TIME PATTERN OF RESPONSE TO STIMULATION OF SHARK RECTAL GLAND BY VIP AND CNP

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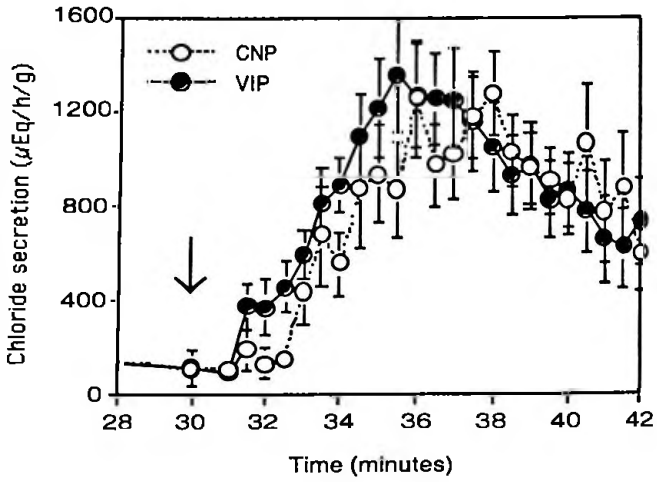
The rectal gland of Squalus acanthias can be stimulated during life by at least two endogenous peptides: the neurotransmitter vasoactive intestinal peptide (VIP) and the cardiac hormone C-type natriuretic peptide (CNP). These agonists entrain quite different intracellular second-messenger cascades to activate secretion. VIP activates adenylate cyclase, whereas CNP stimulates guanylate cyclase. Stimulation by CNP is inhibited by staurosporine (an inhibitor of protein kinase C), cytochalasin D (which binds to the barbed end of actin filaments and prevents their extension) and ML-7, which blocks myosin light chain kinase. None of these agents inhibit secretion induced by VIP. The present experiments were carried out to see whether these two modes of stimulation also differed in the latent time that elapsed between stimulus and response.

Isolated rectal glands of Squalus acanthias were perfused with oxygenated shark Ringer's solution at pH 7.6 as previously described (P. Silva et al. Methods in Enzymol, 192:754-66, 1990). Procaine, 20 mM, was added to all perfusion media in order to block the release of VIP from nerves within the rectal gland, an action of natriuretic peptides that might otherwise complicate evaluation of the direct effect of CNP on rectal gland epithelial cells. Duct fluid was collected at 10 minute intervals in small tared plastic centrifuge tubes and the volume measured at 10 minutes by weighing or at 30 second intervals in 100 ml pipettes, in which the volume secreted during intervals of less than one minute was estimated by measuring the linear progress of the leading fluid meniscus along the pipette. The concentration of chloride in the duct fluid was estimated by amperometric titration.

An initial thirty minutes of control perfusion (three collection periods) allowed the gland to reach a stable basal state. At the end of this control period a 1 ml bolus of either VIP (vasoactive intestinal peptide, V-3628, Sigma Chemical Co.) or CNP (C-type natriuretic peptide, N-8768, Sigma Chemical Co.) was perfused directly into the arterial catheter over one minute, and the rate of secretion was monitored every 30 seconds thereafter until it returned to its basal level. The results are shown in the accompanying Figure.

The time needed to activate secretion was compared in 13 glands stimulated by CNP with 13 glands stimulated by VIP, in which comparable peak rates of secretion were attained. The average time of activation was 3.65±0.46 (mean±SEM) minutes for CNP and 1.69±0.25 minutes for VIP, ($p<0.01$), while the peak rate of secretion of chloride (1728±194 vs 1708±186 mEq/h/g) was similar for both agonists.

Direct stimulation of the rectal gland via CNP takes appreciably longer to be effectuated than via VIP. These results suggest that a different and perhaps more complex pathway of intracellular second-messenger activation is entrained by CNP than by VIP, consistent with the involvement of contractile elements of the cytoskeleton by the CNP cascade.



Time course of rectal gland stimulation by cardiac natriuretic peptide (CNP) and vasoactive intestinal peptide (VIP). The rate of chloride secretion (mEq/h/g) is plotted on the ordinate, vs time (minutes) in the abscissa. The peptide agonists (either CNP, 5 or 10 x 10^{-7} M or VIP 5 x 10^{-8}M) were infused into the rectal gland artery at the time indicated by the arrow. Peak rates of secretion at these concentrations were similar, but activation by CNP takes approximately 2 minutes longer than by VIP (see text).

Supported in part by grant NSF DBI-9820400