Intrarenal Localization of Receptors for α-Rat Atrial Natriuretic Polypeptide: An Autoradiographic Study with [125I]-Labeled Ligand Injected in Vivo into the Rat Aorta

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Abstract—We examined intrarenal localization of receptors for α-rat atrial natriuretic polypeptide (α-rANP) by injecting [125I]-labeled ligand in vivo into the rat aorta. We found that the receptors for α-rANP are distributed also on the vasa recta of the outer and inner medulla in addition to the previously reported sites, i.e., the renal arteries, renal pelvis, glomeruli, and inner medullary tissues including collecting tubules. In the vascular bundle of the outer medulla, the majority of grains was preferentially localized on the arterial vasa recta. The electron microscopic autoradiography of the glomerulus showed that the binding sites were mainly localized on the foot process of the podocyte. Since α-rANP injected into the aorta under physiological conditions was bound to the glomerulus and vasa recta in the kidney, the effect of ANP on these binding sites may be important in the mechanism of natriuresis.

Although atrial natriuretic polypeptide (ANP) is known to cause natriuresis by acting directly on the kidney, the sites and mechanisms of action in the kidney remain to be established. Napier et al. (1) demonstrated the specific binding sites of [125I] labeled ANP in the membrane fraction prepared from the rat kidney cortex. Subsequently, we demonstrated by in vitro autoradiography that the specific binding sites for α-rANP (28 amino acid residues) were localized on the glomeruli, renal artery, renal pelvis, and inner medullary tissues including the collecting tubule (2). More recently, some of our initial observations were confirmed in brief reports of other investigators using the similar technique (3, 4). None of these studies, however, could demonstrate the specific binding of ANP to the intrarenal vessels other than renal arteries. In order to examine detailed localization of the receptors for ANP in the glomerulus and renal vessels, we performed light and electron microscopic autoradiography by injecting the ligand in vivo into the rat aorta. The results indicate that the receptors for ANP are located also on the vasa recta of the outer and inner medulla. The electron microscopic autoradiography shows that the receptors within the glomerulus are distributed mainly on the podocyte.

Materials and Methods

Experimental protocol: Male Sprague-Dawley rats weighing 200–230 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). We administered 10−11 moles of [125I] α-rANP (specific activity: 0.4–0.6 mCi/μg), which had been prepared as previously described (2), into the aorta through a cannula inserted into the carotid...
artery (5). In order to observe the nonspecific binding, an excess of unlabeled α-rANP (10^{-9} moles) was administered two minutes before the injection of [^{125}I] α-rANP. The kidneys were washed by infusing about 10 ml of Hanks’ solution through another cannula inserted into the abdominal aorta distal to the branches of the renal arteries at 15 min after the injection of the ligand, and they were then immediately frozen in dry-ice/n-hexane or fixed with 1% glutaraldehyde followed by a postfixation with 1% osmium tetroxide. For the medullary tissue, the postfixation procedure was avoided, because osmium causes the staining of lipid droplets in the renal medulla which are prone to be misinterpreted as silver grains (6). We selected 15 min as the equilibrium period after injection of the ligand because the most prominent diuresis was observed during this period by intravenous injection of ANP in the rat (7).

Preparation of autoradiography: The cryostat sections (15 μm thickness) prepared from the kidneys were mounted on glass slides and dried. The X ray films (Cronex No. 4 E.I. Dupont de Nemours & Co., Wilmington) were apposed to the sections, exposed for 20 days at room temperature, and developed.

In order to observe the binding sites histologically at the light microscopic level, semithin sections (about 2 μm thickness) from a Spurr’s resin block of the fixed kidney were coated with nuclear track emulsion (NTB-2, Eastman Kodak Company, New York). For the electron microscopic autoradiography, ultrathin sections from the cortex were coated with emulsion (Sakura NR-H2, Konishiroku Photo Ind., Japan) diluted with fifteen times volume of distilled water. After exposure for 30–62 days, the sections were developed by a Kodak D-19 developer.

Results

Autoradiography on X ray film: Figure 1A shows a representative autoradiogram of a kidney section which was prepared after injection of [^{125}I] α-rANP into the aorta. When the kidneys were removed 5 and 10 min after the injection of the ligand, the autoradiogram was less dense, but the distribution of grains were similar to Fig. 1A. The most prominent binding of [^{125}I] α-rANP was seen in the cortex as a punctate pattern of grains. In the inner stripe of outer medulla, the longitudinally organized structures were also

Fig. 1. Autoradiogram of binding sites of [^{125}I] α-rANP in rat kidney. After 10^{-11} moles of [^{125}I] α-rANP was injected into the rat aorta, the kidney was washed, frozen, and cut into cryostat sections as described in the text. The heavy dense silver grains are seen as a punctate pattern in the cortex (A). The spokelike pattern of dense grains is observed in the inner stripe of outer medulla. When rats were administered with an excess of unlabeled α-rANP two minutes before the injection of labeled ligand, the density of grains was diminished in these sites (B).
observed along the corticomedullary axis of the kidney. Much less dense grains were distributed on the renal pelvis, cortex and inner medulla. It should be noted that no grains were observed on the outer stripe of the outer medulla, where dense nonspecific binding was observed by the in vitro autoradiography previously reported by us (2). This would indicate that the nonspecific binding of the outer stripe of outer medulla is an artifact associated with the in vitro procedure. When the 100-fold excess of unlabeled ligand was administered two minutes before the injection of the labeled ligand, the grains were diminished in all sites, except in the cortical area other than glomeruli (Fig. 1B).

**Higher resolution autoradiography of emulsion-coated kidney sections:** In the preparations coated with nuclear track emulsion, we observed under a light microscope either the silver grains or the underlying tissue by altering the plane of focus. In good agreement with our previous in vitro autoradiography (2), we demonstrated that the binding sites in the cortex were localized on the glomeruli. In the previous paper (2), we had already confirmed by reverse-phase high-pressure liquid chromatography that the bound ligand re-extracted from the isolated glomeruli was identical to the authentic αrANP.

Figure 2, A and A', show the phase contrast and dark field photomicrographs of the glomerulus in the same field. As shown in Fig. 2A', grains were distributed on the glomerular network, but not on the Bowman's capsule (the arrow), afferent and efferent arteries, or juxtaglomerular apparatus as indicated by the arrow head. In the electron microscopic autoradiograph of the glomerulus, silver grains were mainly distributed on the footprocess of the podocyte (Fig. 3A, B). When 103 grains were counted in the fields which were randomly photographed, the grains were distributed as follows: mesangial cells (15, 15%), endothelial cells (8, 8%), podocytes (67, 65%), and others (13, 12%).

The comparison between the phase contrast photomicrograph (Fig. 4A) of the vascular bundle in the outer medulla and the dark field one of the same area (Fig. 4A') reveals that the grains are localized on the vasa recta as indicated by the arrow heads. The high magnification photomicrograph of the vascular bundle taken by focusing on the midplane of section and grains showed that the grains are mainly distributed on the arterial vasa recta (Fig. 4B.). The mean values of the grains counted from the arterial and venous vasa recta were 19.1±1.7 (n=9) and 3.3±0.5 (n=9) per cell, respectively. These data indicated that the spokelike binding pattern in the inner stripe of outer medulla seen in Fig. 1A is attributed to the dense grains on the vascular bundle, preferentially on the arterial vasa recta.

In the inner medulla, much more grains...
Fig 3. Electron microscopic autoradiograms of the glomerulus (A) Autoradiogram at the electron microscopic level of the glomerulus (×8000) The grains on the foot process of the podocyte, the mesangial cell (M), and the endothelial (E) cell are indicated by the arrow heads (B) Higher magnification photomicrograph from another field of the glomerulus (×10000) The silver grains are also seen mainly on the foot process of the podocyte.

were also observed on the vasa recta, as shown in the phase contrast (Fig 5A) and dark field (Fig 5A') photomicrographs. Smaller amount of grains was observed also on the collecting tubules. However, the amount of grains was so small that it was difficult to differentiate them from the background under this condition.

Discussion

Since ANP has been shown to exist in the peripheral blood (8), it is expected to be more physiological to administer the ligand into the aorta than to apply it in vitro on the kidney slices. In addition, this procedure may

Fig 4. Autoradiogram of the vascular bundle in the outer medulla. When the phase contrast (A, ×400) and dark field (A', ×400) photomicrographs are compared with the same field, the majority of grains are localized on the vasa recta as indicated by the arrow heads. B shows a high magnification photomicrograph of the vascular bundle focused on the midplane of section and grains (×1000). The grains tended to distributed more on the arterial vasa recta. TAL thick ascending limb, TL thin loop of Henle, A arterial vasa recta, V venous vasa recta
be favorable in detecting the vascular receptors. In the present study, we succeeded to demonstrate that the vasa recta of the outer and inner medulla is also the site of the receptors for ANP in addition to the previously reported sites, i.e., the renal arteries, renal pelvis, glomeruli and inner medullary tissues including the collecting tubule (2). However, in contrast to the previous observation by the in vitro application of the ligand, the binding sites of the ligand were more predominant in the glomerulus and the vasa recta when the ligand was administered in vivo. In the vascular bundle of the outer medulla, it was demonstrated that the grains were preferentially localized on the arterial vasa recta. As in the previous paper (2), we could not detect any specific binding in the arcuate arteries, interlobular arteries, and afferent and efferent arterioles. Furthermore, it was shown in this paper that juxtaglomerular apparatus was devoid of the binding of α-rANP.

Because of the methodological limitations, we could not determine the binding kinetics of ANP to the receptors in the vascular bundles. Nevertheless, the specificity of the binding of ANP to the vascular bundle was confirmed by the observation that the pretreatment of rats with an excess of unlabeled ligand completely abolished the binding of the [125I]-labeled ligand (Fig. 1B). In our previous in vitro autoradiographic study (2), a considerable amount of nonspecific binding was observed in the outer stripe of outer medulla, whereas in the present study, no such nonspecific binding was observed in this area. This would indicate that the in vitro autoradiography might have suffered from a certain artifact, although the reason is unknown at the present time.

Since ANP has been shown to exhibit a marked vasodilating activity (9), it is reasonable to assume that ANP causes the vasodilation of the vasa recta leading to an increase in the renal papillary plasma flow as reported by Borenstein et al. (10) and Hirata et al. (11). Histotopographical studies in the rat showed that the thin descending limb of the short-loop nephron is located so that it is in close contact with the vasa recta (12). A functional study in the hamster (being analogous to the rat) showed that the descending limb of the short-loop nephron was moderately permeable to urea (13). This transport property of the descending limb, in combination with the histotopographical arrangement, is favorable for the urea recycling which is essential for the urine concentration (12, 13). Thus it is highly possible that ANP inhibits the urea recycling process by increasing the blood flow within the vascular bundle, leading to the washout of urea accumulated in the renal medulla. In fact, Hirata et al. (11) found that atrial natriuretic factor decreased urea concentration in the renal medulla. The computer simulation study by Imai et al. (14) suggest that a decrease in urea concentration gradient along the renal papillary interstitium could be associated with a decrease in sodium reabsorption along the descending limb of
the long-loop nephron. Thus the decrease in urea concentration in the renal medulla may be responsible for natriuresis by ANP. Therefore, the existence of the receptors for ANP on the vasa recta in the vascular bundle may, at least in part, play an important role in the mechanism of the natriuretic and diuretic effect of ANP.

The electron microscopic autoradiogram showed that the major binding sites of ANP in the glomerulus were the foot process of the podocyte. The observed pattern is quite different from that of angiotensin II binding where grains are mainly distributed on the mesangial cells (5, 15). Recently, Bianchi et al. (16) who reported similar findings obtained from the light microscopic autoradiography have described that ANP binding sites were localized over endothelial cells of all capillaries in the glomerulus and absent over other glomerular component cells. However, our findings based on the electron microscopic autoradiography seem to be in agreement with the observation of Cantin and Genest (17), who made a similar description in their recent review article. In fact, the identification of each of the glomerular cell components in light microscopy is not always easy and may cause some confusion as described by Osborne et al. (5). Although it is tempting to speculate that the action on the podocyte may be responsible for an increase in glomerular filtration rate caused by ANP (18, 19), we do not have any functional data to support this speculation. Therefore, the functional significance of ANP-binding to podocyte must await further investigations.

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References


