ATRIAL NATRIURETIC PEPTIDE AND ITS BINDING SITES IN KIDNEY AND AORTA OF THE ATLANTIC HAGFISH (MYXINE GLUTINOSA)

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The atrial natriuretic peptide (ANP) is synthesized in cardiac myocytes. In both in vivo and in vitro studies it was shown that the target tissues of this hormone in mammals include the kidney, the adrenal gland, and the vascular smooth muscle. Functional studies in fish have been carried out. ANP causes a vasodilatation in the rectal gland vascular bed and enhances chloride secretion in Squalus acanthias (Solomon et al, Am J Physiol 249: R348 - 354, 1985). These effects are most probably mediated through specific binding sites on the respective target cells. Such binding sites have been demonstrated in rat and human kidneys (Bianchi et al, Histochem 82: 441 - 452, 1985; Brucksch et al, Klin Wochenschr, 66: 303 - 307, 88) and in the vasculature of several organs (Schiffrin et al, Circ Res 56: 801 - 807, 1985). It has been proposed that the binding to renal glomerular capillaries is responsible for the increased glomerular filtration rate induced by ANP (Maack et al, Am J Med 77: 1069 - 1075, 1984). Other experiments indicated that ANP primarily has an effect on renal tubules (Biolaz et al, Kidney Int 32: 537 - 546, 1987).

This study focused on the distribution of ANP binding sites in the Atlantic hagfish Myxine glutinosa. This animal has been shown to be a useful model for studies of renal fluid control mechanism (Alt et al, J Exp Biol 91: 323 - 330, 1980). The existence of ANP in the brain and heart of Myxine has already been demonstrated by immunocytochemistry (Reinecke et al, Histochem 86: 233 - 239, 1987). It was therefore investigated, whether binding sites of ANP (99-126), the peptide which has been shown to be biologically active in other vertebrates, exist in the renal system (i.e. glomeruli, neck segment, archinephric duct) and the aorta of the hagfish. Binding sites were visualized by in vitro autoradiography, a method which allows anatomical localization as well as biochemical characterization (Palacios et al, Neurosci Lett 25: 101 - 105, 1981).

Tissues of three hagfish were dissected and frozen in liquid nitrogen. Frozen 16 um sections were cut on a cryostat at -18°C, mounted onto gelatine coated slides, and dried under vacuum at 4°C over night. Following the method of Quirion et al (Peptides 5: 1167 - 1172, 1984) binding sites for ANP were labeled in vitro by incubation with (3-[^{125}I] -iodotyrosil^{20})- rat ANP (specific activity 2000 Ci/mmol, Amersham-Buchler, Braunschweig, FRG). Non-specific binding was determined on adjacent sections in the presence of a thousand fold excess of unlabeled rat-ANP (99-126), (Peninsula Lab, Inc, Belmont, CA).

For preliminary tests, binding was visualized by autoradiography with ^{3}H-Ultrofilm. In order to localize ANP binding sites more exactly, sections were postfixed with 4 % paraformaldehyde in 0.1 M phosphate buffer pH 7.2 and dipped into photoemulsion (Kodak NTB 3) under safelight. After exposition, the slides were developed photographically, counterstained with hematoxylin, and mounted in Eukitt.

In preliminary experiments, autoradiography with LKB-Ultrofilms showed that ^{125}I-ANP(99-126) binds specifically to structures in all

hagfish organs investigated. The strongest binding was found in the glomeruli. These showed a low unspecific reaction that corresponded to less than 5 % binding of ANP(99-126). In the archinephric duct lower amounts of the labeled ligand were retained, whereas non-specific binding in this organ was similar to that in the glomeruli. Aortic tissue showed a medium binding of the labeled peptide, but non-specific binding was approximately 40 %.

On autoradiograms of sections dipped with photoemulsion, ANP binding sites were localized more exactly. In the glomeruli, arterioles were intensely labeled. However, the densest pattern of silver grains was detected over cells of the inner epithelia of Bowman's capsules and in the neck segment (Fig. 1a). Control sections from incubations with an excess of unlabeled hormone show a random distribution of silver grains over these structures (Fig. 1b).

Labelling in the archinephric duct was observed in the layer of smooth muscle cells which surrounds the intraluminal cells of the tubules. The intraluminal cells themselves did not bind ANP. Sections showing unspecific binding revealed a diffuse pattern of silver grain distribution.

The muscle cell layer as well as the endothelial cell layer of the aorta were heavily labeled by ^{125}I-ANP. But as in the preliminary experiments with H-Ultrofilm these structures showed a high degree of unspecificity.

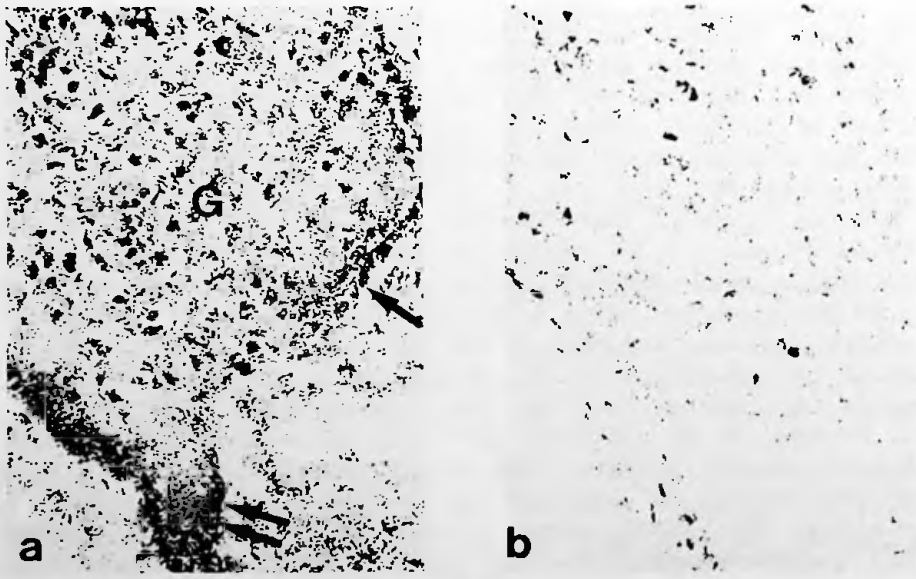

Fig. 1 Binding of ANP to glomeruli of the hagfish. Specific binding in the presence of 76 pM ^{125}I-ANP(99-126) (a), and unspecific binding in the presence of 76 pM ^{125}I-ANP(99-126) and 80nM unlabeled ANP(99-126) (b). Note the dense pattern of silver grains over glomerular arterioles (G), inner epithelia of Bowman's capsule (one arrow), and the neck segment (two arrows).

The present data show for the first time that binding sites for the cardiac hormone ANP(99-126) exist in Myxine. Binding sites have also been

demonstrated in studies on higher vertebrates and it therefore can be assumed that target sites for the hormone are present in all vertebrates. It has been shown that homologous biologically active material from the hearts of Myxine glutinosa exerts ANP-like effects on rat aorta stripes (Forssmann et al, unpublished). It therefore can be assumed that the ^{125}I-rat ANP used in this study autoradiographically monitored comparable binding sites in Myxine.

In contrast to other investigations on rat and man (Bianchi et al, Ibid.; Bruksch at al, Ibid.) binding sites in Myxine were not only detected on glomeruli but in the Bowman's capsule as well. Such a species specific distribution of ANP binding sites has also been shown for adrenal glands (Fuchs et al, Peptides 7: 873 - 876, 1987).

The specific binding of ANP in the archinephric duct may correspond to that found in the collecting duct system of the rat medulla, which is probably involved in the regulation of sodium reabsorption (Sonnenberg et al, Am J Physiol 250, F963 - 966, 1986). ANP may not only have an effect on local transport mechanisms but also an influence on contractile structures surrounding Bowman's capsule, neck segment, and archinephric duct. It is assumed that the peptide is involved in the fluid handling of the renal system of hagfish, which is primarily a volume regulating device (Alt et al, Ibid.)

The presence of ANP binding sites in the aortic walls is in accordance with earlier studies on other species that revealed high affinity binding sites in vascular walls (Schiffrin et al, Ibid.). Experiments with dogfish heart extracts (kindly provided by Dr. P. Silva) showed no vasoactive effects on the isolated perfused single glomerulus of Myxine (Elger, Stolte, Bull Mt Desert Isl Bio Lab 56: 24 - 25, 1984). It has to be further investigated whether ANP affects the hagfish vasculature and whether it has the same hormonal effects in Myxine that it has in higher vertebrates.

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