DISTRIBUTION AND ANALYSIS OF NATRIURETIC PEPTIDE RECEPTOR-A (NPR-A) IN TISSUES OF DOGFISH, SQUALUS ACANTHIAS, AND HAGFISH, MYXINE GLUTINOSA

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In mammals, the receptors for natriuretic peptides (NPs) are now well-characterised (Koller and Goeddel, Circulation 86: 1081-1088, 1992). To date, three types of natriuretic peptide receptor (NPR) have been described: NPR-A (also GC-A) which is activated by ANP and BNP; NPR-B (also GC-B) which is activated by CNP; and NPR-C which is a unique receptor that binds all NP's but does not stimulate cGMP activity, and is thought to function as a mechanism for clearing NPs from the circulation and tissues. Although it is now well-documented that NPs are present in the heart and brain of elasmobranch and cyclostome fishes, information on the distribution and characterisation of their receptors is still rudimentary. A single study in hagfish, Myxine glutinosa, described the presence of binding sites in the aorta and the kidney (Kloas et al., Comp. Biochem. Physiol. 91A: 685-688, 1988), but to date, the only information on the location of NP receptors has been derived with physiological techniques. We have undertaken to study the distribution of NP receptors in fish and to determine if the receptors can be characterised as in mammals. In the present study we have determined the distribution of NPR-A binding sites in target osmoregulatory tissues of the shark, Squalus acanthias, and hagfish, Myxine glutinosa, and begun a characterisation of this binding site by performing competitive displacement studies with other NP ligands.

Tissue section autoradiography was performed according to the method of Konrad et al. (Am. J. Physiol. 259: E246-E255, 1990). Preincubation buffer consisted of 50 mM tris-(hydroxymethyl)aminomethane (Tris).HCl buffer (pH 7.4), 50mM NaCl, 5mM $MgCl_2$, 0.1 % bovine serum albumin (BSA), and 0.05% bacitracin. Sections were incubated in this buffer plus 4μg/ml of leupeptin, 2μg/ml chymostatin, 2μg/ml pepstatin, 10^{-6} M PMFS phenylmethylsulfonylfluoride, and 150 pM or 200 pM of rat (3-[^{125}I]iodotyrosol^{28}) atrial natriuretic peptide (74TBq/mmol; Amersham, Illinois). Nonspecific binding was determined in adjacent sections in the presence of 10^{-6} M unlabelled rat 3-28 ANP (rANP)(Bachem, California). In some tissues, displacement of specific binding was also determined in the presence of porcine CNP (pCNP; Bachem, California), shark CNP (sCNP; kindly donated by Dr. J. Forrest), and rat des[Gln^{18}, Ser^{19}, Gly^{20}, Leu^{21}, Gly^{22}]ANP-(4-23)-NH_2 (C-ANF; Bachem, California), a truncated ANP that, in mammals, binds only to NPR-C (Koller and Goeddel, Op. Cit., 1992). Following incubation, sections were exposed to Hyperfilm autoradiography film. For higher resolution autoradiography some sections were dipped in nuclear track emulsion (Kodak NTB.2) at 43 °C and stored at 4 °C for 7 days before developing, fixing and staining. The observations presented below are based on data generated using both techniques. Crude membranes of the gills and rectal gland of Squalus, and the gills only of Myxine, were prepared and binding assays performed according to the method of Kollenda et al. (Am. J. Physiol. 258:R1084-R1088, 1990). 25-100 μg protein was incubated in buffer (as above) with 25 pM of [^{125}I]-rANP, and increasing concentrations of competing ligands (rANP, C-ANF, pCNP, and sCNP).

Gills: In Squalus, specific [^{125}I]-rANP binding was present on the gill filaments but no binding was observed in the interbranchial septum or the gill arch. The densest distribution of [^{125}I]-rANP binding sites was on the filament body and the secondary lamellae. Binding sites were also observed on intrafilamental blood vessels. In membrane preparations of the gills, 50% of the [^{125}I]-rANP binding was displaced by 0.8 nM rANP and 0.2 nM C-ANF, with all binding being displaced by 10 nM of each peptide. In contrast, 10 nM of pCNP and sCNP displaced only 60% of the [^{125}I]-rANP binding. In Myxine, specific [^{125}I]-rANP binding was present in the

respiratory and efferent zones of the gills. In membrane preparations of the gills, 50% of the [^{125}I]-rANP binding was displaced by 1 nM rANP and 10 nM C-ANF. 30 nM rANP displaced all [^{125}I]-rANP binding, however at 30 nM C-ANF, only 65 % of the [^{125}I]-rANP binding was displaced.

Kidney: Specific [^{125}I]-rANP binding was present in the bundle zone of the Squalus kidney, but the exact locations of the binding have not been precisely determined. Specific binding was not observed on the renal corpuscles nor in the sinus zone. In the kidney of Myxine, binding was observed on the glomeruli and archinephric duct confirming the observations of Kloas et al. (Op. Cit., 1988)). This binding was completely displaced by 1nM rANP, but, significant residual binding was observed in sections incubated with 100 nM of C-ANF or pCNP.

Brain: No specific [^{125}I]-rANP binding was observed in the telencephalon, diencephalon, mesencephalon, and tectum of Squalus. However, [^{125}I]-rANP binding sites were present in the hypophysis. In Myxine, an extensive distribution of [^{125}I]-rANP binding sites were observed in the hemispheres, diencephalon, mesencephalon, rhombencephalon, and in the neurohypophysis. The binding was densest in the peripheral regions of the brain through which many fibre tracts course. The presence of [^{125}I]-rANP binding sites in the hagfish brain is curious since no immunoreactivity was found with antisera that specifically binds to an ANP epitope. However, the distribution of [^{125}I]-rANP binding sites in the brain showed good correlation with that of NP-immunoreactive structures observed using antisera that cross-reacts with BNP and CNP (Donald et al., Cell and Tissue Res. 270:535-545, 1992).

Rectal gland: A dense distribution of [^{125}I]-rANP binding sites was present in the central duct of the rectal gland. Examination of emulsion-dipped sections showed that these binding sites were associated with the luminal epithelium of the duct. In transverse sections of the gland a denser distribution of binding sites was present in the outer third of the gland than in the inner two-thirds. These binding sites could not be directly correlated with the anatomical locations of rectal gland tubules nor with neural structures within the gland. Subsequently, the distribution of [^{125}I]-rANP binding sites was determined in sections of cultured rectal gland tubule cells (kindly donated by Drs K. Karnaky and J. Forrest). Specific binding sites were observed on both apical and basolateral membrane surfaces, which is consistent with electrophysiological studies which reported NP stimulation of chloride secretion in these cultures after exposure to either apical or basal side (Karnaky et al., Amer. J. Physiol. 260:C1125-C1130, 1991). In membrane preparations of the whole rectal gland, 50% of the [^{125}I]-rANP binding was displaced by 0.2 nM rANP, 0.2 nM C-ANF, 1 nM sCNP, and 20 nM pCNP.

These studies show that binding sites for ANP are present in all tissues examined, except for the brain of Squalus, in which binding was confined to the hypophysis. Competition experiments in the gills and rectal gland of Squalus show that C-ANF competes for the binding site with equal or greater affinity than ANP, with the CNP's being less potent. These data is not consistent with the order of potencies for the mammalian NPR-A or NPR-C and suggest that the ANP binding site in Squalus is pharmacologically dissimilar. In contrast, competition experiments in the gills and kidney of Myxine show that C-ANF has lower affinity than ANP for the binding sites; in the kidney pCNP also has much lower affinity than ANP. These data are more consistent with the properties of the mammalian NPR-A receptor. The observation that C-ANF does not fully displace binding in the gill and kidney also suggests that both NPR-A and NPR-C are present in these tissues.

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