IMMUNOREACTIVE ATRIOPEPTIN IN PLASMA AND TISSUES OF FISHES: THE EFFECT OF SALINITY CHANGE

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There is an emerging data set which suggests strongly that a cardiac peptide hormone (atriopeptin) may play a role in fish osmoregulation, as has been demonstrated in recent years in volume-loaded mammals (e.g. Genest, J. & Cantin, M., Rev. Physiol. Biochem. Pharmacol. 110, 2-145, 1988). Synthetic, mammalian atriopeptin (AP) inhibits the Na,K,2Cl cotransporter in the flounder intestine (O'Grady, S.M. et al., Am. J. Physiol. 249, C531-534, 1985), stimulates the same cotransporter in the killifish opercular skin (Scheide, J.I. & Zadunaisky, J.A., Am. J. Physiol. 254, R27-R32, 1988), produces natriuresis in both the freshwater trout (Duff, D.W. & Olson, K.R., Am. J. Physiol. 251, R639-R642, 1986) and the aglomerular, marine toadfish (Lee, J. & Malvin, R.L., Am. J. Physiol. 252, R1055-1058, 1987), and stimulates the dogfish rectal gland indirectly via VIP (Silva, P. et al., Am. J. Physiol. 252, F99-F103, 1987). In addition, we have now demonstrated that aortic rings from the dogfish shark vasodilate in response to mammalian AP (Evans & Weingarten, this volume) and the toadfish (Opsanus beta) aortic ring is also sensitive to mammalian peptide, as well as homologous atrial, ventricular, and brain extracts (Evans, unpublished). Importantly, few studies (Epstein, F.H. et al., Bull. MDIBL, 72-73, 1987-88; Galli, S. et al., FASEB J. 2, A5245, 1988; Westenfelder et al., Am. J. Physiol. 255, F1281-F1286, 1988) have been published demonstrating the presence of immunoreactive atriopeptin in fish (shark and teleost) plasma and tissue extracts. Volume loading the dogfish shark appeared to increase immunoreactive AP levels (Epstein et al., Op. Cit., 1987-88), but transfer of at least four species of teleosts (mullet, marine catfish, toadfish and chub) to lower salinities actually reduced the apparent AP concentration in the plasma (Galli et al., Op. Cit., 1988; Westenfelder et al., Op. Cit., 1988). The present study was undertaken to confirm the earlier work on the dogfish shark, extend these data to other species, and examine the effect of acclimation to reduced salinities.

Hagfish (Myxine glutinosa) were collected by the Huntsman Marine Laboratory, St. Johns, N.B., dogfish shark (Squalus acanthias) adults and pups, long-horn sculpin (Myoxocephalus octodecimspinosus), and winter flounder (Pseudopleuronectes americanus) were collected locally, and all species were maintained for at least 5 days in running sea water at ambient temperature (ca. 15°C). A subset of sculpin and flounder were transferred to seawater diluted to 100 mM Cl (ca. 20% sea water) for one week. This salinity is distinctly hypo-osmotic to marine teleost plasma and so would present a volume load to the fish. Fish in either sea water or 20% sea water were anesthetized in MS222 and blood drawn from the caudal vessel into iced, heparinized plastic syringes, transferred into iced polypropylene microcentrifuge tubes containing EDTA (1 mg/ml blood) and aprotinin (500 KIU/ml blood) and centrifuged at 4°C in an Eppendorf microfuge at 3000g for 15 minutes. Measured aliquots of plasma were then acidified with 2 ml/ml plasma of 0.1% trifluoroacetic acid (HPLC grade). Atria, ventricles, and brain were removed from each fish, combined for 6-30 individuals depending on species, weighed, boiled in excess 1N acetic acid for 15 minutes, cooled, and homogenized in a tissue homogenizer. The tissue homogenates were centrifuged at 4°C in the microfuge or Sorva' RC-5B at 16,000g for 30 minutes. Tissue supernatants, as well as acidified plasma, were then extracted into polypropylene tubes with a Sep-Pak C_{18} cartridge (Waters Associates) using 60% acetonitrile (HPLC grade)

in 0.1% TFA as the eluent. Tissue and plasma extracts were evaporated to dryness on a SpeedVac concentrator (Savant) for 7-12 hours and stored at -70°C until analysis. Extracts were reconstituted in distilled water and radioimmunoassays were run using a kit (Peninsula Laboratories, RIK8798) for alpha human atriopeptin. All data are presented as means ± S.E.

Apparent immunoreactive atriopeptin was found in the plasma, atria, ventricles, and brain of all species examined. Plasma concentrations were as follows: hagfish, 185 ± 15 pg/ml (N = 6 pooled samples from 18 fish); sculpin, 102 ± 8 pg/ml (6); flounder, 32 ± 4.8 pg/ml (5). In addition, both the adult shark (N = 5) and the pup (N = 5 pooled samples from 30 fish) had apparent AP_{ir} > 120 pg/ml, the upper limit of the assay. Tissue apparent AP_{ir} was: hagfish atria = 0.64, ventricles = 0.23, brain = 0.39 ng/g tissue (N = 1, pooled from 18 fish); adult shark atria = 0.66, ventricles = 0.11, brain = 0.09 ng/g tissue (N = 1, pooled from 5 fish); shark pup atria = 1.52, ventricles = 0.38, brain = 0.02 ng/g tissue (N = 1, pooled from 30 fish); sculpin atria = 0.72, ventricles = 0.16, brain = 0.22 ng/g tissue (N = 1, pooled from 6 fish); flounder atria = 0.32, ventricles = 0.06, brain = 0.05 ng/g tissue (N = 1, pooled from 5 fish). When the sculpin and flounder were acclimated for at least one week to 100 mM Cl sea water, the apparent AP_{ir} declined by 90% in both species: Sculpin = 9.6 ± 2.1 pg/ml (6); flounder = 2.5 ± 0.4 (6).

The present data indicate that, not only do members of all three fish classes (viz. Agnatha, Chondrichthyes, and Osteichthyes) appear to have immunoreactive AP in their plasma, the levels, as measured using an antibody raised against the human peptide, are in the same range as those found in humans (e.g. Genest, J. & Cantin, M., Op. Cit., 1988). It therefore appears that a cardiac peptide hormone evolved very eary in vertebrate evolution, and has remained structurally similar for some 400 million years. However, fish cardiac and brain tissue levels of apparent AP_{ir} are quite low; mammalian atria generally have concentrations in the 0.1 to 1 mg/g tissue range, ventricles in the range of 10-100 ng/g, and brain approximately 10 ng/g (Inagami, T., et al., In: Atrial Hormones and Other Natriuretic Factors, ed. by Mulrow and Schrier, APS, Bethesda, pp. 39, 52, 1987). The extremely low apparent AP_{ir} in fish tissue data are not consistent with the data on plasma levels from the same species, probably indicating that the tissue peptide is folded in a way to make it relatively insensitive to the antibody raised against the mammalian AP. The alternatives that the fish peptide in tissues is distinctly different from the mammalian form, or is actually found in such small concentrations seems unlikely, especially since our unpublished data indicate that extracts from the toadfish atria, ventricles, and brain are very vasodilatory to the isolated toadfish ventral aortic ring.

Despite the apparent low levels of AP_{ir} in fish hearts and brain it is interesting to note that the ventricular:atrial concentration ratio in all the fish studied to date is significantly greater than that described for normotensive, adult mammals (viz. 0.23 ± 0.4 vs. ca. 0.001), indicating that the fish ventricle may be a significant source of atriopeptin, as it may be in fetal, newborn, or hypertensive mammals (e.g. Hassall, C.J. et al., Cell Tiss. Res. 251, 161-169, 1988; Edwards, B.S. et al., J. Clin. Invest. 81, 82-86, 1988). However, our data don't indicate that the ventricles of the fetal dogfish produce significantly more AP_{ir} than the adult (see above). Our finding of significant AP_{ir} in the fish ventricle is consistent with our unpublished data indicating that extracts from the toadfish ventricle are even more vasodilatory (mg for mg) than atrial extracts.

Our finding that apparent AP_{ir} in the plasma of both the sculpin and the flounder acclimated to distinctly hypo-osmotic salinity actually increases is especially interesting, and consistent with our data on the toadfish (Galli et

al., Op. Cit., 1988; Evans, unpublished). These data are not consistent with the proposition that teleost atriopeptin plays a role in osmoregulation in the volume-expanding low salinities. In fact, they suggest strongly that teleost AP may be important in marine osmoregulation. The fact that mammalian AP inhibits gut salt uptake (O'Grady et al., Op. Cit., 1985), but stimulates gill salt extrusion (Scheide & Zadunaisky, Op. Cit., 1988) are consistent with this proposition. Moreover, in mammals AP inhibits prolactin secretion (Samson, W.K. & Bianchi, R., Can. J. Physiol. Pharmacol. 66, 301-305, 1988), and cortisol stimulates AP release (e.g. Weidmann, P. et al., J. Clin. Endocrinol. Metab. 66, 1233-1239, 1988). The fact that prolactin is known to be very important in teleost osmoregulation in fresh water, while cortisol is involved in sea-water acclimation (e.g., Evans, D.H., In: Fish Physiology, Vol. XB, Hoar and Randall, eds., Academic Press, Orlando, pp. 239-283, 1984), is certainly consistent with a role of AP in seawater acclimation. Moreover, Na seems to be a potent stimulant of AP release from at least rat hypothalamic fragments (Shibasaki, T. et al., Life Sciences 42, 1173-1180, 1988). The role of atriopeptin in fish osmoregulation seems to be an intriguing area for future research. (Supported by NSF DCB-8801572)