Role of angiotensin II AT_1 receptor activation in cardiovascular diseases

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Numerous clinical studies and experimental investigations using cell culture and animal models suggest that angiotensin II (AngII) via AT_1 receptor activation might induce cardiovascular hypertrophy, fibrosis and atherosclerosis resulting in vascular events such as myocardial infarction, heart failure or stroke and in end-organ damages. However, a question still remains: which part of these damages is due to a direct effect of AngII on its target tissues and which is due to AngII-induced hypertension? In an attempt to answer this question, a new model of transgenic mice, expressing a constitutively activated AT_{1A} receptor instead of the wild type receptor has been obtained by homologous recombination. These mice present with a moderate increase of blood pressure (20 mm Hg), hypertrophy of the small kidney arteries but not cardiac hypertrophy. The major phenotypic trait of these mice is the early and progressive development of a cardiovascular fibrosis. In light of these results and those from the literature, there is more and more evidence that in human hypertension, activation of the renin angiotensin system plays a minor role in the development of cardiovascular hypertrophy, but clearly participates to the development of cardiovascular fibrosis.

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The major consequences of hypertension are end-organ damages and cardiovascular complications, such as myocardial infarction (MI), stroke, and heart failure. Diverse antihypertensive drugs are able to reduce both high blood pressure (BP) and these complications. However, several clinical trials point out that there is an additional benefit for the prevention of cardiovascular diseases to treat patients with inhibitors of the renin–angiotensin system (RAS), that is angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs).[1] This beneficial effect was supposed to be the consequence of the inhibition of specific tissular effects of angiotensin II (AngII) on vascular structure, atherosclerosis, and cardiac remodeling. Starting from this concept, numerous *in vitro* and *in vivo* studies have tried to evaluate the precise physiological impact of AngII on cardiovascular diseases. Most of these studies have limitations due to the impossibility to dissociate between direct tissular actions and hypertensive effects of AngII or to eliminate the involvement of other interfering factors (aldosterone, bradykinin, AT_2 receptor, and so on).

AngII is the major effector of the RAS and exerts its physiological actions through membrane-bound receptors of the G-protein coupled receptor (GPCR) family. There are two types of AngII receptors, called AT_1R and AT_2R. Rodent display two subtypes of AT_1 receptors ($AT_{1A}R$ and $AT_{1B}R$).[2] Without any doubt the AT_1 receptor ($AT_{1A}R$ in rodents) transduces the large majority of the cardiovascular actions of AngII and therefore the putative roles of $AT_{1B}R$ and AT_2R in the cardiovascular system will be evoked briefly in this review. During the past years, a major breakthrough in the understanding of GPCR functions was the identification of artificial or natural mutations of these receptors able to constitutively activate their signaling pathways, independently of the mediator.[3] Using random mutagenesis, we were able to identify the mutations that constitutively activate the angiotensin AT_{1A} receptor.[4] It was then logical to introduce such mutations in the mouse genome in order to analyze the pathophysiological consequences of an activated AT_1 receptor expression. Using homologous recombination, the wild-type (WT) AT_{1A} receptor gene was replaced with a 'gain of function' mutated AT_{1A} receptor gene (AT_{1A}MUT), containing two mutations: N111S mutation, which constitutively activates the receptor and a C-terminal deletion, which

impairs constitutive internalization and desensitization of this receptor.[5] This AT_{1A}MUT mouse model provides new information on the direct role of AT_1 receptor activation in the development of cardiovascular diseases. This review focuses and summarizes the experimental data and present concepts on the role of AT_1 receptor activation in the development of cardiovascular diseases.

Renin–angiotensin system, AT_1 receptors, and hypertension

In human, activation of the RAS leads to hypertension in several situations, including renal artery stenosis.[6] The extensive therapeutic use of RAS inhibitors, such as ACEI and ARB, in essential hypertension sustains the importance of the system in BP regulation.[7] However, no polymorphism of the *RAS* genes (angiotensinogen, renin, and converting enzyme) has been unambiguously demonstrated to be linked or associated to hypertension.[8] Several studies were unable to associate consistently hypertension to polymorphisms of the *AT_1* receptor gene. In addition, no activating mutation of the AT_1 receptor, which was a logical candidate, has been yet described in secondary forms of hypertension, such as primary hyperaldosteronism.[9]

These results obtained in human pathology suggest an apparently modest role of the *RAS* genes in determining monogenic or polygenic forms of human hypertension and contrast with results obtained in genetically modified animal models. Manipulation of the *RAS* genes in mouse and rat has comforted the idea that the system is crucial for BP regulation. Duplications of the angiotensinogen or *AT_{1A}* receptor genes create hypertensive mice.[10,11] Large overexpression of renin and angiotensinogen genes in transgenic rats produced a fulminant hypertension.[12] Conversely, the knockout (KO) of renin, angiotensinogen, converting enzyme or *AT_{1A}* receptor genes in mice result in a 20 mm Hg reduction of BP, whereas AT_{1B}R KO indicates that this receptor is not involved in BP regulation and cardiovascular function.[13] Similar KO experiments suggest that AT_2R counterbalances the AT_{1A}R pressor action and causes a reduction in AngII-induced hypertension.[14] The predominant role of AT_{1A}R was recently and very elegantly explored by kidney crosstransplantation experiments in AT_{1A}KO and WT mice. In these experiments, Crowley *et al.*[15] demonstrated that BP differences between the two strains are equally due to kidney and peripheral AT_{1A} receptors, but that AngII induces hypertension primarily through kidney receptors.

All these data suggest that a permanent and excessive activation of the AT_1 receptor should result in hypertension. The definitive demonstration of this hypothesis was obtained recently in our laboratory. Homozygous knock-in AT_{1A}MUT mice are viable and present with a permanent higher BP (+20 mm Hg) than WT littermates (Table 1). This BP elevation is observed as early as five weeks of age, is moderate and stable during the mouse life and is more pronounced in male. The logical consequences of this elevated BP and AT_{1A} receptor activation are a dramatic reduction of plasma renin concentrations (10% of the WT values) and plasma immunoreactive AngII (50% of the WT values). However and unexpectedly, the plasma levels of aldosterone are identical in WT and AT_{1A}MUT mice and the zona glomerulosa of the adrenal cortex does not present any hypertrophy and expresses normal levels of aldosterone synthase (personal data). As the adrenal cortex is the only organ expressing equal amounts of AT_{1A} and AT_{1B} receptors, one can suppose that these normal aldosterone levels result from equilibrium between a constitutive overactivation of the mutated AT_{1A} receptor and an underactivation of the WT AT_{1B} receptor following downregulation of the RAS. Other regulatory factors independent from the RAS may also be considered. This normal aldosterone production, despite the hypertension and the downregulation of the RAS, defines a relative hyperaldosteronism in AT_{1A}MUT animals (aldosterone/renin ratio 10-fold higher in AT_{1A}MUT mice compared to WT animals).

The reactivity of BP to AngII was investigated in these different models of mice genetically modified for their AT_1 receptors. The amplitude of the BP response is reduced in AT_{1A}KO mice, suggesting a major role of the AT_{1A} receptor in mediating this response. The double AT_{1A}/AT_{1B}KO abolishes the AngII BP reactivity, indicating a compensatory role of the AT_{1B} receptor.[13]

Table 1 | Cardiovascular phenotypes of AT_{1A} transgenic mouse models

Mouse model	Systolic blood pressure (mm Hg)	Cardiac phenotype		Vascular phenotype		Response to MI	Response to pressure overload	Response to atherogenic regimen
		Hypertrophy	Fibrosis	Hypertrophy	Fibrosis			
AT_{1A} knockout	−24	NS[16]	NS[16]	NS	ND	Improved survival	NS[16]	Inhibition of ApoEKO atheroscleroses
Cardiac-specific AT_{1A} overexpression[18]	ND	LV/BW × 2.5	Interstitial	ND	ND	ND	ND	ND
AT_{1A} knock-in mice (AT_{1A}MUT)[5]	+20	NS	Perivascular Interstitial	Media thickness of small renal arteries	Perivascular (myocardium, kidney)	ND	ND	UI (ApoEKO breeding)

NS, not significant compared to age-matched wild-type animals; ND, not determined; UI, under investigation.