Regulation of blood pressure is a complex integrated response involving a variety of organ systems including the central nervous system (CNS), cardiovascular system, kidneys, and adrenal glands. These systems modulate cardiac output, fluid volumes, and peripheral vascular resistance, the key determinants of blood pressure. More than 40 years ago, Guyton and Coleman developed computer models of arterial output, fluid volumes, and peripheral vascular resistance, the kidneys, and adrenal glands. These systems modulate cardiac activity, the central nervous system (CNS), cardiovascular system, and kidneys and adrenal glands. The conclusion of this analysis was that regulation of sodium excretion by the kidney and consequent effects on body fluid volumes made up the critical pathway determining the chronic level of intra-arterial pressure.

The Renin-Angiotensin System and Blood Pressure Control

Our own studies of the physiology of blood pressure regulation have focused on the renin-angiotensin (Ang) system (RAS) using genetically modified mouse models. Highly conserved through phylogeny, the RAS is an essential regulator of blood pressure and fluid balance. This biological system is a multi-enzymatic cascade in which angiotensinogen, its major substrate, is processed in a 2-step reaction by renin and Ang-converting enzyme (ACE), resulting in the sequential generation of Ang I and Ang II. Along with its importance in maintaining normal circulatory homeostasis, abnormal activation of the RAS can contribute to the development of hypertension and target organ damage. The importance of the RAS in clinical medicine is highlighted by the impressive efficacy of pharmacological agents that inhibit the synthesis or activity of Ang II.

At the cellular level, responsiveness to Ang II is conferred by expression of Ang receptors. Ang receptors can be divided into 2 pharmacological classes: type 1 (AT1) and type 2, based on their differential affinities for various nonpeptide antagonists. Studies using these antagonists suggested that most of the classically recognized functions of the RAS are mediated by AT1 receptors. Gene targeting studies confirmed these conclusions. AT1 receptors from a number of species have been cloned, and 2 subtypes, designated AT1A and AT1B, have been identified in rat and mouse. The murine AT1A receptors are products of separate genes and share substantial sequence homology. AT1A receptors predominate in most organs, and the AT1A receptor is considered the closest murine homologue to the single human AT1 receptor. Understanding the physiological functions of this predominant murine AT1 receptor has been a major focus of our work.

Gene targeting using homologous recombination in embryonic stem cells provides an avenue for the direct application of precise molecular genetic interventions to the study of complex systems in whole animals. As such, it represents a powerful approach for physiological investigation. The series of studies performed using mice in which genes in the RAS have been altered by gene targeting illustrate both the feasibility and the utility of this technique for addressing physiological issues. One result of these studies was the finding that a targeted null mutation of the AT1A receptor results in a marked reduction in resting blood pressure and sodium sensitivity, reinforcing the potent capacity of the pathway of Ang II acting via the AT1A receptor in blood pressure homeostasis.

The Central Role of the Kidney in Blood Pressure Regulation

As discussed above, Guyton clearly articulated the argument for the central role for the kidney in BP control, and the relationship between alterations in systemic blood pressure and changes in renal sodium excretion is well documented. For example, an elevation in perfusion pressure in the renal artery results in a rapid increase in sodium and water excretion by the kidney, so-called “pressure natriuresis.” Based on such observations, Guyton et al suggested that whenever arterial pressure is elevated, activation of this pressure-natriuresis mechanism will cause sufficient excretion of sodium and water to return systemic pressures to normal. They further hypothesized that the substantial capacity for sodium excretion by the kidney provides a compensatory system of virtually infinite gain to oppose processes, including increases in peripheral vascular resistance, which would tend to increase blood pressure. It follows then that defects in renal excretory function would, therefore, be a prerequisite for sustaining a chronic increase in intra-arterial pressure.

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The RAS has potent actions to modulate pressure-natriuresis relationships in the kidney,20,21 and these actions shape the characteristics of RAS-dependent blood pressure regulation in normal physiology and in disease states. For example, chronic infusion of Ang II causes a shift of the pressure-natriuresis curve to the right, suggesting that when the RAS is activated, higher pressures are required to excrete an equivalent sodium load.20 Conversely, administration of ACE inhibitors or Ang receptor blockers shifts the curve to the left, meaning that natriuresis is facilitated at lower levels of blood pressure. The basic features of endogenous control of the RAS are consistent with these homeostatic functions. The system is activated at low levels of salt intake stimulating renal sodium reabsorption and conservation of body fluid volumes and blood pressure. In contrast, with high sodium intake, the system is suppressed, facilitating natriuresis.

Direct evidence for the powerful capacity of renal excretory functions to modulate blood pressure in humans is provided by the genetic studies of Lifton et al.22 In a series of elegant articles, these investigators have shown that virtually all of the Mendelian disorders with major impact on blood pressure homeostasis are caused by genetic variants affecting salt and water reabsorption by the distal nephron.22 On the other hand, this concept has been challenged recently, and several recent studies have suggested that primary vascular defects may cause hypertension by impacting peripheral resistance without direct involvement of renal excretory functions.23–26 However, in each of these studies, it is possible that renal vascular function might have been affected, consequentially modifying the pressure-natriuresis mechanism and thereby raising blood pressure through a primary mechanism involving altered salt and water excretion by the kidney.

**AT1 Receptors Are Expressed in Tissue Compartments Involved in Blood Pressure Control**

AT1 receptors are prominently expressed in organ systems with key roles in blood pressure homeostasis, including the heart, kidney, blood vessels, adrenal glands, and cardiovascular control centers in the brain.8 In the vascular system, stimulation of AT1 receptors causes potent vasoconstriction.14 In the adrenal cortex, their activation stimulates the release of aldosterone,27 thereby promoting sodium reabsorption in the mineralocorticoid-responsive segments of the distal nephron.28 In the brain, intraventricular injection of Ang II causes a dramatic pressor response mediated by AT1 receptors.29 In the kidney, activation of AT1 receptors is associated with renal vasoconstriction and antinatriuresis.30,31 However, because AT1 receptors are ubiquitously expressed, dissecting their physiological actions in individual tissue compartments including the kidney has been difficult with conventional pharmacological or gene targeting experiments.

**Kidney Cross-Transplantation Model**

As an approach for defining the physiological contributions of AT1 receptors in the kidney compared with other organ systems, we used a kidney cross-transplantation strategy to separate the actions of AT1 receptor pools in the kidney from those in systemic tissues. This approach has been used productively in previous studies to delineate the role of the kidney in various models of hypertension.32–34 In our studies, kidney transplantation was carried out between genetically matched F1(C57BL/6×129) wild-type mice and F1(C57BL/6×129) mice homozygous for a targeted disruption of the Agtr1a gene locus encoding the AT1A receptor.14 There are strong genetic modifiers on certain backgrounds including C57BL/6 that can adversely affect kidney structure in mice lacking AT1A receptors.35 However, on the F1(C57BL/6×129) background, deficiency of the AT1A receptor is not associated with abnormalities of renal structure, either in the vasculature or in the inner medulla.35 Accordingly, this model has been useful for studying the physiological functions of the AT1A receptor in the absence of potentially confounding effects of developmental abnormalities in the kidney. Except for the presence or absence of AT1A receptors, the donors and recipients are genetically identical, so there is no rejection and no need for immunosuppressive therapy.

By varying the genotype of the transplant donor and recipient, we generated 4 groups of animals shown in Figure 1. In this model, both native kidneys were removed so that renal function was provided entirely by the single transplanted kidney. The wild-type group consisted of wild-type mice transplanted with kidneys from wild-type donors and, therefore, expressing AT1A receptors normally in the kidney transplant and in all systemic tissues. For the systemic knockout (KO) group, AT1A receptor-deficient recipients were transplanted with kidneys from wild-type donors; these animals lacked AT1A receptors in all of the tissues except the kidney. Kidney KO animals were wild-type recipients of AT1A receptor-deficient kidneys, thus lacking expression of AT1A receptors only in the kidney but with normal expression of receptors in all of the systemic, nonrenal tissues including the adrenal gland. Finally, the total KO group consisted of AT1A receptor–deficient recipients of AT1A receptor–deficient kidneys and, therefore, were completely lacking AT1A receptors in all of the tissues.36
Distinct Roles for AT$_1$ Receptors in the Kidney and Systemic Tissues in Blood Pressure Regulation

We first examined the consequences of removal of AT$_{1A}$ receptors from the kidney or from systemic tissues on basal blood pressure control. Absence of AT$_{1A}$ receptors from the kidney alone in kidney KO mice was sufficient to reduce blood pressure by $\approx 20$ mm Hg (Figure 2), despite normal expression of receptors in all of the other tissues. These findings indicate that renal AT$_{1A}$ receptors have unique and nonredundant actions in blood pressure homeostasis. Aldosterone levels are unaffected in the kidney KO animals, indicating that blood pressure is regulated by direct effects of AT$_1$ receptors on kidney cells. We concluded, as predicted by Guyton’s hypothesis, that this reduction in blood pressure was a direct consequence of interrupting AT$_1$ receptor actions in the renal vasculature and/or renal epithelia that would otherwise promote renal sodium reabsorption and reduce urinary sodium excretion. This was supported by our finding that reduced blood pressures in the kidney KO mice could be substantially reversed by feeding a high-salt diet.

Although our findings in the kidney KOs supported a critical role for the kidney in regulation of blood pressure, we found that systemic KO mice lacking AT$_{1A}$ receptors in extrarenal tissues, but with the normal complement of receptors in the kidney, also have blood pressure reductions of $\approx 20$ mm Hg (Figure 2). Thus, AT$_1$ receptors outside the kidney also make a unique contribution to blood pressure homeostasis that is virtually equivalent in magnitude and independent of intrarenal actions of Ang II. We considered the possibility that alterations of aldosterone release because of the absence of AT$_1$ receptors in the adrenal gland and/or reduced renal nerve activity as a consequence of the lack of AT$_1$ receptors in the CNS might be responsible for the low blood pressures in systemic KO mice. Either of these possibilities would be consistent with a pathway involving control of renal sodium handling, in this case affected by alterations in aldosterone levels and/or renal nerve activity, as the final common pathway for chronic blood pressure homeostasis.

Indeed, urinary aldosterone excretion was reduced by $\approx 50\%$ in systemic KO mice. However, after adrenalectomy and clamping aldosterone at supraphysiological levels while also infusing glucocorticoids, blood pressures remained lower in systemic KO mice than in similarly manipulated wild-type mice, indicating that reduced aldosterone levels do not explain the reduced blood pressures. Moreover, norepinephrine contents in the transplanted kidneys were markedly and similarly reduced in both systemic KO and wild-type mice, suggesting that altered renal nerve activity also could not explain the reduced blood pressures in the systemic KO mice. This finding also suggested that significant reinnervation of the transplanted kidneys had not occurred over the period of study. Therefore, the effects of neural pathways emanating from AT$_{1A}$ receptors in the CNS to modulate renal nerve activity are likely underestimated in our model. Based on these results, we speculated that the major actions of extrarenal AT$_1$ receptors to regulate blood pressure in our model are likely to be a consequence of effects on the vasculature. These might be direct actions of AT$_{1A}$ receptors on vascular smooth muscle cells or indirect effects of AT$_{1A}$ receptors in the CNS to modulate vascular resistance.

Our finding that elimination of extrarenal AT$_1$ receptors alone is sufficient to lower resting blood pressure seems to contradict Guyton’s hypothesis. However, it is clear that the renal-pressure-natriuresis mechanism is reset to a lower blood pressure in mice without extrarenal AT$_1$ receptors. If this were not the case, the reduced blood pressure would cause continued sodium retention. The mechanism responsible for this resetting does not seem to be a direct effect of Ang II on the kidneys, nor an effect mediated by the CNS or by aldosterone, and remains to be identified.

In any case, an intact response to Ang II in the kidney was not sufficient to compensate for the absence of receptors in vasculature and/or the CNS. However, in the mouse, the RAS has a much more important role in normal maintenance of blood pressure than in humans or even rats. For example, administration of an ACE inhibitor or Ang receptor blocker to euvoolemic humans or rats typically has little effect on blood pressure. On the other hand, administration of these agents to a euvolemic mouse causes a marked fall in blood pressure, similar to that seen when key RAS genes such as Agtr1a are eliminated by gene targeting. Thus, the RAS in the mouse appears to normally operate near its maximal compensatory range, similar to the circumstance of a salt-depleted human or rat. Accordingly, our findings indicate that when circulatory volumes are threatened, the full range of AT$_1$ receptor actions at key tissue sites are activated and apparently necessary to protect against circulatory collapse. Their relative contributions might be quite different in hypertension.

Ang II Causes Hypertension Through AT$_1$ Receptors in the Kidney

With regard to basal blood pressure homeostasis, our studies demonstrated that AT$_1$ receptor actions in the kidney and
extrarenal tissues made virtually equivalent contributions to preventing hypotension and supporting normal blood pressure. Thus, when circulatory volumes are threatened, the full range of AT₁ receptor actions at key tissue sites are activated and apparently necessary to protect against circulatory collapse. On the other hand, we reasoned that these relative roles of renal and extrarenal AT₁ receptors might be quite different in hypertension. Accordingly, we next used the kidney of renal and extrarenal AT₁ receptors were only minimally affected by Ang II infusion (\( \text{P}\times 0.03 \) vs wild-type; \( \text{SP}<0.008 \) vs systemic KO; \( \text{TP}<0.006 \) to 0.0001 vs wild-type; from Crowley et al[43]).

As shown in Figure 3, mean arterial pressure in the wild-type transplant group rose dramatically on initiation of Ang II infusion to \( \approx 160 \) mm Hg and remained elevated throughout the infusion period.\(^43\) By contrast, blood pressures in the total KO animals that are completely devoid of AT₁ receptors were only minimally affected by Ang II infusion (Figure 3), reflecting the key role of AT₁ receptors in the development of hypertension in this model. The degree of hypertension was markedly attenuated in the kidney KOs compared with the wild-type group. Despite the early and transient increase in blood pressure presumably because of peripheral vasoconstriction, the absence of AT₁ receptors in the kidney alone was sufficient to protect from Ang II–dependent hypertension despite their full complement of AT₁ receptors in the brain, heart, systemic vasculature, and adrenal glands. In contrast, in the systemic KO animals expressing AT₁A receptors only in the kidney, mean arterial pressure rose progressively over the first 2 weeks of Ang II infusion. By day 12, blood pressures in the systemic KOs converged with and were virtually identical to the wild-type group. Thus, the presence of AT₁A receptors in the kidney alone in systemic KOs is sufficient to recapitulate the hypertension phenotype of the wild-type group. Together, these data show that Ang II causes hypertension primarily through AT₁A receptors expressed in the kidney.

The mechanism for the distinct blood pressure responses in the 2 groups appears to be related to differences in renal sodium handling. Ang II infusion is associated with reduced renal sodium excretion and weight gain in the systemic KO group, whereas the absence of renal AT₁ receptors in the kidney KOs is associated with enhanced natriuresis, no change in weight, and resistance to hypertension.\(^43\) Moreover, administration of a low-salt diet to systemic KO animals during the Ang II infusion period dramatically attenuates the hypertensive response (authors’ unpublished data). Importantly, these effects on blood pressure and sodium excretion are determined by direct actions of AT₁ receptors within the kidney, independent of any contribution of Ang II–dependent aldosterone release.\(^43\)

**Cardiac Hypertrophy Follows Hypertension, Not AT₁ Receptors in the Heart**

A major goal of hypertension treatment is to prevent or ameliorate injury to key target organs, including the heart, kidney, and brain. One of the most prevalent manifestations of end-organ damage in hypertension is the development of left ventricular hypertrophy (LVH)\(^44\) and its presence confers substantial cardiovascular risk.\(^44,45\) Although pressure load from elevated blood pressure clearly contributes to LVH, several lines of evidence suggest that activation of the RAS also plays a role. For example, Ang II, acting through AT₁ receptors, stimulates hypertrophy of cardiac myocytes in culture.\(^6,47\) In addition, clinical studies have demonstrated actions of ACE inhibitors and Ang receptor blockers to cause regression of LVH more effectively than other classes of antihypertensive agents with apparently similar levels of blood pressure control.\(^48–50\)

Although distinguishing the relative contributions of hypertension and cardiac AT₁ receptor activation to the development of LVH in vivo has been difficult, we reasoned that our model might be useful for this purpose. The extent of blood pressure elevation was very similar in the wild-type and systemic KO groups, but they differ in their expression of AT₁A receptors in the heart. As shown in Figure 4, although they lack cardiac AT₁A receptors, the systemic KO group developed robust LVH with heart weights that were not significantly different from those of the wild-type group. By contrast, the kidney KO group with a full complement of cardiac AT₁A receptors did not develop significant hypertension with chronic infusion of Ang II and, likewise, had no appreciable change in their heart weights. Across the different experimental groups, there was a tight linear correlation between heart weight and blood pressure irrespective of the presence or absence of cardiac AT₁A receptors.\(^43\)Thus, in a simple model of Ang II–dependent hypertension, the severity...
of cardiac hypertrophy was exclusively dependent on blood pressure. We found no evidence for a contribution of direct actions of AT1 receptors in the heart to promote LVH. Although our data are clear cut, they do not necessarily obviate the results of well-designed prospective clinical trials demonstrating beneficial effects of ACE inhibitors or Ang receptor blockers on regression of LVH.\textsuperscript{49,50} Rather, they suggest that the benefits of these agents in LVH cannot be explained by inhibition of cellular actions of Ang II in the heart, but instead may be because of differences in the degree or pattern of blood pressure control that was achieved.\textsuperscript{51,52}

**Conclusions**

We have combined gene targeting with renal cross-transplantation to examine the role of AT1 receptors in the kidney and their contribution to blood pressure regulation and to the development of hypertension. With regard to normal blood pressure homeostasis, we find distinct and virtually equivalent contributions of AT1 receptor actions in the kidney and in extrarenal tissues to determine the level of blood pressure. The contribution of extrarenal AT1 receptors cannot be explained by altered aldosterone generation or changes in renal nerve activity, suggesting that AT1 receptor actions in systemic tissues, such as the vascular and/or central nervous systems, make nonredundant contributions to blood pressure regulation. In hypertension, the population of AT1 receptors in the kidney assumes a pre- eminent role. Our studies show that Ang II causes hypertension primarily through effects on AT1 receptors in the kidney associated with reduced urinary sodium excretion, independent of actions of the sympathetic nervous system or aldosterone. We also find that renal AT1 receptors are absolutely required for the development of Ang II–dependent hypertension and cardiac hypertrophy. When AT1 receptors are eliminated from the kidney, the residual repertoire of systemic, extrarenal AT1 receptors is not sufficient to induce hypertension or cardiac hypertrophy. Our findings highlight the critical role of the kidney in the pathogenesis of hypertension and its cardiovascular complications. In addition, they suggest that the major mechanism of action of RAS inhibitors in hypertension is attenuation of Ang II effects in the kidney. Finally, our present findings confirm the critical role of altered renal sodium handling in Ang II–dependent hypertension and are completely consistent with Guyton’s hypothesis.\textsuperscript{19}

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**Disclosures**

None

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Kidney in Hypertension: Guyton Redux
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