# Contribuição Internacional

# The renin-angiotensin system in hypertension: a review of studies using transgenic animals

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**Abstract**

Essential hypertension is an extremely prevalent disorder in western societies and thus, has received a great deal of attention by the research community. Hypertension is a polygenic and multi-factorial disorder, and transgenic animals have been very helpful in studying specific systems and their implication in hypertension. Because the renin-angiotensin system has a strong impact on the control of blood pressure both in the short and long term, it has been one of the most extensively studied physiological systems. Nevertheless, despite decades of research, the specific mechanisms implicated in its action on blood pressure and electrolyte balance, as well as its integration with other cardiovascular pathways remains incomplete. Recently, the production of transgenic models either over-expressing or knocking-out specific components of the renin-angiotensin system has given us a better understanding of their role in hypertension. In addition, recent attention has turned from the endocrine to local tissue renin-angiotensin systems and their physiological effect on blood pressure and end-organ damage. Herein, we will review studies using genetic manipulation of animals to determine the role of the endocrine and tissue renin-angiotensin system in hypertension.

**Keywords:** Hypertension; Renin-angiotensin system; Transgenic animals; Gene targeting.

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## The renin-angiotensin system

The renin-angiotensin system (RAS) consists of an enzymatic cascade in which renin, an aspartyl protease, cleaves angiotensinogen to form the decapeptide angiotensin I (Ang I). Ang I is further cleaved by angiotensin-converting enzyme (ACE), a dipeptidyl carboxypeptidase, to produce the octapeptide angiotensin II (Ang II), the physiologically active component of the system. The actions of Ang II are stimulated by its binding to specific receptors (AT-1 and AT-2), classified by their differential affinities for various nonpeptide antagonists[1]. Both of these cell surface receptors belong to the large family of G-protein associated receptors. These receptors have a wide tissue-specific distribution, and are both present in the kidney, brain and adrenal gland. In general, AT-1 receptors are

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present in adult cardiovascular tissues whereas AT-2 is highly expressed during fetal development[2].

Pharmacological studies using specific antagonists have determined that most of the physiological actions of Ang II are mediated through the AT-1 receptor[1]. Two subtypes of this receptor, AT-1a and AT-1b, have been identified in the rat[3], mouse[4] and an AT-1b receptor has been reported in humans[5], although it is generally accepted that humans express only one type of AT-1 receptor. These receptor subtypes are pharmacologically indistinguishable but are the product of different genes (Agtr1a and Agtr1b) that are differentially expressed and regulated[6,7]. Gene-targeting experiments have been useful to determine the individual role of the AT-1a and AT-1b in the periphery[8,9] and in the central nervous system[10]. Indeed, it has been shown that AT-1a receptors are predominantly involved in the regulation of vascular tone in the periphery as well as the pressor response in the central nervous system (CNS), while the AT-1b receptors seem to be necessary for the drinking response to Ang II in the CNS. On the other hand, AT-2 receptor function has not yet been fully determined. Recent studies have suggested that it might oppose the actions of the AT-1 receptor with respect to blood pressure and cellular proliferation[11-13]. It has also been suggested that AT-2 receptor stimulation enhances renal tubular sodium reabsorption[14].

## Transgenic animals to model the endocrine RAS

### The TGR(mREN2)27 transgenic rat

One of the most extensively studied transgenic models is the TGR(mREN2) transgenic rat which expresses the murine Ren-2 gene cloned from the DBA/2J mouse strain[15]. They chose the mouse Ren-2 renin gene because it had already been characterized in transgenic mice and was expected to be highly expressed in certain tissues[16]. As expected, these rats highly express the transgene in the adrenal gland, and at lower levels in the thymus, small intestine, testis, ovary, coagulation gland, kidney, brain, lung, blood vessels, pituitary and thyroid[17]. Because transgene expression in the kidney, adrenal gland and several brain areas precedes the development of hypertension, it has been suggested that there may be a causal relationship between expression of renin in those tissues and the development of hypertension[18]. Interestingly, Ren-2 expression is down-regulated in kidney, medulla oblongata, and cortex, but not in the adrenal gland and hypothalamus. Surprisingly, circulating levels of the different components of the RAS are normal or in some cases suppressed in heterozygous TGR(mREN2)27 rats as compared to their negative littermates. The only exception is markedly elevated levels of prorenin, the inactive form of renin[19]. The development of hypertension in these mice is clearly due to the over-activity of RAS since they can be treated by ACE inhibitors and AT-1 receptor antagonist[17]. These data suggest the possibility that the primary cause of hypertension in this model may not be due to increased endocrine RAS, but possibly the result of high tissue-specific RAS activity.

Recently, high levels of renin have been detected in the amniotic fluid of pregnant TGR(mREN2)27 rats[20]. Moreover, low birth weight in the resultant offspring was also reported. It is interesting to note that epidemiological studies have lead to the hypothesis that reduced birth weight increases the likelihood of cardiovascular disease later in life. Also of interest in these animals is a marked sexual dimorphism with respect to the degree of hypertension. The effects appears to be mediated by androgens since either castration of males or treating females with androgens can abolish this effect[21]. It has also been suggested that estrogen may be protective against hypertension by amplifying the vasodilator contributions of angiotensin (1-7) while reducing the formation and vasoconstrictor actions of Ang II[22].

One particularly interesting observation is that the severity of hypertension depends partly upon the genetic background of the rats used for maintaining the TGR(mREN2)27. An accelerated and malignant form of hypertension occurs when these rats are bred with a specific strain of Sprague-Dawley rat, but does not occur if Lewis rats are used for breeding[23]. Because they were able to exclude many environmental factors, this suggests that even when a specific genetic modification leads to hypertension, there are other genes in the background which can modify the phenotype. Such genetic background effects have been observed in numerous transgenic and knockout models[24].

Similar to the TGR(mRen2)27 rats, transgenic rats having an inducible form of hypertension using the same mouse Ren-2 gene but driven by the cytochrome P450 promoter have been developed[25]. In these rats, the transgene is expressed primarily in the liver and is rendered inducible by xenobiotics such as indole-3 carbinol. The hypertension developed is dose-dependent and reversible. Indeed, continuous dietary administration of indole-3 carbinol caused a marked hypertension, increase in the RAS activity, and clinical manifestations of malignant hypertension such as polyuria and weight loss. This model could thus be useful to study the initiation

events of pathological processes such as hypertension. Indeed, if the time of onset and the extent of hypertension can be controlled, then the cellular and molecular events involved in the initiation of the vascular and organ injury can be more easily determined. The two main limitations of this model are the insertion of the transgene in the Y chromosome, and the ectopic production of renin in the liver.

## Transgenic models expressing the human renin and angiotensinogen gene

In order to try to better emulate human hypertension, many transgenic models have used the insertion of human renin (hRen) and/or angiotensinogen (hAGT) in the mouse or rat genome. Since the renin-angiotensinogen reaction is species-specific, human renin cannot cleave mouse or rat angiotensinogen and vice versa. Consequently, both the renin and the AGT genes are required from the same species. In hAGT transgenic rats, acute and 10-day infusion of recombinant human renin caused a significant increase in blood pressure[26]. These rats express high levels of hAGT in the liver, brain, kidney, gastrointestinal tract, and aorta whereas rat angiotensinogen can be detected in the liver and brain. Similarly, in double transgenic rats (dTGR) expressing both hRen and hAGT genes, the hypertension observed is dependent on the human RAS components[27]. In these models, both hRen and rat renin are physiologically regulated and thus, are expressed at low levels, probably as a result of the feedback inhibition caused by high blood pressure and Ang II. Long-term treatment with an ACE-inhibitor and AT-1 receptor antagonist caused an increase in renin levels in parallel with the decrease in blood pressure[28]. This is in contrast to studies done in the TGR(mREN2)27 where there is no affect of ACE inhibitors on renin levels despite a concomitant normalization of the blood pressure[29]. Endogenous rat angiotensinogen expression in the kidney was decreased, and both rat and hAGT are increased by treatment with ACE inhibitor and AT-1 receptor antagonist[28]. This in agreement with previous studies suggesting that angiotensinogen is regulated by both Ang II and blood pressure in the kidney[30].

The hypertension that developed in the double transgenic animals greatly depends on the Ang II-induced reduction in sodium and water excretion, which is intrinsic to the kidney[31]. Both submaximal doses of ACE inhibitor and AT-1 receptor antagonist cause a significant decrease in blood pressure while the use of both types of drugs together totally normalizes blood pressure[28]. In this model, ACE inhibitors seem to decrease blood pressure by increasing sodium excretion through increased renal blood flow and glomerular filtration, rate while AT-1 receptor blockers seem to decrease tubular sodium and water reabsorption.

Interestingly, during the breeding of the hAGT to the hRen rats, female hAGT rats developed hypertension on the fifth day of pregnancy[32]. Indeed, the hRen transgene is expressed in the placenta, which causes a significant increase in plasma hRen. However, female hRen transgenic rats bred with male hAGT exhibited a decrease in blood pressure, and no plasma hAGT could be detected in these rats. Similar results have been observed in female hAGT transgenic mice that are mated with male hRen mice[33]. Thus these animals may be good models for the study of preeclampsia. Recently a novel mouse model of genetic preeclampsia was described[34].

In addition to rats, many mouse models expressing human components of the RAS have been developed. For instance, double transgenic mice overexpressing hAGT and poorly regulated human renin transgenes (3 kb-hRen or 0.9 hRen transgenes) are markedly hypertensive, have elevated levels of plasma Ang II and have many hallmarks of hypertensive-induced end-organ damage including endothelial dysfunction[35-39]. When hAGT mice were mated with mice expressing larger and better regulated 140 kb or 160 kb hRen transgenes encoded on P1 artificial chromosomes, plasma Ang II levels and blood pressure were only moderately elevated[40]. Indeed, hREN expression in these, and other similarly generated mice, was restricted to the kidney, and was appropriately regulated by physiological stimuli such as ACE inhibition, Ang II infusion and high salt diet[40,41]. Therefore, there is compensatory down-regulation of hRen mRNA in these double transgenic mice, which would account for the lower blood pressures observed in them. Contrary to these findings, double transgenic model containing the same human angiotensinogen gene and a 45 kb-hRen transgene were reported to have normal blood pressure[42]. The authors suggested that the normal pressure measured in these mice was due to the appropriate regulation and expression of the renin transgene. Indeed, similar to the PAC hRen mice, the 45 kb-hRen transgenic mice exhibit transgene expression only in the kidney, and expression responds adequately to physiological stimuli. It is possible that the authors did not observe an increase in blood pressure due to their method of measurement. A tail-cuff sphygmomanometer, rather then direct measurement by indwelling catheter, was used in this study to assess blood pressure. In our laboratory, only direct measurement of blood pressure uncovered the modest increase in blood pressure observed in the PAC double transgenic

mice while no difference could be detected by tail-cuff[43].

Although renin is regulated appropriately in the double transgenic mice containing the PAC hRen transgene, a modest increase in blood pressure can still be observed. It has been suggested that this might be due to the high levels of hAGT present in these mice, and that even small amounts of hRen may be sufficient to cleave this additional angiotensinogen. This is further supported by studies where transgenic mice containing three and four copies of the endogenous angiotensinogen gene caused a small increase in circulating mouse angiotensinogen which was sufficient to cause significant increases in blood pressure[44]. Hence, individuals with elevated angiotensinogen levels might be predisposed to hypertension. Genetic evidence suggests that patients carrying a variant of the angiotensinogen gene may have elevated circulating angiotensinogen and hypertension[45]. The same variant was reported to be linked or associated with preeclampsia[46].

## RAS gene knockout models

Many genes of the RAS have been knocked out in transgenic animals. For instance, the complete absence of angiotensinogen[47,48], ACE[49,50] or AT-1a receptors[51,52] causes a significant decrease in blood pressure. In the case of ACE, it seems that specific knockout of the tissue-bound ACE without alteration in the circulation ACE also causes similar decreases in blood pressure[53]. These results support the notion that tissue-bound ACE is critical in the physiologic generation of Ang II, and are in agreement with studies showing a lack of correlation between the hypertensive effect of ACE inhibitors and circulating levels of ACE[54]. Because the decrease in blood pressure in the AT-1a knockout is similar to that of the angiotensinogen and ACE knockout, it has been suggested that most of the effects on blood pressure that Ang II exerts is mediated by the AT-1a receptors.

Mice lacking the AT-1b receptor have normal blood pressure and thus it was suggested that this receptor was not implicated in the regulation of resting blood pressure[9]. However, studies conducted on the AT-1a knockout mice uncovered a small pressor response to Ang II infusion that could be completely blocked by losartan, an AT-1 receptor antagonist[51]. This suggested that the pressor effect of Ang II in these mice was mainly due to stimulation of the AT-1b receptor. As indicated above, using AT-1a and AT-1b receptor deficient mice, we demonstrated that AT-1a receptors mediate the central pressor effect of Ang II, while AT-1b receptors mediate the central dipsogenic effect of Ang II[10].

In contrast, a modest elevation in blood pressure in mice lacking the AT-2 receptor has been reported[11]. AT-2 receptor deficient mice also exhibited an increase sensitivity of blood pressure to Ang II infusion[13,55]. These observations provide further support for the notion that AT-2 receptors counteract the effects of the AT-1 receptor. These effects may be important during AT-1 receptor blockade, when renin and Ang II dramatically increase. Indeed, studies done in AT-1 knockout mice have demonstrated that captopril administration can cause an increase in blood pressure, presumably by preventing the production of Ang II which would bind and activate AT-2 receptors[52]. However, Ang II infusion in these mice did not cause a decrease in blood pressure as might be expected, and thus the role of AT-2 receptors remains controversial and unclear.

Complete absence of individual RAS components can have profound effects on blood pressure and cause lethality thus limiting the use of homozygous knockout mice. However, heterozygous knockouts can provide important tools for the study of blood pressure regulation. For instance, mice that are heterozygous for null mutations in the angiotensinogen[44] or AT-1a receptor[56] genes have reduced blood pressure, although not as drastic as in the homozygous knockouts. Furthermore, insertion of a functional angiotensinogen gene into the angiotensinogen knockout mice restores blood pressure[57,58]. In contrast, heterozygous ACE knockout mice, which have a 50% reduction in ACE activity, do not exhibit a decrease in blood pressure[49]. Similarly, increasing ACE levels by increasing the number of functional ACE gene copies does not affect blood pressure[59]. These authors thus suggested that arterial pressure was not influenced by partial reduction in ACE levels, which is consistent with the common notion that ACE is not rate limiting in the RAS. However more recently, Carlson et al. observed a copy-dependent decrease in blood pressure in mice with complete or partial deletion of the ACE gene using radiotelemetry measurements of blood pressure[60]. These results might again, be due to the higher sensitivity of the technique used in this study. In previous studies, tail-cuff[50] or acute catheterization[61] were used to measure blood pressure. Both of these methods require restraints, heating, and/or tethering and are typically used to measure blood pressure only during the day, the normal rest (sleep) period for the mouse, when arterial pressure is the lowest. Thus, these methods might not be able to uncover a smaller variance in blood pressure that might occur during the nocturnal period when the mice are

awake, active and have higher blood pressure.

## Local RAS transgenic animals and hypertension

Typically, the classic effects of the RAS, such as increased blood pressure and blood volume have been thought to be due to the systemic RAS, where circulating renin processes circulating angiotensinogen to ultimately generate blood borne Ang II, which acts as an endocrine hormone. However, in the last few years, more attention has been brought to local RAS. Tissue RAS are defined as existing in tissues with the capacity for the local generation and action of Ang II. All components of the RAS can be found in the brain, heart, vasculature, and kidney among others, and could be implicated in the specific effects that have been attributed the systemic RAS. For instance, it has been suggested that the intrarenal RAS can regulate systemic blood pressure and aspects of renal function such as blood flow and sodium reabsorption[62], whereas in brain it may facilitate neurotransmission and stimulate vasopressin release and sympathetic outflow[63,64].

### Targeting the brain

Our laboratory has produced models to examine the effect of an intrinsic RAS in the brain, both in neurons[65] and glia[66], and in kidney[67,68]. There has been substantial interest in the brain RAS because of evidence implicating its contribution to the hypertensive state in many animal models such as the spontaneously hypertensive rat (SHR), DOCA-salt hypertensive rat, Dahl-salt sensitive rat, and renal hypertensive rat[69,70]. For instance, acute and chronic intracerebroventricular (ICV) injection of an ACE inhibitor, angiotensin antagonist, and antisense oligonucleotides to AT-1 receptors or angiotensinogen mRNA attenuates the development of hypertension in SHR[71,72]. Also, we have reported a significant decrease in blood pressure with ICV injection of losartan, an AT-1 receptor antagonist, in double transgenic mice expressing both hRen and hAGT[73,74]. This effect seems to be mediated, at least in part, by an increase in vasopressin since a bolus injection of AVPX, an arginine vasopressin inhibitor, caused a greater decrease in blood pressure in the double transgenic mice compared to control mice, whereas hexamethonium, a ganglionic blocker, caused an equal decrease in both double transgenic and control mice. Similarly, decreases in blood pressure have been observed in TGR(mRen2)27 rats with microinjections of CV-11974, another AT-1 antagonist, into the RVLM[75].

To better assess the role of primary production of renin and angiotensinogen in the brain, we developed transgenic mice expressing hAGT and/or hRen driven by either the synapsin I (SYN I) promoter, a neuronal promoter, or the glial fibrillary acidic protein (GFAP) promoter, a glial promoter. Transgene expression in the GFAP-hAGT mice occurred mainly in astrocytes in the brain, but hAGT could also be detected in neurons in the subfornical organ[66]. When crossed with mice expressing hRen systemically, a 15 mmHg increase in blood pressure was observed. In addition, these double transgenic mice exhibited an increased preference for drinking saline. These results are in accordance with studies done in transgenic rats expressing an antisense RNA against angiotensinogen mRNA driven by the GFAP promoter, TGR(ASrAOGEN), where a significant decrease in blood pressure was observed[76]. We also produced transgenic mice expressing hRen under the control of the GFAP promoter (GFAP-hRen)[77]. These transgenic mice expressed hRen in the brain, specifically in glia, with some ectopic expression in lung and adipose tissue, but no detectable plasma hRen. When these mice were bred with GFAP-hAGT mice, the double transgenic animals had an increase in blood pressure and an increase in drinking volume and salt intake. The increase in blood pressure observed was normalized by ICV injection of losartan while the same dose given IV (intravenous) had no effect. This suggests that the observed increase in blood pressure was due to the local production and action of Ang II in the brain. This pressor effect may be mediated by an increase in sympathetic activity, since hexamethonium, a ganglionic blocker, caused a greater fall in blood pressure in the double transgenic mice than negative littermates. This is in contrast with our previous study using the systemic hAGT and hRen double transgenic mice where blood pressure was significantly lowered by AVP receptor blocker[73]. Taken together, these data suggest that the mechanisms underlying increased blood pressure may differ among models. Of course, it is possible that vasopressin release, and therefore the response to vasopressin receptor blockade, is blunted in the glial double transgenic mice due to negative feedback caused by the increased drinking.

We also produced Syn I-hAGT transgenic mice, which expressed the transgene highly in the brain, at low levels in the kidney and heart, but had no detectable plasma angiotensinogen[65]. The hAGT was present solely in neurons in the brain. A pressor response could be observed in these mice with ICV, but not IV, injection of purified hRen. This response could be prevented by ICV injection of losartan which suggested an AT-1 receptor-

dependent role of neuron-derived AGT in the regulation of blood pressure. In contrast, when these mice were bred with the systemic hRen or the PAC hRen mice, no increase in blood pressure could be observed, although they had an increased salt-preference. It is possible that this is due to low levels of hRen in critical blood pressure regulation centers in the brain present in both the systemic and PAC hRen mice. In contrast, when the Syn I-hAGT mice were bred with Syn I-hRen mice they were moderately hypertensive as well as had increased drinking volume and salt preference[77].

Recently, we and others have reported an altered form of renin mRNA derived from the utilization of an alternative transcription start site in the brain[78,79]. If translated, this mRNA would encode an intracellular (non-secreted) and constitutively active form of the protein suggesting the possibility of an intracellular pathway of Ang II production in the brain. Studies are presently underway to examine the regulation of blood pressure and fluid homeostasis in new transgenic models expressing this intracellular form of renin driven by either the GFAP or Syn I promoters.

Recently, a transgenic mice model over-expressing the rat AT-1a receptor driven by the neuron-specific enolase promoter (NSE-AT1a) was reported[80]. Although these mice had normal resting blood pressure, they exhibited an increased sensitivity to ICV Ang II. Central AT-1 blockade caused a significant decrease in blood pressure in the NSE-AT1a mice but had no effect on their negative littermates. This suggests that although there seems to be increased AT-1 contribution to blood pressure regulation in these transgenic mice, particularly effective baroreflex buffering might prevent hypertension in this model.

## Targeting the kidney

Our laboratory has also produced a kidney-specific model of hypertension. We produced transgenic mice expressing hAGT driven by the kidney androgen-regulated protein (KAP) promoter, which is expressed specifically in the kidney proximal tubule and is very androgen responsive[68]. Elevated hAGT was observed in urine reflecting its elevated production in proximal tubule cells and its release into the tubular lumen. Double transgenic mice expressing KAP-hAGT and systemic hRen had increased blood pressure but normal circulating Ang II levels[67]. The increase in blood pressure could be induced in the female double transgenic by treatment with testosterone. Presumably, high concentrations of Ang II were present in tubular fluid. Accordingly, the increased blood pressure could be reduced by the use of high concentrations of losartan, whereas low concentrations of losartan effectively lowered blood pressure in the systemic hAGT/hRen double transgenic mice. This observation is consistent with the filtering actions of the kidney, where only a fraction of the blood is filtered during a single pass through the nephron. Thus, it is possible that an effective intratubular concentration of losartan may have been reached with higher concentrations. Ang II has direct effects on sodium transport in the early nephron by stimulating sodium-hydrogen exchange in proximal tubule, and indirect effects in the late nephron by regulating synthesis of epithelial sodium channels by aldosterone[81]. This supports the hypothesis that hypertension in these mice may be caused by alterations in sodium or fluid homeostasis, perhaps through alterations in these transport mechanisms. Such affects appear to be a common underlying mechanism causing high blood pressure in a number of human genetic syndromes[82].

## Conclusions

The use of transgenic animals to study the RAS has lead to a better understanding of its importance in hypertension. Moreover, the recent demonstration that local RAS exists and is physiologically active in many tissues pointed to the importance of the tissue pathway pf Ang II generation and action. Further studies will undoubtedly include the use of tissue-specific knockouts to dissect the function of these system in each tissue. For instance, single intracardiac administration of a retroviral vector containing AT-1 receptor antisense gene causes a prolonged anti-hypertensive actions in the spontaneously hypertensive rat[83]. Our laboratory has demonstrated that the absence of hAGT in the liver, induced by the use of the Cre-loxP recombinase system causes a loss of hepatic and circulating hAGT[84]. These data directly demonstrate that extra-hepatic sources of angiotensinogen do not significantly contribute to the circulating pool of angiotensinogen. Infection of double transgenic mice containing hRen and a floxed hAGT transgene with an adenovirus encoding cre-recombinase (Adcre) reduced blood pressure significantly[85]. Using cre-recombinase in conjunction with cell-specific promoters will be of great use to study role of different tissue specific RAS components. We are currently examining mice which contain tissue-specific deletion of AGT expression in the kidney, liver and brain to assess the importance of AGT production in each tissue. We anticipate that a better understanding of these systems might lead to more specific and accurate treatment of certain types of human hypertension.

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