SHARK HEART C-TYPE NATRIURETIC PEPTIDE IS A POTENT CHLORIDE SECRETAGOGUE IN MONOLAYERS OF CULTURED SHARK (SQUALUS ACANTHIAS) RECTAL GLAND CELLS

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Elasmobranchs utilize the rectal gland to regulate plasma ion concentrations and fluid volume (Solomon et al., Am. J. Physiol. 248:R638-R640, 1985). In one model, atrial natriuretic peptide, released from the heart of the shark, causes the release of vasoactive intestinal peptide from peritubular nerve endings which results in the stimulation of chloride secretion (Silva et al., Am. J. Physiol. 252:F99-F103, 1987). We reported that atriopeptins (AP's) act directly on cultured shark rectal gland cells from apical or basolateral sides to stimulate sodium chloride secretion and elevate the second messenger, cGMP (Karnaky et al., Am. J. Physiol. 260:C1125-C1130, 1991).

Recently, C-type natriuretic peptides (CNP's) have been demonstrated in the heart of Scyliorhinus canicula (Suzuki et al., FEBS 282:321-325, 1991) and Squalus acanthias (Schofield et al., Am. J. Physiol. 261:F734-739, 1991). Since these CNP's differ by only several amino acids from killifish brain CNP (kCNP: Price et al., Biol. Bull. 178:279-285, 1990) we tested the effects of the latter on the cultured shark rectal gland. We found that kCNP is approximately 10-100 times more potent than rat AP III in stimulating chloride secretion and in elevating cGMP in the cultured SRG (Karnaky et al., Bull. Mt. Des. Isl. Bio. Lab. 31,122-123, 1992). In the present study we examined the effects of species-specific Squalus acanthias CNP (sCNP) on cultured shark rectal gland cells.

Monolayer cultures of dogfish shark rectal gland epithelium maintained on collagen-coated nylon mesh were used for measuring short-circuit current (I_{sc}) in Ussing chambers (Valentich, Bull. Mt. Des. Isl. Biol. Lab., 26:91-94, 1986). Shark heart CNP was prepared at the Yale Protein and Nucleic Acid Chemistry Facility by the solid-phase method using an Applied Biosystems 430A peptide synthesizer (Schofield et al., Am. J. Physiol. 261:F734-739, 1991).

Basolateral exposure to 10^{-9} M sCNP markedly stimulated bumetanide-inhibitable, I_{sc} from a control value of 1.1 ± 0.02 to 17.8 ± 7.55 μamp/cm^2 (mean $\pm$ S.E.; n=10). By comparison, 10^{-9} M rat AP III caused no increase in I_{sc}. The dose-response curve relating stimulation of I_{sc} and concentration of sCNP revealed an EC_{50} of approximately 4 nM. The

I_{sc} stimulated by basolateral 10^{-8} M sCNP was completely abolished by removal of chloride from the Ringer solutions bathing the cultures, and returned to the original value upon readdition of chloride. sCNP was equally effective from basolateral and apical sides. In the presence of maximal concentrations of sCNP, bumetanide (10^{-4} M) reduced I_{sc} from 75.9 $\pm$ 10.6 to 10.4 $\pm$ 2.2 μamp/cm^2.

In summary, sCNP is more potent than rat AP III in stimulating chloride secretion and receptors for sCNP appear to be present on both basolateral and apical membranes of cultured cells. The potency, coupled with the presence of sCNP in the dogfish's heart, strongly suggests that a C-type natriuretic peptide plays a direct role in controlling chloride secretion in the shark rectal gland. The availability of this potent cardiac natriuretic should permit further studies on the synthesis, regulation, sites of action, and signal transduction mechanisms of CNP in the regulation of chloride transport in elasmobranchs.

Supported in part by NIH EHS-P30-ESO3828 to the Center for Membrane Toxicity Studies and NIH RO1-DK-34208 (JNF) and grants from the University Research Committee of MUSC and the Cystic Fibrosis Foundation (KK). Stacey French was a recipient of a Hearst Student Fellowship. Cynthia Kelmenson was the recipient of a Student Research Fellowship for the American Heart Association. Publication No. 107 of the Grice Marine Biological Laboratory, Charleston, SC.