AN ATRIAL NATRIURETIC PEPTIDE-LIKE FACTOR ISOLATED FROM THE DOGFISH SHARK (SQUALUS ACANTHIAS) RECTAL GLAND: INITIAL CHARACTERIZATION

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The elasmobranch rectal gland helps regulate plasma ion concentrations and fluid volume (Solomon et al., Am. J. Physiol. 248:R638-R640, 1985). We have reported that atriopeptins (AP's) act directly on cultured shark rectal gland cells from apical or basolateral sides to stimulate chloride secretion and elevate the second messenger, cGMP (Karnaky et al., Am. J. Physiol. 260:C1125-C1130, 1991). More recently, we have shown that C-type natriuretic peptide is a potent stimulator of chloride secretion when added to either apical or basolateral side (Karnaky et al., this Bull., 1992). C-type natriuretic peptides 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).

The ability to regulate cell function from the apical side of secretory cells in the shark rectal gland suggests novel new control mechanisms which involve local secretion of autocrine or paracrine factors. These findings raise interesting questions concerning the origin and nature of peptide hormones which carry out this function. We have prepared extracts of the shark rectal gland and tested these with three different bioassays.

Extracts were made in the following manner: shark rectal glands (a kind gift of Dr. Michael Field) were minced, boiled, acidified, homogenized, and centrifuged. The supernatant was then applied to a C18 Sep-Pak cartridge. Two partially-purified fractions were obtained by successive rinses in 10% and 40% acetonitrile, producing SP10 and SP40 fractions, respectively. Our preliminary data has shown that the SP40 fraction has proven the best fraction for isolating natriuretic factors from dogfish shark heart and we and others have routinely used this type of protocol to partially purify atrial natriuretic peptide (ANP) and ANP-like peptides from hearts and plasma of many different species.

In a competition binding assay we utilized bovine pulmonary endothelial cells (BPAE cells), which are rich in ANP-binding sites, to examine the

competition between our extract and ^{125}I-ANP for binding. ^{125}I-ANP (^{125}I-rAPIII) binds to these cells in a highly specific manner (specific binding $\geq$ 95% of total binding) with a K_D=0.2 nM. Unrelated peptides such as insulin, bradykinin, angiotensin II, and dynorphin do not displace ^{125}I-ANP from these sites at concentrations as high as 1 μM. These cultures were treated with ^{125}I-ANP only or with this isotope and varying concentrations of the SP10 or SP40 fractions. Non-diluted SP40 fractions displaced 83% of the control ^{125}I-ANP binding (2590 ± 180 c.p.m. bound with the SP40 present versus a control value of 15,260 ± 690 c.p.m. bound; N= average of two samples). A dose-response was demonstrated as serially-diluted SP40 fractions were correspondingly less effective at competing for the ANP-binding sites on the endothelial cells. The resultant displacement curve roughly paralleled that obtained using unlabeled synthetic ANP. As expected the SP10 fraction was less effective in displacing ^{125}I-ANP from its binding sites with only the undiluted SP10 fraction demonstrating activity (23% displacement). An equivalent displacement was obtained with a 16-fold dilution of the SP40 fraction.

C18 Sep-Pak purified extracts were also tested for their effects on intracellular cGMP levels in shark rectal gland (SRG) cells. ANP is known to increase cGMP in these cells. The SP40 fraction increased intracellular cGMP levels over 10 fold (from a control value of 66.0 ± 8.2 to 794.0 ± 23.7; N=3), whereas the SP10 fraction had minimal effect. In our experience with shark heart, this SP10 fraction does not contain ANP-like molecules. Moreover, in a parallel purification protocol, an equivalent amount of the original acid extract was precipitated with 75% acetone and the supernatant from this fraction was found to elevate cGMP over 6 fold (from 66.0 ± 8.2 to 418.0 ± 54.0; N=2). In our experience, ANP-like peptides are typically found within this supernatant under these conditions. These results are consistent with the presence of an ANP-like peptide in SRG extracts; however, the possibility that an unrelated molecule is responsible for this increase in cGMP cannot be excluded.

Lastly, we tested the SP40 extract for possible stimulation of chloride secretion (as measured by the short-circuit current [Isc]) in cultured shark rectal gland cells. Eight shark rectal gland cultures showed significant increases in short-circuit current when the extract was added to the **APICAL** side of the cultures (Isc increased 8.8 ± 2.3 μamp/cm^2, $P < 0.005$, N=9). VIP and 2-chloro adenosine were not active from the apical side but were very potent secretagogues from the basolateral side in these cultures (VIP: 36.0 ± 6.5, $P < 0.01$, N=5; 2-chloroadenosine: 24.6 ± 3.5, $p < 0.005$, N=5).

In summary these data, taken together, strongly suggest that the SRG contains an ANP-like molecule which can control chloride secretion from the apical side of the epithelium. At present, it is unclear whether this ANP-like activity represents ANP-like molecule(s) synthesized within the SRG, synthesized at distant sites, e.g. heart, and sequestered in the SRG, or blood contamination. Regardless of tissue origin, the observations that the SRG contains extractable ANP-like activity and that these extracts are capable of stimulating Isc to the apical surface of SRG cells suggests that unique mechanisms for regulating Cl^-

secretion may be at work in the dogfish SRG. It has only recently been appreciated that several hormones work from not only the basal (blood) side of the epithelium, but also from the apical (lumenal) side as well. The ability to regulate cell function from the apical side suggests novel new control mechanisms which involve local secretion of autocrine or paracrine factors. Apical membrane hormone receptors have been described in a number of epithelia: 1) adenosine in the colonic epithelial cell line T_{84} (Barrett et al., Am. J. Physiol. 256:C197-C203, 1989); 2) lysylbradykinin in cultured rat epididymal epithelium (Cuthbert and Wong, J. Physiol. (Lond). 378:335-345, 1986; 3) insulin and insulin-like growth factor in colon (Pillion et al., Am. J. Physiol. 257:E27-E34, 1989; 4) vasoactive intestinal peptide in bovine tracheal epithelium (Elgavish et al., Life Sci. 44:1037-1042, 1989); 5) ANP in SRG epithelium (Karnaky et al., Bull. Mt. Des. Isl. Biol. Lab. 29:86-87, 1990); 6) bradykinin in canine trachea (Leikauf et al., Am. J. Physiol. 248:F48-F55, 1985). Two interesting recent papers suggest an autocrine or paracrine role for ANP in the kidney. McKenzie et al. (Amer. J. Anat. 190:182-191,1991) have used immunocytochemical techniques and revealed ANP within intercalated cells of the outer medullary and cortical collecting tubules and ducts of several adult mammals, including humans. The authors suggested that the intracellular localization of ANP may be the result of endogenous synthesis and that following secretion, ANP may be available to receptors in the inner medullary collecting ducts. Ritter et al. (J. Clin. Invest. 87:208-212, 1991) have constitutive secretion of an atriopeptin-like prohormone in the cortical tubule fraction in primary adult rat kidney cultures. These authors hypothesize that the renal ANP may be important as an autocrine or paracrine regulator of renal function. The cultured SRG epithelium should prove to be an excellent model system with which to understand these novel mechanisms.

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