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SHORT COMMUNICATION

RAT ATRIOPEPTIN DILATES VASCULAR SMOOTH MUSCLE OF THE VENTRAL AORTA FROM THE SHARK (*SQUALUS ACANTHIAS*) AND THE HAGFISH (*MYXINE GLUTINOSA*)

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Recent evidence suggests that atriopeptin (AP) may play a role in osmoregulation in fishes (Evans, 1990) *via* hemodynamic and ionic transport effects analogous to those described for mammalian volume regulation (e.g. Genest and Cantin, 1988). Specifically, heterologous mammalian AP has now been shown to produce natriuresis (Duff and Olson, 1986), even in an aglomerular teleost (Lee and Malvin, 1987), inhibit intestinal salt uptake (O'Grady *et al.* 1985) and stimulate gill sodium extrusion (Scheide and Zadunaisky, 1988) in teleosts, all responses that favor osmoregulation in dehydrating sea water, rather than volume-loading fresh water. The proposition that AP may be primarily involved in salt extrusion rather than volume regulation is supported by recent studies showing that plasma immunoreactive atriopeptin (AP_{ir}) levels are higher when euryhaline teleost fishes are acclimated to high salinities (Westenfelder *et al.* 1988; Evans *et al.* 1989). The recent finding that mammalian AP produces vasodilation in the ventral aorta and branchial vasculature of the marine toadfish (Evans *et al.* 1989) suggests that AP may also play a role in controlling gill hemodynamics. However, an increase in gill perfusion would presumably exacerbate the osmoregulatory problems in either the marine or freshwater environment, since the gill is the site of passive ionic and water movements in teleost fishes (Evans, 1979).

Most studies on the presence and function of AP in fishes have dealt with teleosts, but a limited data base indicates that AP probably plays some physiological role(s) in the two other major groups of fishes, Chondrichthyes (sharks, etc.) and Agnatha (hagfishes and lampreys). The plasma of representatives of both groups contains AP_{ir} (Evans *et al.* 1989) and it is clear that AP may be a major stimulant of spiny dogfish shark (*Squalus acanthias*) rectal gland salt secretion *via* both indirect (Silva *et al.* 1987) and direct (Karnaky *et al.* 1990) means. AP has also been found to lower dorsal aortic blood pressure in *S. acanthias* (Solomon *et al.* 1985; Benyajati and Yokota, 1990), but either it does not change (Solomon *et al.* 1985) or it reduces (Benyajati and Yokota, 1990) urinary glomerular filtration rate

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and sodium excretion in this species. No physiological studies of the effects of AP on Agnatha have been published, but AP receptors have been identified autoradiographically in the ventral aorta and kidney of the Atlantic hagfish (*Myxine glutinosa*; Kloas *et al.* 1988), and AP_{ir} has been described in the plasma of the sea lamprey (*Petromyzon marinus*; Freeman and Bernard, 1990). AP_{ir} levels are significantly higher in seawater-adapted than in 10% seawater-adapted lampreys (Freeman and Bernard, 1990). To explore the potential for AP to affect gill hemodynamics in non-teleostean fishes, we have examined the sensitivity of isolated, aortic vascular smooth muscle of an elasmobranch and agnathan species to mammalian AP.

Spiny dogfish sharks were collected in Frenchman Bay, Maine, and maintained in submerged, seawater tanks at the Mount Desert Island Biological Laboratory (MDIBL). Atlantic hagfish were purchased from the Huntsman Marine Laboratory, St Andrews, NB, Canada, transported to the MDIBL and maintained in running seawater tanks. Endothelium-free, vascular smooth muscle rings from the shark aorta were prepared and mounted as previously described (initial tension=500 mg; Evans and Weingarten, 1990). Hagfish were anesthetized in 1% MS222 and the ventral aorta was dissected free proximal to the last branchial arch nearest to the heart. The vascular endothelium was removed with a roughened polyethylene tube under iced hagfish Ringer's solution (HRS; in $mmol\,l^{-1}$: NaCl, 501; KCl, 8.7; $CaCl_2$, 5.4; $MgCl_2$, 12.6; Na_2SO_4, 3.2; NaH_2PO_4, 0.4; $NaHCO_3$, 11; glucose, 0.7) bubbled with 1% CO_2 in air. Rings were mounted in 5 ml of HRS in the same system (12°C) as that used for the shark rings (Evans and Weingarten, 1990); initial tension was set at 50 mg and maintained for approximately 30 min until stable tensions were achieved. Preliminary experiments indicated that it was unnecessary to preconstrict shark aortic rings before application of AP, but it was necessary to preconstrict the hagfish rings by the addition of $10^{-4}\,mol\,l^{-1}$ carbachol, which produced an initial tension of 114±7.3 mg (N=8). We assume that this was necessary because of the extremely low initial tensions maintained in the hagfish rings. Low initial tensions were chosen because of the extremely low blood pressures normally found *in vivo* in the hagfish ventral aorta (approx. 0.67 kPa; Satchell, 1986; D. H. Evans, unpublished results). Synthetic rat AP (101–126) was dissolved in $0.2\,mol\,l^{-1}$ acetic acid, sampled into polyethylene microfuge tubes, lyophilized in a Speedvac (Savant, Farmingdale, NY) and stored at −70°C until used. Rings of both species were exposed to the cumulative addition of AP producing a concentration range of 10^{-11} to $2\times10^{-7}\,mol\,l^{-1}$. Endothelium removal was judged complete because both rings responded to carbachol addition with contraction. Maximal actual tension changes were 120±15 mg (11) for the shark and 69.7±7.4 mg (6) for the hagfish.

Synthetic rat AP (101–126) was purchased from Bachem, Inc. (Torrance, CA). Graphics were performed on a Macintosh II microcomputer using Cricket Graph (1.3; Cricket Software, Inc., Malvern, PA). All data are expressed as means±S.E. Curve fitting and apparent EC_{50} values of AP effects were estimated using Superpaint (2.0; Silicon Beach Software, San Diego, CA).

The isolated, endothelium-free, aortic vascular smooth muscle from both the spiny dogfish shark and Atlantic hagfish is very sensitive to heterologous mammalian AP (Fig. 1). The apparent EC_{50} values of the vasodilatory effect of rat AP are 7×10^{-9} mol l^{-1} (shark) and 4×10^{-9} mol l^{-1} (hagfish), nearly identical to that previously described for the aortic vascular smooth muscle of the teleost *Opsanus beta* (4×10^{-9} mol l^{-1}; Evans *et al.* 1989) and in the same range as that described for various mammalian vessels (Genest and Cantin, 1988; Winquist, 1985). Even greater sensitivity might be expected with homologous (at least in terms of vertebrate class) peptide, but we have recently found that the aortic vascular smooth muscle from *O. beta* is actually slightly less sensitive (Price *et al.* 1990) to eel (*Anguilla japonica*) AP (Takei *et al.* 1989) and a killifish (*Fundulus heteroclitus*) brain AP (Price *et al.* 1990) than to mammalian AP. Whether an AP-induced dilation of the ventral aorta in sharks and hagfish *in vivo* results in alteration of perfusion pressures and patterns in the branchial vasculature remains to be determined, but in *O. beta* AP does produce a net fall in branchial resistance of the perfused head (Evans *et al.* 1989). Moreover, several hormones (Oduleye *et al.* 1982) and neurotransmitters (Nilsson, 1984) and metabolites such as adenosine (Colin *et al.* 1979; Evans and Walton, 1990) have been shown to control gill hemodynamics. Nevertheless, the present data and our earlier study (Evans *et al.* 1989) certainly suggest that AP may also play a role in controlling gill hemodynamics in all three major groups of extant fishes.

Therefore, it is instructive to consider the theoretical effects of an AP-induced increase in branchial perfusion. Sharks are slightly hyperosmotic to sea water because of the retention of high concentrations of urea and trimethylamine oxide (TMAO) (approx. 350 and 70 mmol l^{-1}, respectively) in the plasma. The resulting

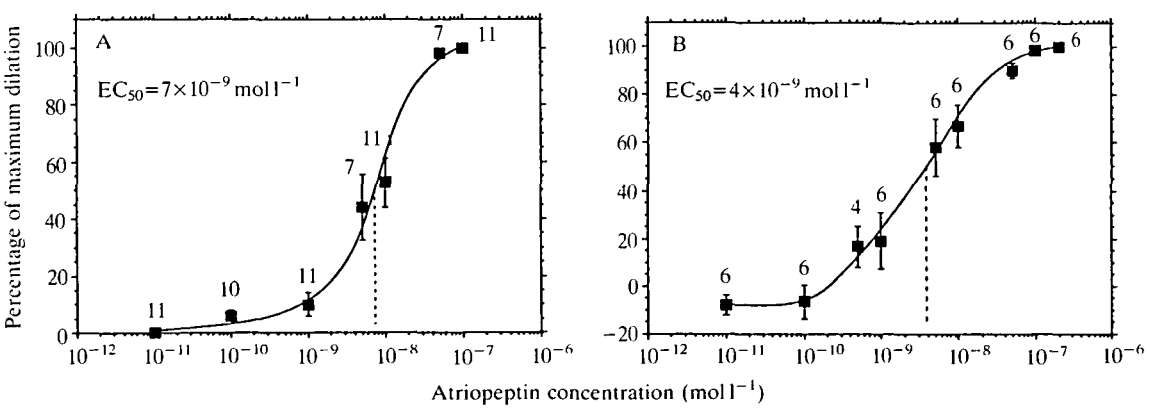

Fig. 1. (A) Concentration–response curve of the effect of rat atriopeptin [AP (101–126)] on isolated ventral aortic rings of the dogfish shark (*Squalus acanthias*). Rings were not pre-constricted before addition of AP. (B) Concentration–response curve of the effect of rat atriopeptin on isolated ventral aortic rings of the Atlantic hagfish (*Myxine glutinosa*). Rings were pre-constricted with 10^{-4} mol l^{-1} carbachol before addition of AP. Bars show ±S.E. where it is greater than the size of the point. Numbers of experiments are indicated above each point.

osmotic influx of water takes place across highly water-permeable gills, but is balanced by production of significant volumes of urine and rectal gland fluid. Since the plasma NaCl content is approximately 50 % that of sea water, there is also a net influx of Na^+ and Cl^- across the gills (despite a very low ionic permeability), which is balanced by rectal gland, and possibly gill, salt excretion (Evans, 1979). A theoretical increase in gill perfusion produced by the vasodilatory action of atriopeptin, as found in the present work, would presumably increase both net water uptake and net ionic influx, thereby exacerbating rather than reducing the osmoregulatory problems faced by sharks.

The agnathan hagfishes are iso-osmotic to sea water, solely by the maintenance of plasma NaCl concentrations near to those of sea water; urea and TMAO are not retained in the plasma. However, plasma Na^+ concentrations are slightly above those in sea water (486 mmol l^{-1} *vs* 439 mmol l^{-1}; Robertson, 1966). Gill water permeabilities are quite high, presumably because no significant net osmotic fluxes of water occur, but gill ionic permeabilities appear to be rather low (Evans, 1979; Evans and Hooks, 1983). In these fishes, a theoretical increase in gill perfusion could potentially increase net loss of Na^+, but would have little effect on volume regulation since no substantial gradients exist.

Thus, marine chondrichthyan fishes which are presumably slightly volume- and salt-loaded, hagfishes which are neither volume- nor salt-loaded in sea water and teleosts which are volume-depleted and salt-loaded in sea water all have aortic vascular smooth muscle which dilates in response to heterologous AP (present study and Evans *et al.* 1989), presumably leading to an increase in gill perfusion, and have AP_{ir} in their plasma (Evans *et al.* 1989). It is difficult to propose a common osmoregulatory parameter in these distinct fish groups associated with a hormone that has the array of known effects elicited in mammals and lower vertebrates by AP (Evans, 1990). However, they all share the need to maximize gas exchange by maintenance of consistent gill perfusion. One might propose, therefore, that, in the earliest vertebrates, AP played a major role in control of gas exchanger hemodynamics, rather than osmoregulation. This very tentative suggestion can only be supported or rejected by a careful assessment of the presence and role of AP in the primary aquatic vertebrates.

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