Chapter I: Preface

The renin angiotensin system (RAS) plays a key role in cardiovascular homeostasis, and metabolism of fluid and electrolytes [1]. A potent vasoconstrictor octapeptide, angiotensin II (AII), also exerts numerous physiological responses including secretion of aldosterone from adrenal cortex, inhibition of renin release from the juxtaglomerular apparatus (JGA) of the kidney and development of cardiac hypertrophy and myocardial fibrosis [1]. Pharmacological study has revealed that there are two different types, AT1 and AT2, of AII receptors in mammalian, and that above responses are mainly mediated through AT1 receptors [2]. In rodents, AT1 receptors are situated as two isoforms in two different locus. Both types of recepters, designed AT1a and AT1b, are 94% identical at amino acid level and pharmacologically indistinguishable to each other [3].

Previous studies have reported that the overproduction of angiotensin II in transgenic mice carrying both the human renin and human angiotensinogen genes (Tsukuba Hypertensive Mice) leads to a sastained increase in blood pressure [4]. Furthermore, we have recently reported that a null mutation of the angiotensinogen gene displays chronic hypotension [5]. These observations indicate that the upstream components of RAS play an important role in the maintenance of blood pressure under physiological condition, which can't be compensated by other homeoststic systems such as autonomic nervous system.

In the present study, we generated mutant mice in which the AT1a receptor gene was disrupted by replacing with ß-galactosidase (LacZ) gene to clarify the cellar localization of the receptor, that is, one of downstream components of RAS. We demonstrated that the AT1a receptor localized in the mesangial cells and JGA of renal cortex and the homozygous mutant

mice display hyperreninemia and hypotension together (chapter II).

AT1 receptor is expressed abundantly in the mesangial cells of the kidney [19,20,33,39]. The administration of angiotensin II has been reported to stimulate the synthesis of components of the extracellular matrix in mesangial cells and smooth muscle cells in vitro [41,49]. Ray et al. suggested that angiotensin II binding to AT1 receptors directly stimulates fibronectin production and cellular proliferation in cultured human fetal mesangial cells, and that the AT1 receptor subtype may well be involved in the regulation of fibronectin synthesis during development and in diseases [41]. Recent studies have suggested that some of the pathophysiologic actions of angiotensin II result from its stimulation of TGF-ß expression [28, 35, 44], which has multiple effects on glomerular cell functions including modulation of the synthesis of extracellular matrix proteins [23, 26, 36]. However, these results were obtained from in vitro studies, and the in vivo action of angiotensin II on the mesangial cells via AT1a receptor has not been clarified. Indeed it is impossible to clarify in vivo actions directly. Using angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists, it is possible to suppress angiotensin II action in the tissue, but not to block it totally. Therefore, in this study, we used homozygous AT1a null mutant mice as a tool to gain an understanding of the in vivo action of angiotensin II on mesangial cells (chapter III).

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[54,60]. 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 (chapter IV).

We also investigated the expression of neuronal type nitric oxide synthase and renin in the juxtaglomerular apparatus of angiotensin type-1a receptor gene-knockout mice. Angiotensin type-1a (AT1a) receptor gene-knockout (AT1a-/-) mice exhibit chronic hypotension and renin overproduction. The synthesis and secretion of renin are thought to be regulated by physiologic parameters such as tubular sodium chloride delivery, renal perfusion pressure, and plasma level of angiotensin II [61]. The concentrations of sodium chloride at the distal tubules are monitored by the macula densa and the information is transferred to the juxtaglomerular renin-producing cells of the afferent arterioles [62-64]. Although the signal transduction mechanisms within this system are not yet well understood, it has recently been proposed that nitric oxide (NO) derived from the macula densa is an important candidate for the local intercellular mediator in the juxtaglomerular apparatus [65-72]. Histologic studies have demonstrated the localization of neuronal isoform of NO synthase (N-NOS) in the macula densa [73, 74]. When applied to the distal tubules, L-arginine enhances renin secretion from the microperfused glomerulus, and this effect is abolished by perfusing with a high-salt medium or by the blockade of NOS with NG-nitro-L-arginine [75]. In vivo studies have demonstrated a parallel increase in renal N-NOS and renin production by various experimental manipulations such as salt restriction and furosemide treatment [69, 76, 77]. Renin synthesis and secretion induced by these treatment are abolished by a selective inhibition of N-NOS by 7-nitroindazole [67, 71]. These lines of evidence suggest that NO derived from macula densa N-NOS is inversely

regulated by sodium chloride delivery at the distal tubules and participates in the stimulatory control of renin, although this notion remains controversial [78, 79]. The present study was designed to provide evidence for the possible involvement of macula densa N-NOS in the renin overproduction in AT1a-/- mice. We investigated whether the levels of renal N-NOS and renin expression are enhanced and if so, whether dietary salt loading produces a parallel decrease in these enzymes in AT1a-/- mice[45]. For comparison, the levels of renal N-NOS and renin expression in Atg-/- mice were determined (chapter V).

In human AngII receptors are separated into two major subtypes, designated as type 1 receptor (AT1-R) and type 2 receptor (AT2-R) and most of the effects of Ang II in the renal and cardiovascular systems are mediated by Ang II receptor type 1 (AT1-R) [101-103].Analyses using Northern blot [119] or in situ hybridization [115] indicated that AT2-R mRNA was not detected in the rat or mouse kidneys. However, autoradiography revealed that in the rabbit the fibrous sheath around the kidney contained AT2-R binding sites [120] and in the rhesus monkey AT2-R binding sites were present on the juxtaglomerular apparatus and vasculature in the renal cortex [106]. With respect to localization of Ang II receptor subtypes in the adult human kidney, all studies were performed on the protein level by the autoradiography using [125I]-Ang II as a ligand[108,110,111] and distribution patterns at mRNA levels have not yet been analyzed. Grone et al. [110] and Goldfarb et al. [111] demonstrated that AT1-R binding sites were predominantly present in the glomeruli, while AT2-R is the major subtype in large cortical blood vessels. In contrast, Sechi et al. reported that AT1-R is present primarily in both glomeruli and cortical blood vessels and AT2-R protein is not expressed in the human kidney [108].

In this study, we examined for the first time the cellular localization of AT1-R and AT2-

R in the adult human kidney at mRNA levels using in situ hybridization and its expression pattern determined by RNase protection assay was compared with distribution patterns in other human tissues such as atrium, liver or aorta[118]. RNase protection assays and in situ hybridization established that both AT1-R and AT2-R mRNAs were present in the human kidneys (chapter VI)