Hypertension Highlights

Cardiovascular and Renal Regulation by the Angiotensin Type 2 Receptor
The AT2 Receptor Comes of Age

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The renin-angiotensin system is a coordinated hormonal cascade of major importance in cardiovascular and renal regulation. The principle effector of this system is the octapeptide angiotensin II (Ang II), which acts at 2 cell membrane receptors, AT1 and AT2. The majority of the actions of Ang II have been demonstrated to be mediated by the AT1 receptor, including growth promotion, vasoconstriction, antinatriuresis, aldosterone secretion, salt appetite, thirst, sympathetic outflow and inhibition of renin biosynthesis, and secretion. The AT2 receptor has been less well understood. Past studies, however, clearly demonstrated that the AT2 receptor mediates cellular differentiation and growth, opposing the actions of Ang II through the AT1 receptor.

Studies in the mid to late 1990s demonstrated that AT2 receptor stimulation engenders an autacoid vasodilator cascade composed of bradykinin (BK), nitric oxide (NO), and guanosine cyclic 3′, 5′-monophosphate (cGMP). This discovery turned attention to the possibility that the AT2 receptor may mediate vasodilation, opposing the vasoconstrictor actions of Ang II at the AT1 receptor. Parallel cell signaling studies indicated that the AT2 receptor is G-protein–coupled (through Gia) and that receptor stimulation is accompanied by an increase in phosphotyrosine phosphatase activity and an inhibition of MAP kinase enzymes (p42 and p44) composing the extracellular signal-related kinase (ERK1/2). AT2 receptor-mediated inhibition of the extracellular signal-regulated kinase pathway opposed the actions of Ang II, resulting in extracellular signal-regulated kinase phosphorylation via the AT1 receptor.

Work during the late 1990s through 2002 suggested that the AT2 receptor might serve as a vasodilator counterforce to the AT1 receptor. However, vasorelaxation was difficult to elicit in some experimental models, attributed to the relatively low level of AT2 receptor vascular expression compared with that of the AT1 receptor. To unmask the vasodilator action of the AT2 receptor, experiments began to focus on eliminating the vasoconstrictor action of the AT1 receptor with an AT1 receptor blocker before and during AT2 receptor stimulation. These studies demonstrated that AT2 receptor activation by Ang II clearly dilated blood vessels and reduced blood pressure. Studies also demonstrated that at least part of the depressor action of AT1 receptor blockade is mediated by AT2 receptor stimulation, in acute and chronic experimental models. The vasodilator action of the AT2 receptor was easier to detect during conditions in which the renin-angiotensin system was upregulated, such as sodium restriction, Ang II infusion, or renal vascular hypertension. Most studies pointed to the role of BK, NO, and cGMP in mediating the observed vasodilator response via the AT2 receptor.

So, is there anything new about the AT2 receptor during the past 2 years? Yes, in many ways these have been “banner years” in promoting our understanding of the AT2 receptor, validating previous observations, and elucidating new roles for the receptor in cardiovascular and renal function. Studies have unequivocally confirmed the role of the AT2 receptor as a vasodilator mediator in resistance microvessels as well as in large capacitance vessels. AT2 receptor stimulation of BK, NO, and cGMP formation is a consistent signaling pathway for vasodilation. An additional cell signaling pathway, phospholipase D, has been identified for the AT2 receptor. The AT2 receptor inhibits renin biosynthesis and release. The AT2 receptor appears to have a protective role in ischemic nephropathy. The AT2 receptor is cardioprotective after myocardial infarction and during states of Ang II excess; these beneficial responses also are mediated by BK and NO. An AT2 receptor gene polymorphism is associated with coronary disease in men.

Vasodilation Mediated by BK, NO, and cGMP

During the past 2 years, several convincing studies have been reported confirming the vasodilator action of the AT2 receptor. Hannan et al reported that AT2 receptors mediate vasodilation in the rat uterine artery. AT2 receptor antagonist PD-123319 (PD) induced a 4-fold leftward shift (more sensitive) in the Ang II concentration–response curve for vascular contraction. Thus, the AT2 receptor inhibited Ang II–induced constriction in these vessels. Because the action of PD was duplicated by the BK-B2 receptor antagonist icatibant and independently by the NO synthase inhibitor N-nitro-L-arginine, and arterial cGMP concentrations were increased by Ang II, the vasodilatory action of the AT2 receptor was considered to be mediated by BK, NO, and cGMP. Because...
normal pregnancy is accompanied by enhancement of AT1 receptor expression, it is likely that the AT2 receptor mediates counter-regulatory vasodilation in pregnancy. If this is so, then abnormalities in AT1 receptor expression and function are candidates for vasoconstriction in pregnancy-induced hypertension (preeclampsia).

The AT2 receptor dilates mesenteric arterial segments under flow conditions. In the presence of AT1 receptor blockade, Ang II dilated these vessels via endothelial AT1 receptors. BK-B2 receptor blockade substantially inhibited this response, and AT2 receptor-mediated vasodilation was markedly reduced in kininogen-deficient Brown Norway Katholick rats compared with wild-type (WT) controls. These studies demonstrate that the AT2 receptor mediates vasodilation via local BK production in mesenteric resistance vessels in a flow-dependent manner. These observations suggest that shear stress caused by increased blood flow may be important in AT2 receptor-mediated vasodilation. Similarly, Bergaya et al showed that to observe the contribution of the vascular kallikrein/kinin system to flow-dependent dilation, functional AT2 receptors are required. AT1 receptor inhibition with PD decreased flow-induced dilation in WT mice, but not in mice lacking kallikrein. In these studies, the BK-B2 receptor antagonist icatibant reduced the vasodilatory response to flow in WT mice, but not in mice lacking the AT2 receptor.

Taken together, this new information strongly suggests that resistance microvessels are a major site of AT2 receptor action. However, evidence accumulated during the past year also documents a role of AT2 receptors in large capacitance vessels. Yayama et al tested AT2 receptor expression in the rat thoracic aorta subjected to pressure-overload by means of suprarenal abdominal aortic banding. There was a 3-fold upregulation of AT2 receptor mRNA in the pressure-overloaded aorta 4, 7, 14, and 28 days after banding. In the pressure-overloaded vessels, Ang II constrictor responses were abrogated but were restored to control levels by AT2 receptor antagonist PD. Removal of the endothelium nullified the differences in Ang II responsiveness previously observed between sham control and pressure-overloaded aortas. Therefore, pressure-overload of large capacitance vessels upregulates AT2 receptor expression, which appears to limit Ang II-mediated vasoconstriction. Because renal perfusion pressure and blood flow were reduced in this model, the endogenous renin-angiotensin system was activated, which may have induced increased AT2 receptor expression and function. In this regard, this study found that pressure-induced AT2 receptor expression was dependent on Ang II activation of AT1 receptors. Thus, a counter-regulatory positive feedback loop may be established wherein AT1 receptor-induced vasoconstriction is automatically counterbalanced by AT2 receptor upregulation and consequent vasodilation. The mechanism of AT1 receptor-induced dilation in the pressure-overload model is BK-dependent, because the contractile response was restored with PD or icatibant in mice. Ang II invoked a 9-fold increase in aortic cGMP that was abolished by either PD or icatibant. Functional vasodilator AT2 receptors also have been demonstrated in the fetal aorta, where AT2 receptor expression is extremely high. These findings may be important in pathophysiology, because normal counter-regulatory upregulation of the AT2 receptor in certain disease states may be rendered sluggish or inoperative, lending to unmitigated vasoconstriction.

Two studies during the past year have clarified AT2 receptor-mediated vascular actions in the coronary circulation. Thai et al found in the normal and failing rat hearts that chronic candesartan-induced coronary vasodilation was abolished with NO synthesis inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) or PD, which reduced acetyl choline-induced vasodilation to baseline levels. Also, in bovine pulmonary endothelial cells, candesartan increased NO synthase expression, but this was eliminated by PD. Thus, AT2 receptor blockade induced endothelium-dependent coronary vascular relaxation that was mediated by Ang II stimulation of the AT2 receptor. Batenburg et al studying human coronary microarteries, demonstrated that Ang II-induced contraction was prevented by AT1 receptor blockade and potentiated by AT2 receptor blockade. The augmentation of coronary vasoconstriction by PD was abolished by icatibant, L-NAME, or removal of the endothelium. In the presence of AT1 receptor blockade, Ang II elicited vascular relaxation, which was eliminated by PD. These studies demonstrate functional AT2 vasodilator receptors in the coronary microcirculation that use a BK-NO-cGMP signaling pathway. Because the expression of AT2 receptors is quantitatively similar to that of AT1 receptors in the human heart, these findings invite further exploration of the normal physiological balance between these 2 receptors and their relative roles in cardiac pathophysiology.

The vasodilator action of AT2 receptors may be mediated not only by BK and NO but also by inhibition of phospholipase D. In pregumellar vascular smooth muscle cells from spontaneously hypertensive rat kidneys in which AT1 receptors were expressed 3:1 over AT2 receptors, Ang II activated phospholipase D more powerfully than in vessels from Wister Kyoto rats in which the receptor expression ratio was 1:1. Thus, AT2 receptors inhibit the ability of Ang II to activate phospholipase D via AT1 receptors.

**Inhibition of Renin Biosynthesis and Secretion**

The AT2 receptor is expressed in renal juxtaglomerular (JG) cells of the afferent arteriole. JG cell AT2 receptors inhibit renin biosynthesis and secretion, providing a short-loop negative feedback mechanism to dampen Ang II production. Angiotensin-converting enzyme inhibition and/or AT1 receptor blockade unmask this short-loop negative feedback inhibition of renin secretion resulting in increased circulating active renin concentrations and Ang I production. AT2 receptor blockade in JG cells increased renin secretion rate and Ang II levels in the media and reduced active, but not total, renin content. The decrease in active renin content was blocked by PD. AT2 receptor agonist CGP42112A (CGP) increased renin secretion rate and decreased active renin content, and these actions of CGP were blocked by PD. These findings suggest that AT1 receptor blockade inhibited prorennin processing in JG cells via AT2 receptors.

In conscious rats, plasma renin activity and intrarenal levels of Ang II were increased by direct renal cortical...
administration of either AT₁ receptor blocker valsartan or AT₂ receptor antagonist PD.²⁵ Both receptor blockers independently increased renal renin mRNA and renin concentration. In response to valsartan and PD, renin immunoreactivity was markedly increased in both JG and tubule cells.²⁵ Therefore, renal renin biosynthesis and secretion are inhibited by a novel AT₂ receptor negative feedback mechanism in parallel with that of AT₁ receptors in JG cells. This inhibitory action of AT₂ receptors illustrates that not all AT₂ receptor actions oppose those of AT₁ receptors. Further studies are required to determine the physiological and pathophysiological significance and the cell signaling mechanism(s) of AT₂ receptor-mediated renin suppression.

Renal Protection
The AT₂ receptor has a protective role in the renal wrap model of angiotensin-dependent hypertension.¹⁰ However, the more general protective role of the AT₂ receptor in the kidney has not been well-understood. AT₂ receptor transgenic (AT₂R-Tg) and WT mice were subjected to 5/6 nephrectomy, a model of ischemic renal injury.²⁶ In AT₂R-Tg mice, glomerular expression of the AT₂ receptor was upregulated by 5/6 nephrectomy. Urinary albumin excretion was decreased by approximately one-third in AT₂R-Tg compared with WT mice. In response to 5/6 nephrectomy, AT₂R-Tg had decreased transforming growth factor-β and platelet-derived growth factor, and urinary excretion of NO metabolites was increased 2.5-fold. All of these responses were abolished by the AT₂ receptor antagonist PD.²⁶ The 5/6 nephrectomy model develops a time-dependent increase in AT₂ receptor expression at 7, 15, and 30 days after renal ablation.²⁷ Animals pretreated with AT₁ receptor antagonist losartan demonstrated a further increase in AT₂ receptor expression. AT₂ receptor antagonist PD downregulated the AT₂ receptor and increased blood pressure and renal damage in this model.²⁷ All of these studies demonstrate that the AT₂ receptor is protective in ischemic renal injury.¹⁰,²⁶,²⁷

Potential Role in Renal Function
As stated, AT₂ receptor-mediated vasodilation can oppose AT₁ receptor-mediated vasoconstriction. However, the role of the AT₂ receptor in the renal microcirculation has not been investigated systematically. Duke et al²⁸ explored the independent contributions of AT₁ and AT₂ receptors in the control of regional kidney blood flow in the rabbit. These authors showed that under basal conditions, Ang peptides do not significantly influence medullary blood flow. In the renal cortical circulation, as in systemic microvessels, AT₂ receptor activation counteracted AT₁ receptor-mediated vasoconstriction. However, in the renal medulla, AT₂ receptor stimulation engendered vasodilation that also was opposed by AT₁ receptor activation.²⁸ These observations may be important in the pathogenesis of hypertension because renal medullary blood flow has been recorded as reduced in certain animal models of hypertension,²⁹ in which the renin-angiotensin system is activated.

A paucity of studies are available on the potential actions of AT₂ receptors on renal sodium excretion. Hakan and Hussain³⁰ recently reported that AT₁ receptor blockade with candesartan produced natriuresis to a greater degree in obese Zucker than in lean rats. Candesartan-induced natriuresis was abolished by AT₂ receptor antagonist PD in obese, but not in lean, rats and infusion of AT₁ receptor agonist CGP-42112A induced a natriuresis to a greater extent in obese than in lean rats. AT₂ receptor protein was upregulated both at basolateral membrane and brush border sites of renal proximal tubule cells of obese but not lean rats. The results of these studies suggest that, especially under conditions leading to renal tubule upregulation of the AT₂ receptor, this receptor might play a role in renal sodium transport. However, the renal tubule actions of AT₂ receptors are far from clear and this is an area of intense future interest.

Cardioprotective Actions of AT₂ Receptors
The cardiac AT₂ receptor has been the subject of much interest during the past decade. Studies have shown that the AT₂ receptor inhibits growth of cardiac myocytes. In the failing heart, AT₁ and AT₂ receptor expression is decreased and increased, respectively. However, the precise role of the AT₂ receptor in cardiac hypertrophy and left ventricular (LV) remodeling has been controversial and may depend on the AT₁/AT₂ receptor ratio in a physiological/pathophysiologic context-specific manner.³¹ Several recent articles have helped clarify the role of the AT₂ receptor in cardiac hypertrophy and remodeling after experimental myocardial infarction (MI). Brede et al³² studied AT₂ receptor-deficient mice after myocardial infarction, which led to increases in heart:body weight ratios and myocardial cross-sectional areas in mice lacking the AT₂ receptor (AT₂-null) compared with WT mice. The AT₂-null mice had downregulation of myocardial endothelial nitric oxide synthase and reduced cGMP levels. In isolated cardiomyocytes, Ang II induced endothelial nitric oxide synthase expression via AT₂ receptors, and NOS blockade abolished the antihypertrophic effects on cardiac myocytes in vivo. Oishi et al³³ also demonstrated that AT₂-null mice have greater cardiac remodeling/hypertrophy, systolic and diastolic dysfunction, and increased mortality after MI compared with WT mice. In contrast to the previous study, the cardioprotective role of the AT₂ receptor was independent of myocyte hypertrophy. However, both studies demonstrate cardioprotection by the AT₂ receptor after MI.

In an earlier study, Bove et al³⁴ had demonstrated using functional magnetic resonance imaging techniques that AT₂ receptor overexpression in the mouse heart preserves LV structure and function during LV remodeling after MI. This group of investigators has recently extended this study to demonstrate that the cardiac NO pathway is largely, if not exclusively, responsible for the beneficial effects of AT₂ receptor expression.³⁵ NOS inhibitor L-NAME was able to abrogate virtually all of the protective actions of cardiac AT₂ receptor overexpression after MI LV remodeling.³⁵ Beneficial effects of cardiac-specific AT₂ receptor overexpression included reduced LV wall thickening, decreased end-diastolic- and end-systolic volume indices, and increased ejection fraction after MI compared with WT mice. These results are for the most part consonant with the work of Xu et al,³⁶ who demonstrated that the cardioprotective effects of AT₁ receptor...
activation are mediated by the AT1 receptor, even though the AT2 receptor may not play a role in the regulation of normal (basal) cardiac function.

Application of gene transfer technology in spontaneously hypertensive rats also has demonstrated cardioprotective actions of the AT2 receptor.37 Administration of a lentiviral vector encoding the AT2 receptor resulted in cardiac AT2 receptor overexpression without altering the expression of the AT1 receptor. AT2 receptor gene transfer reduced LV wall thickness and heart/body weight ratios compared with controls. It is interesting that the reduction in cardiac hypertrophy occurred in the absence of a reduction of blood pressure.

Another compelling study focused on the effects of AT2 receptor activation on myocardial collagen deposition and fibrosis.38 WT mice were infused with Ang II for 2 weeks, at which point prominent perivascular fibrosis of the intramural coronary arteries developed. However, mice selectively overexpressing AT2 receptors in cardiac myocytes had a markedly reduced fibrotic response. Furthermore, the inhibition of perivascular myocardial fibrosis in the transgenic mice was abolished by co-administration of BK-B2 receptor antagonist icatibant or NOS inhibitor L-NAME. The cardioprotective effect of AT2 receptor overexpression was accompanied by increased cardiac kininogenase activity, suggesting that the BK pathway had been activated with resultant stimulation of NO production. These results have important clinical ramifications, because the beneficial action of AT1 receptor antagonist treatment on fibrosis also may be mediated by AT2 receptor stimulation.

**The Significance of AT2 Receptor Gene Polymorphisms**

Recent evidence suggests an association in the presence of linkage for the AT2 receptor (A1332G) and premature coronary artery disease in men.39 Because the AT2 receptor gene is located on the X-chromosome, hemizygotic men would have a higher probability of showing the effects of a recessively acting X-chromosome susceptibility gene than would dizygotic women. Women with the AT2 receptor polymorphism do not have any alteration in the frequency of coronary disease compared with those without this genetic marker.39 These results are consistent with those of an earlier study.40

**Conclusions and Future Directions**

The AT2 receptor is now clearly established as vasodilator receptor with the majority of studies implicating the BK-NO-cGMP signaling cascade in its vasorelaxant action. The AT2 receptor is counter-regulatory to the vasoconstrictor actions of Ang II engendered by the AT1 receptor and may provide at least some of the hypotensive benefit of AT1 receptor blockers in hypertension. The AT2 receptor mainly dilates small resistance arteries, although the receptor also has a vasorelaxant action on large capacitance vessels. One remaining question is whether the vasodilator action of the AT2 receptor can be observed chronically, as suggested by past studies.8,9 Another question is whether the AT2 receptor is vasodilator in humans. If so, development of an AT2 receptor agonist for therapeutic purposes would probably be useful.

Although the AT2 receptor opposes the AT1 receptor in vascular reactivity, these 2 receptors operate similarly to inhibit renin biosynthesis, processing, and secretion. We need to learn more about the signaling mechanisms involved in AT2 receptor-induced renin inhibition and to determine whether the AT1 and AT2 receptors act synergistically in this action. In any case, AT1 receptor blockade should increase renin secretion and Ang II formation, which would limit further renin generation by stimulation of the AT2 receptor.

Evidence is mounting that the AT2 receptor is protective against ischemic renal injury. However, the effects of the AT2 receptor on renal function, and particularly renal sodium excretion, remain largely unknown. Most studies have been conducted in AT2-null mice, but these animals have increased AT1 receptor expression, complicating interpretation.41 These studies suggest that absence of the AT2 receptor shifts the pressure-natriuresis curve to the right (less sensitive) and induces antinatriuretic hypersensitivity to Ang II.42,43 Consistent with these studies, in the renal proximal tubule cell membrane, AT2 receptors seem to inhibit Na+K+ATPase activity44 and mediate a similar inhibitory effect on bicarbonate reabsorption in this nephron segment.45 Much more information is needed, however, on the role of AT2 receptors in renal tubule sodium transport and renal sodium excretion.

The AT2 receptor appears with increasing certainty to be cardioprotective, inhibiting detrimental cardiac remodeling after MI. The AT2 receptor also inhibits coronary perivascular fibrosis in response to increased circulating Ang II. The cardioprotective effect seems to result from a combination of improved cardiac systolic and diastolic function and an inhibition of fibrosis and is mediated by NO. These observations are strong indicators that AT2 receptor agonist therapy may be beneficial in minimizing or preventing left ventricular dysfunction after MI. A major unanswered question is whether the AT1 receptor is important in the regulation of normal cardiac function or whether the receptor only becomes a positive factor in the presence of a cardiac insult such as MI. Also, the precise intracellular signaling mechanisms whereby the receptor exerts these actions need further clarification.

The past 2 years have witnessed major advances in our understanding of the function of the AT2 receptor, which appears to perform important cardiovascular actions of Ang II. The stage is set for closing the gaps in our basic knowledge and determining the cardiovascular and renal actions of this receptor in humans.

**References**


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