

# C-TYPE NATRIURETIC PEPTIDE IS A POTENT SECRETAGOGUE FOR THE CULTURED SHARK (SQUALUS ACANTHIAS) RECTAL GLAND

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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 um 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 have tested the effects of the latter (a kind gift of Dr. D. H. Evans) on the cultured shark rectal gland.

Monolayer cultures of spiny dogfish (Squalus acanthias) shark rectal gland epithelium in 6-well culture plates were equilibrated with a low bicarbonate, HEPES-buffered Ringer solution containing 1 mM 3-isobutyl-1-methyl xanthine (IBMX) for 20 min. Cells were incubated for 10 min with varying concentrations of KCNP. 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).

Basolateral exposure to  $10^{-10}$  M KCNP markedly stimulated bumetanide-inhibitable  $I_{sc}$  [from a control value of  $10.0 \pm 2.0$  to  $40.0 \pm 5.0$   $\mu\text{amp}/\text{cm}^2$ ;  $n=6$ ]. Higher doses did not increase  $I_{sc}$  appreciably. KCNP was also effective from the apical side. By comparison,  $10^{-9}$  M rat AP III causes a only few  $\mu\text{amp}/\text{cm}^2$  increase. In 4 experiments,  $10^{-10}$  M KCNP addition resulted in elevated cGMP concentrations (from a control value of  $5.0 \pm 0.5$  to a value of  $12.0 \pm 1.0$  pmoles

cGMP/mg protein). By comparison,  $10^{-9}$  M rat AP III has no effect on cGMP in the cultured shark rectal gland. KCNP did not stimulate chloride secretion in the killifish operculum (N=6). In summary, KCNP is approximately 100 times more potent than rat AP III in stimulating chloride secretion and in elevating cGMP in the cultured SRG. The potency suggests that a C-type natriuretic peptide plays a role in controlling chloride secretion in the shark rectal gland.

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