NATRIURETIC PEPTIDE (CNP) STIMULATION OF CHLORIDE CONDUCTANCE IN XENOPUS OOCYTES EXPRESSING mRNA FROM RECTAL GLAND OF THE DOGFISH SHARK SQUALUS ACANTHIAS

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The family of natriuretic peptides derived from atrial extracts includes CNP, a major natriuretic peptide found in the brain of bony fish (Evans, D.H., Rev. Physiol. 52:43, 1990). CNP and its homologues are thought to serve an osmoregulatory role in fish affecting both absorptive and secretory salt transport processes. In perfused shark rectal gland, atrial peptides are thought to act secondary to stimulation of release of the neuropeptide VIP (vasoactive intestinal peptide) from nerve terminals (Silva et al., AJP, 252:F99, 1987). Other studies indicate that natriuretic peptides can stimulate chloride secretion from shark rectal gland cells in primary culture, and the stimulation is thought to involve an increase in cellular cGMP (Karnaky et al., MDIBL Bul., 29:86,1990). Although the cultured cell studies suggest that natriuretic peptides can have a direct effect on chloride secretory cells, they do not rule out the possibility that a paracrine stimulation of secretion exists in the cultures due to contamination by other cell types. We approached the question of direct versus indirect stimulation by expressing shark rectal gland mRNA in Xenopus oocytes with the assumption that the oocyte would express both receptor-mediated and second messenger-activated conductance properties of the epithelial cells.

The methods are similar to those described in the accompanying paper Worrell et al. (this issue). For these experiments, polyA$^+$ mRNA was derived from freshly isolated shark rectal gland. Oocytes were injected with 50 ng mRNA and oocyte current assayed 2-3 days later.

Figure 1 shows that the basal currents were low (<0.2 μA) at ± 80 mV relative to the resting membrane potential. Addition a cAMP cocktail containing 10 μM forskolin, 200 μM 8-(4-chlorophenylthio)-adenosine (3':5'-cyclic monophosphate) (cpt-cAMP), and 1 mM 3-isobutyl-1-methyl xanthine (IBMX) stimulated a marked elevation of membrane currents, a depolarization of the resting membrane potential, and a large increase in the membrane conductance. The stimulated currents were chloride selective since reducing bath chloride from 96 to 13 mM shifted the current-voltage (I-V) relation to the right as anticipated from the change in chloride equilibrium potential and reduced the conductance. These effects were entirely reversible. Similar findings have been reported by Sullivan et al. (AJP, 260:C664, 1991) in oocytes expressing rectal gland mRNA. As illustrated in Figure 2, current was also stimulated by bath addition of 10^{-8} M CNP derived from killifish brain (CNP was kindly provided by Drs. D. Evans and K.J. Karnaky). Activation by CNP was also reversible (Fig. 2), and the CNP-stimulated currents shifted as anticipated for stimulation of a chloride conductance pathway in a manner similar to that shown in Fig.1 (data not shown).

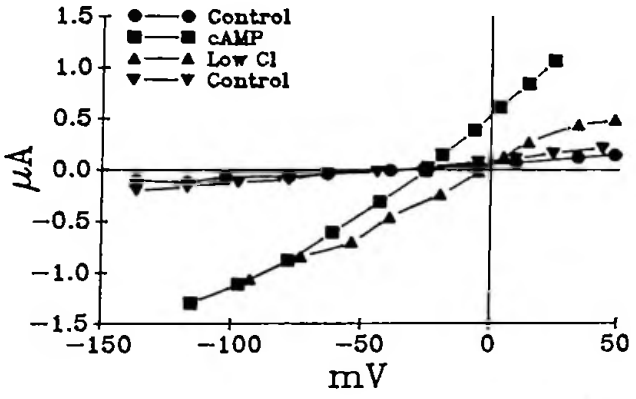

Figure 1. Current-voltage relations of an oocyte injected with 50 ng shark rectal gland mRNA in the presence and absence (control) of cAMP cocktail or cAMP cocktail + low Cl media (13mM). Currents represent an average of the values obtained from 100 to 200 ms during a 250 ms voltage pulse.

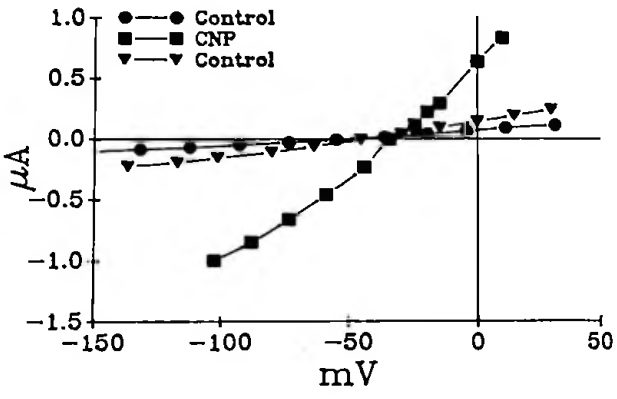

Figure 2. Current-voltage relations of an oocyte injected with 50 ng shark rectal gland mRNA in the presence or absence (control) of 10^{-8} M killifish CNP. Current determination was as in Fig. 1.

These findings are consistent with the idea that natriuretic peptides can act directly on shark rectal gland epithelial cells to stimulate chloride secretion since the effect of CNP on oocytes expressing shark rectal mRNA cannot involve a paracrine (cell-to-cell) pathway. The currents stimulated by CNP are similar to those arising from cAMP stimulation. However, the CNP effect may involve an increase in oocyte cGMP level since increased cGMP in response to CNP has been shown to occur in isolated shark rectal gland cells (Karnaky et al., MDIBL Bul., 29:86, 1990). Thus, there are probably two mechanisms by which natriuretic peptides stimulate chloride secretion; one involving a direct stimulation of the epithelial cells by CNP and another involving VIP release from CNP stimulated peritubular nerve terminals.

Supported by grants from the NIH (DK35815 to RAF & DK29786 to DCD), NIEHS Biomedical Science Center Grant, and the Cystic Fibrosis Foundation.