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DIFFERENCE IN MOLECULAR SIZE OF RECEPTORS FOR α-RAT ATRIAL NATRIURETIC POLYPEPTIDE AMONG THE KIDNEY, AORTA, AND ADRENAL GLAND AS IDENTIFIED BY DIRECT UV-PHOTOAFFINITY LABELING

Chizuko Koseki, Yujiro Hayashi*, Norio Ohnuma*, and Masashi Imai

Department of Pharmacology, National Cardiovascular Center Research Institute, Osaka 565, Japan

** Department of Clinical Pharmacology, Suntory Institute for Biomedical Research, Osaka 618, Japan*

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Summary: In order to identify the molecular size of receptors for α-rat atrial natriuretic polypeptide (α-rANP), we utilized the direct UV irradiation method for photoaffinity labeling with the biologically active [^{125}I]α-rANP. In the preparation of isolated glomerulus and the inner medullary collecting duct (IMCT)-rich fraction, the autoradiograms of the electrophoresed sodium dodecyl sulfate (SDS)-polyacrylamide gels showed a single radioactive band which is displaceable with unlabeled α-rANP. The dose-dependent displacement fit very well with a binding-inhibition curve representing the binding affinity of 6.5×10^{-10}M. The molecular size of the ligand-receptor complex was about 65,000 daltons for both glomerulus and IMCT-rich fraction. In contrast, in homogenate of the aorta and adrenal gland, the ligand-receptor complex was 140,000 daltons.

Autoradiographic studies have shown that the binding sites of atrial natriuretic polypeptide (ANP) are widely distributed in many tissues (1,2). Binding studies demonstrated the specific receptors for ANP in the kidney, adrenal cortex, and aorta (3-10). The binding sites within the kidney have been shown to be localized on the glomerulus (1,3,9,11-13), vasa recta (1,11), inner medullary collecting tubule (IMCT) (3,11,13), renal artery (1,3,11,13), and renal pelvis (3).

Recently, several groups of investigators (14-18) determined the molecular size of the ANP receptor-ligand complex in various tissues either by the photoaffinity labeling method with an aid of a photoreactive ligand (14,16,17) or by the cross-linking method using bifunctional agents (15,17,18). All the investigators identified a molecular species with the size ranging 120,000-180,000 daltons. But some investigators reported that other molecular species with smaller size (60,000-70,000) can also be identified, suggesting that the receptors for ANP consist of subunits (15,17,18).

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Although we have also identified the molecular size of ANP receptors by using direct photoaffinity labeling method, the mildest cross-linking method, we have reached the different conclusions. In this communication, we report that the receptors in the kidney consist of a smaller molecular species (65,000 daltons) whereas those in the aorta and the adrenal gland are larger (140,000 daltons).

MATERIALS AND METHODS

The bioactive [^{125}I]α-rANP was prepared by the lactoperoxidase method as described previously (3). The specific activity of [^{125}I]α-rANP was 400-600 μCi/μg.

Tissue preparations Male Sprague-Dawley rats weighing 200-250g were anesthetized with sodium pentobarbital (50mg/kg i.p.). After the blood was washed out by Hanks' solution through the cannula inserted into the abdominal aorta, the aorta, adrenal and kidney were removed. Then, the aorta, adrenal, and outer stripe of outer medullary tissue (OMo) from the kidney slice were homogenized at 50 power for 1min with Physcotron (Niti-on, Chiba) in 0.25M sucrose including 0.04% trypsin inhibitor, 1mM phenylmethyl sulfonyl fluoride, and 1mM EDTA. Isolated glomeruli and IMCT-rich fraction were prepared by the sieving and the Percoll density gradient method, respectively as described previously (3). The tissue homogenates and isolated glomeruli and IMCT-rich fraction were stored at -80°C until affinity labeling.

Photoaffinity labeling The binding assay was carried out with or without 4×10^{-6}M unlabeled peptide. For the equilibrium binding samples were incubated with 4×10^{-10}M [^{125}I]α-rANP, in 50mM Tris·HCl, 10mM $MgCl_2\cdot6H_2O$, pH7.4 including 0.2% bovine serum albumin at 4°C for 10min. After the radio activity of bound ligand was measured by a gamma counter, the preparations were irradiated for 30min on ice with a 18W Blak Ray UV lamp at a distance of about 5cm. Prior to application to acrylamide gel electrophoresis, the cross-linked samples were solubilized with 1% SDS with 5% β-mercaptoethanol and boiled for 3min.

Electrophoresis and autoradiography Each solubilized sample (about 0.01mg protein) was electrophoresed with 10% SDS-polyacrylamide gel by the method of Laemmli (19). After the electrophoresis the gels were stained with 0.25% Coomassie brilliant blue, dried, and exposed to X ray films (Dupont Cronex No.4) intensifying by a Dupont Cronex Lightning Plus screen at -20°C for 10 days. Molecular weight standards were ferritin half unit (220,000), phosphorylase b (94,000), albumin (67,000), catalase (60,000), ovalbumin (43,000), lactate dehydrogenase (36,000), and carbonic anhydrase (30,000).

Measurement of protein Protein was determined by Lowry's method (20) with bovine serum albumin as the standard.

RESULTS

Figure 1 shows the autoradiograms of the photoaffinity labeled [^{125}I]α-rANP-receptor complex in the glomerulus, the homogenate of OMo, and IMCT rich fraction. Lane A is Coomassie brilliant blue staining pattern of the glomerulus. In lane B, the ligand-receptor complex was clearly shown in the position of about 65,000 daltons. This band was displaced in the presence of 4×10^{-6}M unlabeled α-rANP (lane C). In IMCT-rich fraction, the complex was also observed in the position of 65,000 daltons (lane F) and the binding was displaced with 4×10^{-6}M unlabeled α-rANP (lane G). OMo had no prominent labeled molecules either in the presence (lane D) or absence (lane E) of unlabeled α-rANP. In the glomerular preparation, the cross-linked [^{125}I]α-rANP-receptor complex corresponding to 65,000 daltons was inhibited in a dose-dependent manner when concentration of unlabeled α-rANP was increased (Fig. 2, lane B-E). In contrast, 4×10^{-6}M angiotensin Ⅱ was without an inhibitory effect (lane F). The inhibitory effect of unlabeled α-rANP was analysed quantitatively by measuring the peak area of the densitogram at 65,000 daltons (Fig. 3b). The percent bound [^{125}I]α-rANP was plotted against the concentration of unlabeled α-rANP (Fig. 3a). The data were compared with the displacement curve for [^{125}I]α-rANP binding to the isolated glomerulus. It is

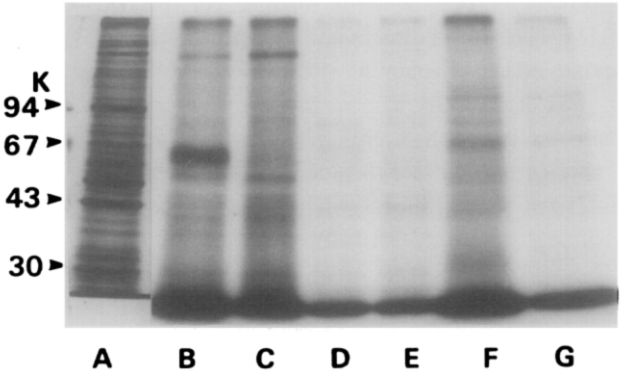

Fig.1 The autoradiogram of the photoaffinity labeled [^{125}I]α-rANP-receptor complex in the glomerulus (lanes A-C), homogenate of OMo (lanes D,E), and IMCT-rich fraction (lanes F,G). Lane A is Coomassie brilliant blue staining pattern of the glomerulus. Lanes B, D, and F; Preparations were incubated with 4×10^{-10}M [^{125}I]α-rANP; Lanes C, E, and G; The binding was displaced with 4×10^{-6}M unlabeled α-rANP. The cross-linked ligand-receptor complex in the position of about 65,000 daltons was visualized in lanes B and C but not in lane D.

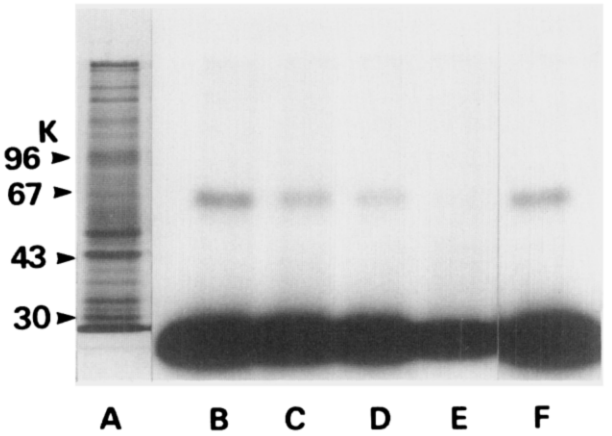

Fig.2 The autoradiogram of the photoaffinity labeled [^{125}I]α-rANP-receptor complex in the glomerulus. The cross-linked ligand-receptor complex corresponding to 65,000 daltons was inhibited in a dose-dependent manner in the presence of unlabeled α-rANP. Lane B; None, Lane C; $4x10^{-9}$M, Lane D; $4x10^{-8}$M, Lane E; $4x10^{-7}$M. As shown in the lane F, $4x10^{-6}$M angiotensin II did not affect the labeling of band of 65,000 daltons.

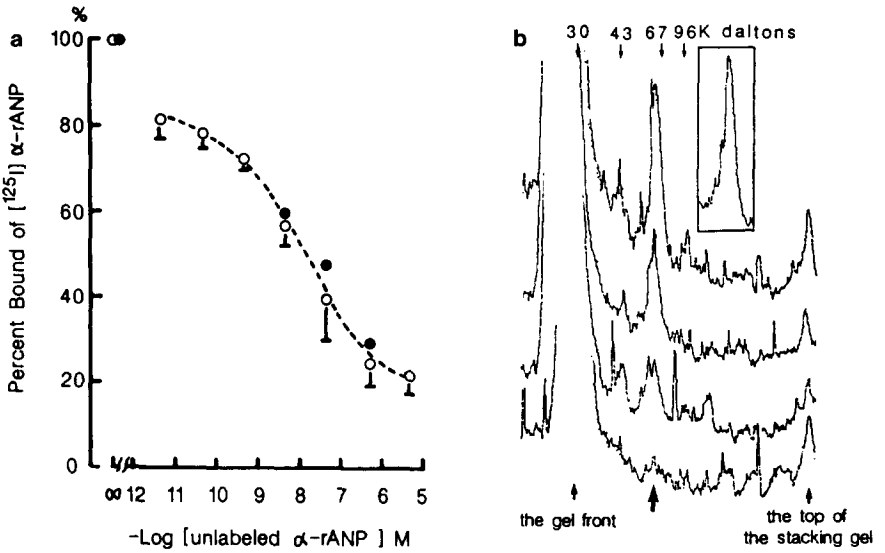

Fig.3 Displacement curve of binding of [^{125}I]α-rANP in the isolated glomerulus. (a) Percent bound of [^{125}I]α-rANP in the presence of unlabeled α-rANP was plotted against the concentration of unlabeled α-rANP (open circle). (b) Densitometry of the gel which is presented in Fig.2. The large arrow shows the band of 65,000 daltons. From the top to the bottom, densitograms are corresponding to from lane B to lane E. The inset indicates the peak of 65,000 daltons from lane F in Fig.2. When each peak area of 65,000 daltons were expressed as percentage to the peak area from Lane B (the top), the cross-linked molecule of 65,000 daltons (closed circle) was well consistent with the displacement curve as shown in Fig.3a.

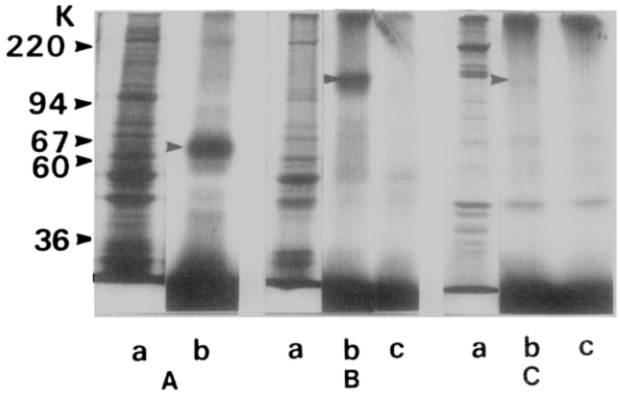

Fig.4 The autoradiogram of the photoaffinity labeled [^{125}I]α-rANP-receptor complex in the glomerulus (A), adrenal gland (B), and aorta (C). Lane a in A, B, and C is Coomassie brilliant blue staining pattern of each preparation. The samples were incubated with 4×10^{-10}M [^{125}I]α-rANP, in the absence (lanes Ab, Bb, and Cb) or presence (lanes Bc and Cc) of an excess unlabeled α-rANP, followed by the photoaffinity labeling. In the adrenal gland (lane B) and aorta (lane C), the cross-linked molecule of about 140,000 daltons is distinct from that of 65,000 daltons in the glomerulus (lane A).

clear that the data exhibit a good fit to the displacement curve, with the affinity binding 6.5×10^{-10}M. The cross-linked band of 65,000 daltons represents the binding site specific for α-rANP.

In the homogenates of the adrenal gland (Fig. 4, lane B) and the aorta (Fig. 4, lane C), the cross-linked ligand-receptor complex was observed as a band of an apparent molecular size of 140,000 daltons (lane Bb and lane Cb), which was displaced with an excess of unlabeled α-rANP (lane Bc and lane Cc). As shown in Fig. 4, lane Ab, a band of 65,000 daltons, was demonstrated in the glomerular preparation, indicating that this molecular species was not an artifact caused by cleavage of a dimer or splitting of subunits of a large molecular species. In order to exclude the possibility that the glomerular preparation contains some factors generating a small molecular species, we conducted a cross-linking study by mixing the glomerular preparation with the adrenal or aortic preparations. We could not find any increase in the amount of the smaller molecular species (data not shown).

DISCUSSION

The cross-linking method to identify receptor-ligand complexes by direct UV-irradiation has been applied for identifying receptors for various ligands

including glucagon (21), cholecystokinin (22), α-bungarotoxin (23), and cytochalasin B (24). Since this method is simple and eliminates any chemical modification of ligands which might loose their biological activity, we utilized this method to identify receptors for α-rANP in the kidney, aorta, and adrenal.

In this study, we demonstrated that the molecular size for α-rANP receptors differs depending on tissues: The components in the glomerulus and IMCT-rich fraction were 65,000 daltons, whereas those in the aorta and adrenal gland were 140,000 daltons. The latter findings are comparable to those reported by others in the bovine (14,15) and rat adrenal cortex (14), rabbit aorta (17), cultured bovine aortic smooth muscle and endothelial cells (18) and liver (16). However, our findings in the glomerulus and IMCT-rich fraction are seemingly contradictory to the observation of Yip et al. (16) that 140,000 dalton protein was labeled in the plasma membrane from rat kidney cortex. Although the origin of their membrane preparations is uncertain, it is possible that glomerular components were not included. Therefore the 140,000 dalton protein might represent the ANP-receptors in the renal cortical plasma membranes other than the glomerulus. A closer examination of their autoradiograms, however, revealed a faint band at 65,000 daltons which was displaceable with unlabeled ANP suggesting a small contamination of glomerular membranes, which include a large amount of receptors for α-rANP consisting of smaller molecular species (3,11).

It has been suggested that ANP-receptors in bovine adrenal cortex (15) and rabbit aorta membrane (17) are composed of subunits. However, it is unlikely that the ANP-binding protein of 65,000 daltons in the glomerulus and IMCT simply reflects splitted subunits, because only a single band was demonstrated in these preparations. One might argue that the kidney might contain some factors which convert all larger molecular receptors to smaller ones. This possibility was excluded by the observation that an addition of the aortic or adrenal preparation to the glomerular membrane did not increase the binding at 65,000 daltons. Since no displaceable binding of α-rANP was demonstrated at 65,000 daltons in the aortic or adrenal preparetion even in the presence of albumin, the receptor protein of 65,000 daltons in the glomerulus and IMCT is clearly not albumin.

In conclusion, the molecular species of α-rANP receptors in the glomerulus and IMCT are distinct from those of other tissues.

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