minute bolus injection of 10^{-7}M VIP, from 1052 $\pm$ 272 (n=10) to 366 $\pm$ 104 mEq/g/hr (mean $\pm$ s.e.). At the same concentration of H-89, CNP-induced secretion of chloride (5 x 10^{-7}M CNP given over one min. in the presence of 10^{-2}M procaine) was almost completely inhibited (780 $\pm$ 152 to 60 $\pm$ 8 mEq/g/hr.

These experiments in intact perfused rectal glands suggest that PKA activity is necessary for direct stimulation of chloride secretion by CNP in the shark rectal gland. They support earlier findings

that H-89 (at a concentration of 10^{-6}M) suppressed stimulation by CNP as well as by VIP in slices of shark rectal gland.[5] The results are compatible with the notion that a major action of CNP is to sensitize the CFTR chloride channel to its activation by small amounts of VIP, cAMP, or activated PKA.

1. **Epstein, F.H., C. Sighinolfi, K. Hessler, A. Verkman, J. Riordan, P. Silva.** A synthetic CFTR inhibitor blocks stimulation of *Squalus acanthias* rectal gland secretion by CNP and VIP. *Bull MDIBL* 43, 13-14, 2004.
2. **Gunning M., C. Cuero, R. Solomon, P. Silva.** C-type natriuretic peptide receptors and signaling in rectal gland of *Squalus acanthias*. *Am J. Physiol* 264:F300-F305, 1993.
3. **Penn R.B., J.L. Parent, A.M. Pronin, R.A. Panetteri, J.L. Benovic.** Pharmacologic inhibition of protein kinases in intact cells. *J Pharmacol Exp Ther* 288:428-37, 1999.
4. **Silva P., R.J. Solomon, F.H. Epstein.** Shark rectal gland. In: *Methods in Enzymology*, vol 192. pp 754-766, 1990, Academic Press.
5. **Solomon R., L. Castelo, E. Franco, M. Taylor, P. Silva, F.H. Epstein**. Preliminary data on intracellular signaling mechanism in the rectal gland of *Squalus acanthias*: A pharmacologic approach. *Bull MDIBL*. 34: 47-48,1995.
6. **Stoff J.S., P. Silva, R. Lechan, R. Solomon, F.H. Epstein**. Neural control of shark rectal gland. *Am J. Physiol* 255:R212-R216, 1988.