Chapter IV: Glomerular Function of the AT1a Receptor Deficient Mice

Summary

To localize angiotensin II type 1a (AT1a) receptor and to reveal the physiological roles of angiotensin II in the renal microcirculation, we investigated the AT1a gene deficient mice, generated by a targeted replacement of the AT1a receptor loci by the lacZ gene[19]. Immunohistochemical localization of β-galactosidase was performed in the heterozygous mutant mice to reveal the expression sites of AT1a. AT1a receptor (=β-galactosidase) was expressed both in the afferent and efferent arteriolar smooth muscles and also in the mesangial cells. Angiotensin II action against arterioles was directly observed using the hydronephrotic mice. Angiotensin II similarly constricted both the afferent and efferent arterioles in the wild-type and heterozygous mutant mice in a dose-dependent manner. This constriction was completely abolished by an AT1 antagonist, CV-11974. In the homozygous null mutant mice, however, angiotensin II did not affect the arterioles at all. The electron microscopy revealed that the mesangial cells made contact with the glomerular basement membrane (GBM) at the capillary neck and also with each other in the wild-type mice. However, in the homozygous null mutant mice, the mesangial cells lost the contact either with GBM or with each other and thus the capillary neck became remarkably wider. The mesangial matrix area appeared loose and enlarged, suggesting impaired mesangial matrix formation. In conclusion, via AT1a receptor, angiotensin II equally constricts both the afferent and efferent arterioles and plays an
essential role in maintaining the normal glomerular function and structure.
Introduction

Angiotensin II is a potent vasoconstricting peptide and also a growth factor to promote protein synthesis in various kinds of cells[51]. In the kidney, angiotensin II has been assumed to constrict the efferent arterioles more intensely than the afferent arterioles[52,53], so that the glomerular pressure is elevated. Moreover, angiotensin II has been considered to constrict the glomerular mesangial cells and thus to reduce the filtration surface area[4]. However, as the phenotypes of the mesangial cells is different from that of the smooth muscles[55], it is questionable that the mesangial cells are constricted by angiotensin II like smooth muscles. Therefore, in the present study, we first clarified the expression sites of angiotensin II type 1a (AT1a) receptor and, then, investigated the physiological actions of angiotensin II in the renal microcirculation.
Materials and Methods

Animals.

AT1a deficient mice generated by a targeted replacement of the AT1a receptor loci by the lacZ gene[6]. In the heterozygous mutant mice, both AT1a and β-galactosidase are expressed where AT1a is expressed in the wild-type mice. C57BL/6 mice were used as control.

Immunohistochemistry of β-galactosidase

Immunohistochemical localization of β-galactosidase was performed in the heterozygous mutant mice to reveal the expression sites of AT1a. The renal tissue was fixed by perfusion of 4% paraformaldehyde and embedded into paraffin. Localization was performed by the immunoperoxidase method[55], where the monoclonal antibody against β-galactosidase was used as primary antibody.

Angiotensin II action on renal arterioles

Using the hydrenephrotic mice, a direct action of angiotensin II on the arterioles was determined[57,58]. Hydrenephrosis was induced by permanent ligation of the left ureter 8 weeks prior to the experiments. Under Inactin anesthesia (100 mg/kg, i.p.), the ventral side of the hydrenephrotic left kidney was spread out as a thin sheet, and placed in the water bath filled with saline kept at 37 °C. Microscopic examination of the renal arterioles was performed by transillumination. Angiotensin II was administered to the water bath at concentration of $10^{-14}M$, $10^{-12}M$, $10^{-10}M$, $10^{-8}M$, and $10^{-6}M$. In another series of the experiment, an AT1 receptor antagonist, CV-11974, 10-5M was administered prior to angiotensin II. The arteriolar
Diameters were measured and analyzed (NH image 1.60, State View 4.02) after taking the video images into the computer (Power Macintosh 7500). Results are expressed as mean ± SE. Changes in arteriolar diameters were evaluated by one-way analysis of variance.

Ultrastructure of the mesangium

The kidneys were fixed by perfusion of 2% glutaraldehyde in 0.1M cacodylate buffer at pH 7 and small pieces of cortical tissue were processed by a cold-dehydration technique in order to well visualize extracellular matrices and the intracellular fibrillar structure[59,60].
Results

**Immunohistochemistry of β-galactosidase**

AT1a (=β-galactosidase) was expressed both in the afferent and efferent arteriolar smooth muscles, being more intensely in the afferent arterioles. AT1a was also abundantly expressed in the mesangial cells.

**Angiotensin II action on renal arterioles**

Angiotensin II similarly constricted both the afferent and efferent arterioles in the wild-type mice (Fig. 9a) and in the heterozygous mutant mice (Fig. 9c) in the dose-dependent manner. CV-11974 completely abolished the arteriolar constriction by angiotensin II in the wild-type mice (Fig. 9b). In the homozygous mutant mice, angiotensin II did not affect either the afferent or efferent arterioles at all (Fig. 9d).

**Ultrastructure of the mesangium**

Electron microscopy of the mesangium in the wild-type mice revealed a few mesangial cells with a small amount of mesangial matrix around them and narrow capillary necks (Fig. 8c). Mesangial cell processes anchored to the glomerular basement membrane (GBM) and thus kept the capillary necks restricted. In the homozygous null mutant mice, the mesangial enlargement was observed together with widening of the capillary neck (Fig. 8d). The mesangial matrix area was enlarged and contained loosely packed extracellular matrices. Mesangial cell processes lost their anchoring to the GBM.
Discussion

It has been assumed that angiotensin II predominantly constricts the efferent arterioles, which was shown in in vitro studies[52,53]. In vivo action of angiotensin II on the arterioles, however, has been unknown. The present study clearly showed that angiotensin II equally acts against the afferent and efferent arterioles via AT1a receptor. We utilized the AT1a receptor gene deficient mice[19]. AT1a receptor was expressed both in the afferent and efferent arteriolar smooth muscles. Although the density of AT1a receptor was rather higher in the afferent arterioles than in the efferent arterioles, angiotensin II equally constricted both the afferent and efferent arterioles dose-dependently, which was shown to be mediated exclusively via AT1a receptor.

It has been hypothesized that angiotensin II constricts the mesangial cells and reduce the filtration area so that angiotensin II regulates the glomerular filtration[54]. However, as the phenotype of the mesangial cells is different from that of the smooth muscles[55], it is unlikely that the mesangial cells is constricted like smooth muscles and play physiological roles by changing glomerular hemodynamics. On the other hand, in cultured mesangial cells, angiotensin II has been shown to promote extracellular matrix formation[51]. However, in vivo action of angiotensin II on mesangial cells has not been elucidated. We, therefore, utilized the AT1a receptor gene deficient mice in order to obtain insight into in vivo angiotensin II action on the mesangial cells. The electron microscopy revealed that angiotensin II action via AT1a receptor is essential to maintain the normal mesangial structure by promoting extracellular matrix formation and also by making the mesangial processes anchor the GBM at capillary necks[59,60].
The results of the present study show that angiotensin II equally acts against the afferent and efferent arterioles and thus regulate the glomerular hemodynamics and that angiotensin II plays essential roles in maintaining the normal glomerular structure. These angiotensin II actions are mediated exclusively by the AT1a receptor.
Fig. 8. Expression sites of AT1a receptors and ultrastructure of the glomerular mesangium. The expression of AT1a was performed by immunohistchemistry of β-galactosidase in the heterozygous mutant mice. AT1a is expressed in the afferent and efferent arteriolar smooth muscles and in the mesangial cells (a and b). The mesangial cells made contact with the GBM at the capillary neck and also with each other in the wild-type mice (c). In the homozygous null mutant mice, the mesangial cells lost the contact either with GBM or with each other and thus the capillary neck became remarkably wider (d). The mesangial matrix area was enlarged and contained a small amount of fibrillar components.
Fig. 9. Angiotensin II action on the arteriolar diameters. Angiotensin II similarly constricted both the afferent (●) and efferent (○) arterioles in the wild-type mice dose-dependently (A; N=9) and this constriction was completely abolished by an AT1 antagonist, CV-11974 10-5M (B). In the heterozygous mutant mice, angiotensin II constricted the afferent and efferent arterioles to the same extent as in the wild-type mice (C). In the homozygous null mutant mice, angiotensin II did not affect the arterioles at all (D).