Immunohistochemical localization of atrial natriuretic peptide binding sites in juxtaglomerular cells and vascular walls of rat kidney

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Abstract. Since the kidney is one of the major sites of action for atrial natriuretic peptide (ANP) and immunoreactive ANP has been detected in tissue extract by radioimmunoassay, we have applied the immunohistochemical technique by using the avidin-biotin complex method to investigate ANP binding sites in the rat kidney. Although no immunostaining was observed in the kidney of control rats, immunoreactive ANP was present in the juxtaglomerular cells, the vascular walls of interlobular arteries, arcuate arteries, arterioles including vas afferens and vas efferens, and the medullary peritubular capillary of ANP-pretreated rats. In contrast, no tubular structure was stained. These results suggest that ANP may affect renin secretion via its direct action on the juxtaglomerular cells and that it predominantly induces natriuresis by its effects on renal hemodynamics.

Rat atrial natriuretic peptide (ANP) was purified and the sequence of several fragments have been reviewed by Cantin & Genest (1985) and Cole & Needleman (1985). Synthetic peptides have been reported to mimic the actions of the endogenous factors, resulting in powerful natriuresis, vasorelaxation (Cantin & Genest 1985; Cole & Needleman 1985) and inhibition of aldosterone secretion (Atarashi et al. 1984). In addition, ANP has been shown to inhibit renin secretion both in vivo (Burnett et al. 1984; Maack et al. 1984) and in vitro (Obana et al. 1985). The interaction of ANP with the renin-angiotensin-aldosterone system may therefore represent another important mechanism for the regulation of body-fluid homeostasis. The mechanistic details of renin inhibition, however, are not elucidated.

Immunoreactive ANP has been detected by radioimmunoassay method in the rat kidney tissue (Sakamoto et al. 1985). Because of its low molecular weight form and a substantial decrease after perfusion with saline, it is suggested that the immunoreactive ANP in the kidney is receptor-bound. For a further clarification of this point, we applied the immunohistochemical technique to demonstrate ANP sites of action in rat kidney tissue with and without prior administration of synthetic ANP.

Materials and Methods

Male Sprague-Dawley rats weighing 250–350 g were used in the study. They were fed regular rat chow and had access to water ad libitum. Under ketamine hydrochloride (50 mg/kg im) anesthesia, a PE-20 polyethylene catheter (Clay Adams, Paraippany, NJ) filled with...
Immunohistochemical staining of paraffin section of control rat heart. ANP immunoreactivity is localized in the atrial cells but not in the ventricular cells (a). High power view of right atrial myocardium of control rats. Most cardiocytes show an intensive paranuclear reaction (b). Magnification: a, × 44; bar 200 μm. b, × 218; bar 40 μm.

RA, right atrium; RV, right ventricle.

0.9% saline containing 100 kU/l of heparin was placed in the right femoral vein. After equilibration for 30 min, 5 μg/kg body weight of 28 amino acids synthetic rat ANP (arANP, Peninsula Laboratories, Belmont, CA) was injected as a bolus through the catheter. Alternatively, the same volume of physiological saline was administered in the control rats. Ten minutes after the injection, both kidneys were perfused with 200 ml of saline through the cannula inserted into the abdominal aorta, according to the method of Curthoys & Belleman (1979) with some modifications. After perfusion, the kidneys and heart were removed and sliced. They were fixed in cold Bouin’s fixative, dehydrated, and embedded in paraffin. Sections, 3 μm, were cut, attached to glass, deparaffinized, and rehydrated.

Immunostaining, using anti-ANP antibody as first antiserum, was carried out by the avidin-biotin complex method (Hsu et al. 1981) using Vectastain ABC kits (Vector Laboratories, Inc, Burlingame, CA). Diaminobenzidine tetrahydrochloride was used as peroxidase substrate. The sections were treated with 0.3% H₂O₂ in methanol and 3% normal goat serum to reduce non-specific background staining and block endogenous peroxidase activity.

To test the specificity of the immunohistochemical reactions, a 0.1 mol/l phosphate buffer containing...
sodium chloride or the following control sera were substituted for the primary antiserum: normal rabbit serum and anti-ANP antiserum pre-absorbed with synthetic rat ANP. Pre-incubation was performed with an excess of synthetic rat ANP overnight at 4°C.

Results

In control rats, granules which specifically stain for ANP were found in almost every portion of the atrium, whereas no staining was observed in the ventricles (Fig. 1a,b). There was no immunostaining in any structure of the kidney.  

In ANP-pretreated rats, however, immunoreactive ANP was demonstrated in the juxtaglomerular cells (Fig. 2), in the wall of interlobular arteries (Fig. 3), arcuate arteries, arterioles including vas afferens (Fig. 4) and vas efferens. In the renal medulla, immunostaining was observed in the peritubular capillary of the inner medulla (Fig. 5). On the contrary, no staining was observed in the glomeruli and tubules.

When the antiserum was pre-absorbed with synthetic ANP, the specific immunostaining in the atrium of control rats and in the kidney of ANP-pretreated rats was abolished. Replacement of primary antibody, either by normal rabbit serum or phosphate buffer, gave a completely negative result.

Discussion

We have here demonstrated the presence of immunoreactive ANP in the juxtaglomerular cells and in various sites of the vascular wall, including in the vas afferens, in ANP-pretreated rats, whereas no such immunostaining was observed in the kidney of control rats. This finding therefore suggests the existence of ANP binding sites in those kidney structures.

Using rat kidney slices, we have previously shown an inhibitory effect of ANP on renin secretion in vitro (Obana et al. 1985), suggesting a possible direct action of ANP on juxtaglomerular cells. Although this effect of ANP was further confirmed by Henrich et al. (1986), the kidney slices utilized in these studies obviously contain nephrone elements and therefore, participation of tubular factors could not have been completely excluded (Burnett et al. 1984; Maack et al. 1984). In addition, Antonipillai et al. (1986) failed to see an inhibitory effect of ANP on basal renin release.

Recently, Kurtz et al. (1986) reported that ANP inhibits renin release from cultured rat juxtaglomerular cells, strongly suggesting the direct action of ANP on juxtaglomerular cells. Our results provide further evidence for the hypothesis that ANP inhibits renin secretion via direct action on juxtaglomerular cells.

In anesthetized animals, ANP has been shown to produce a remarkable increase in glomerular

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**Fig. 4.** Immunohistochemical staining of paraffin section of ANP-pretreated rat kidney. ANP-specific staining is seen in the wall of vas afferens, but no staining is observed in the tubular cells. Magnification, × 420, bar 20 µm. VA, vas afferens.

**Fig. 5.** Immunohistochemical staining of paraffin section of ANP-pretreated rat kidney. ANP-specific staining is seen in the peritubular capillary of the inner medulla. Magnification, × 420, bar 20 µm.
filtration rate with relative constancy of the renal plasma flow rate (Burnett et al. 1984; Maack et al. 1984; Beasley & Malvin 1985; Huang et al. 1985). In studies using the isolated perfused rat kidney, the increase in glomerular filtration rate was attributed to its renal vasoconstrictive effect, expressed preferentially in the efferent arteriole (Atlas et al. 1984). This view was further supported in a study using the isolated perfused glomerulus technique (Fried et al. 1985). Glomerular micropuncture studies, however, have indicated that the alterations in glomerular filtration dynamics are mainly the result of afferent arteriolar dilatation, accompanied by a small rise in efferent arteriolar resistance (Ichikawa et al. 1985). All these findings suggest the vascular vessels as major sites of action for ANP.

Supporting this concept, we found ANP immunostaining in the vascular wall of interlobular arteries, arcuate arteries, and arterioles, including vas afferens and vas efferens. On the other hand, no tubular structure was ever found to be stained. These results coincide with the autoradiographic localization of binding sites for ANP (Bianchi et al. 1985). It is therefore assumed that ANP increases renal solute excretion predominantly by hemodynamic actions as implied by others (Beasley & Malvin 1985; Huang et al. 1985; Atlas et al. 1984; Fried et al. 1985; Ichikawa et al. 1985). In addition, we observed immunostaining in the inner medullary peritubular capillary. This is in close agreement with previous reports demonstrating ANP binding sites in the medullary vasculature (Koseki et al. 1986a; Mantyh et al. 1986; Bianchi et al. 1987). ANP may affect tubular transport of electrolyte and water through its binding to the vascular receptors in the renal medulla.

No immunostaining was observed in the glomerular structure where numerous binding sites have been demonstrated by autoradiography (Bianchi et al. 1985; Koseki et al. 1986b; Lynch et al. 1986). Although the exact reason for this remains unclear, it may be partly attributed to methodological differences. Koseki et al. (1986b) showed that both binding and dissociation of labelled ANP in the isolated glomeruli are rapid. Therefore, it is also likely that ANP bound to the glomerulus has already been dissociated and/or degraded 20 min after the administration of ANP when the kidney tissue was fixed.

The specificity of the ANP binding to certain kidney structures is yet to be elucidated. However, the localization, which is well in agreement with the renal effect of ANP, is characteristic enough to support its relevance to physiological functions.

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