ATRIOPEPTIN STIMULATES CHLORIDE SECRETION BY CULTURED SHARK (SQUALUS ACANTHIAS) RECTAL GLAND EPITHELIUM

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The potent diuretic, natriuretic, and vasodilatory hormone, atrial natriuretic peptide (AP), is thought to serve as an osmoregulatory hormone in fish (Evans, Ann. Rev. Physiol., in press, 1990). Known actions of AP in fish include: 1)inhibition of NaCl absorption in flounder intestine (O'Grady et al., Am. J. Physiol. 249:C531-C534, 1985); and 2) stimulation of chloride secretion in both the isolated killifish opercular epithelium (Scheide and Zadunaisky, Am. J. Physiol. 254:R27-R32, 1988) and isolated, perfused dogfish shark rectal gland [SRG] (Solomon et al. Am. J. Physiol. 249:R348-R354, 1985). In the perfused SRG AP is thought to act indirectly by stimulating the release of the neurotransmitter vasoactive intestinal peptide (VIP) from peritubular nerve terminals (Silva et al. Am. J. Physiol. 252:F99-F103, 1987; Stoff et al. Am. J. Physiol. 255:R212-R216, 1988). The purpose of the present study was to use monolayer cell cultures to determine if AP can activate chloride secretion by a direct effect on SRG epithelial cells. We report here that exposure of cultured SRG epithelium to AP-I or AP-III (rat synthetic) from either the apical or basolateral surfaces stimulates bumetanide-sensitive chloride secretion (measured as short-circuit current (Isc)) and elevates intracellular cGMP.

Monolayer cultures of dogfish (Squalus acanthias) SRG epithelium maintained on collagen-coated nylon mesh or in collagen coated Millipore CM Millicells were used for measuring Isc in Ussing chambers (Valentich, Bull. Mt. Des. Isl. Biol. Lab., 26:91-94, 1986). For cGMP assay, cultures in 35mm plastic dishes were exposed for 10 min to either 10^{-3} M IBMX (isobutylmethylxanthine) in shark Ringer (control Ringer) or in control Ringer plus 10^{-7} M AP-III. The experiment was terminated with the addition of cold HCl and cGMP was extracted and measured by RIA. We also prepared a shark heart extract containing bioactive AP. Briefly, hearts were rapidly removed and frozen in liquid nitrogen. The tissue was pulverized and then placed in 1M acetic acid, boiled for 10 min and subsequently extracted on a C18 SEP-PAK cartridge. The eluate was used in our experiments.

In Ussing chamber experiments, basolateral exposure to 10^{-7} M AP-III increased Isc (data = mean ± S.E.; 11.2 ± 2.0 to 43.2 ± 5.2 μamp/cm^2; $P < 0.001$; N=15 epithelia). Stimulation was characterized by Isc oscillations having a periodicity of 10-15 minutes before reaching a steady-state. As stated above, it is thought that AP acts in the perfused SRG by stimulating the release of VIP (Silva et al. Am. J. Physiol. 252:F99-F103, 1987; Stoff et al. Am. J. Physiol. 255:R212-R216, 1988). VIP-stimulated SRG cultures are characterized by a transient peak in Isc followed by a decline to a steady state value less than half of the maximum Isc. This characteristic VIP response was not observed in cultures stimulated with AP which displayed oscillations in Isc followed by a sustained Isc. Isc dropped from 59.9 ± 7.1 to 7.9 ± 2.8 μAmp/cm^2 ($P < 0.001$; N=5) after chloride was removed from the apical and basolateral baths, suggesting that the stimulated Isc is chloride-dependent. Basolateral exposure to 10^{-4} M bumetanide following stimulation with 10^{-7} M AP-III caused a significant inhibition of Isc (43.9 ± 6.5 to 7.6 ± 3.2 μAmp/cm^2; $P < 0.002$; N=5) demonstrating that chloride secretion required Na/K/Cl co-transport activity. Apical exposure to 10^{-7} M AP-I or III also stimulated chloride secretion to a degree indistinguishable from that observed following basolateral exposure. It is important to note that the time of onset of the Isc response was the same whether AP was added to the apical or the basolateral side. This suggests that AP receptors are located on both the apical and basolateral surfaces. If receptors were located only on the basolateral side then one would expect a delay in the response to apically-applied hormone as it diffused through tight junctions or other paracellular leak pathways. Such a delay was not observed. In addition, cultures which responded to basolateral but not apical VIP exhibited both apical and basolateral responses to AP-I.

Basolateral 10^{-3} M 8-bromo-cGMP also elevated Isc (9.8 ± 2.6 to 24.6 ± 3.1 μAmp/cm^2; $P < 0.002$; N=7). Even at this very high cGMP concentration the Isc was only about half that following 10^{-7} M AP addition to the basolateral side. Importantly, 8-bromo cGMP-stimulated cultures did not exhibit the

characteristic oscillations in Isc observed following AP stimulation. Intracellular cGMP levels increased about 5 fold (206.6 ± 41.2 to 1011.0 ± 184.3 fmoles/mg protein; $P < 0.01$; N=7) after a 10 min exposure to 10^{-7} M AP-III. These observations suggest that other signalling mechanisms in addition to elevated cytosolic cGMP are involved in the chloride secretory response of SRG epithelial cells to AP. AP stimulation of chloride secretion is dose-dependent, with a statistically significant ($P < 0.01$) response beginning at the lowest concentration tested (10^{-9} M). Stimulation with AP-I or AP-III over the concentration range of 10^{-9} M to 10^{-6} M produced identical Isc responses. The eluate isolated from shark heart was tested for its effects on Isc and intracellular cGMP accumulation. At an estimated concentration of 3 X 10^{-9} M the eluate stimulated Isc (7.8 ± 3.2 to 55.9 ± 7.4 μAmp/cm^2; $P< 0.01$; N=3) and at a concentration of 10^{-8} M elevated cGMP levels from 150.0 ± 20.0 to 2255.5 ± 396.0 fmoles/mg protein ($P< 0.001$; N=3).

Taken together, these findings suggest that AP can act directly on SRG epithelial cells to stimulate chloride secretion and elevate intracellular cGMP levels. In addition to helping us understand osmoregulatory mechanisms in elasmobranchs, this marine model system from the spiny dogfish shark has important relevance for biomedicine and human disease. The control of salt and water transport across epithelia by AP may play a critical role in the etiology and therapy of hypertension (Genest and Cantin, eds. Rev. Physiol. Pharmacol. 110:1-145, 1988). Chloride secretion in normal, but not cystic fibrotic human intestine, is stimulated by exogenous cGMP (DeJonge, et al., Ped. Pulmon. Supplement 1,93-95, 1987). Cultured SRG epithelium represents an ideal model system for examining the role of cGMP in the control of chloride secretion in general, as well as in intestinal tissues specifically. Cultured SRG epithelium has the advantage that it exhibits only one functional cell type and only one function, NaCl secretion, rather than the dual functions of NaCl secretion and absorption, as is characteristic of intestine. Furthermore, because this model system is a primary cell culture, it offers the advantage of minimal perturbation of intracellular regulatory mechanisms as is possible in transformed epithelial cell lines such as T-84. Using cultured SRG cells, combined physiological, biochemical and morphological approaches should help define the role of AP and cGMP in the regulation of epithelial chloride secretion.

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