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The angiotensin II type 2 receptor in cardiovascular disease

Catherine A Lemarié, Ernesto L Schiffrin

Abstract
Angiotensin II (Ang II) is considered the major final mediator of the renin-angiotensin system. The actions of Ang II have been implicated in many cardiovascular conditions, such as hypertension, atherosclerosis, coronary heart disease, restenosis, and heart failure. Ang II can act through two different receptors: Ang II type 1 (AT\(_1\)) receptor and Ang II type 2 (AT\(_2\)) receptor. The AT\(_1\) receptor is ubiquitously expressed in the cardiovascular system and mediates most of the physiological and pathophysiological actions of Ang II. The AT\(_2\) receptor is highly expressed in the developing foetus, but its expression is very low in the cardiovascular system of the normal adult. Expression of the AT\(_2\) receptor can be modulated by pathological states associated with tissue remodelling or inflammation such as hypertension, atherosclerosis, and myocardial infarction. The precise role of the AT\(_2\) receptor remains under debate. However, it appears that the AT\(_2\) receptor plays a vasodilatory role, and may be enhanced as a countervailing mechanism in cardiac hypertrophy, and in presence of vascular injury in hypertension and atherosclerosis. Signalling pathways induced by the stimulation of the AT\(_2\) receptor are poorly understood, but three main mechanisms have been described: (a) activation of protein phosphatases causing protein dephosphorylation; (b) activation of bradykinin/nitric oxide/cyclic guanosine 3’,5’-monophosphate pathway; and (c) stimulation of phospholipase A\(_2\) and release of arachidonic acid. Vasodilatory effects of the AT\(_2\) receptor, probably the only well-established role of the AT\(_2\) receptor, have been attributed to the second of these mechanisms. The participation of the AT\(_2\) receptor in cardiovascular remodelling and inflammation is more controversial. In vitro, AT\(_2\) receptor stimulation clearly inhibits cardiac and vascular smooth muscle proliferation, and stimulates apoptosis. In vivo, the situation is less clear, and depending on the studies, the AT\(_2\) receptor appears to be required for cardiac hypertrophic growth or contrariwise, the AT\(_1\) receptor has demonstrated no effects on cardiac hypertrophy. Similar controversial findings have been reported in atherosclerosis. Here we discuss the role of the AT\(_2\) receptor on cardiovascular structure and disease, and the signalling pathways induced by its activation.

Introduction
Angiotensin II (Ang II), the major effector of the renin-angiotensin system (RAS), acts mainly via two different receptors: Ang II type 1 (AT\(_1\)) receptor and Ang II type 2 (AT\(_2\)) receptor. These two receptors were initially defined on the basis of their differential pharmacological and biochemical properties, and later cloned. The AT\(_1\) receptor mediates most of the well-known effects of Ang II, which include vasoconstriction, cell growth, generation of oxidative stress and inflammation, vascular and cardiac hypertrophy, and stimulation of aldosterone secretion among others. Actions of the AT\(_2\) receptor are less clear, but seem to counterbalance some of the actions of the AT\(_1\) receptor. The AT\(_1\) receptor is involved in physiological processes such as development and tissue remodelling (by inhibition of cell growth and stimulation of apoptosis), regulation of blood pressure (BP) (vasodilatation), natriuresis and neuronal activity. The AT\(_2\) receptor shares 34% sequence homology with its AT\(_1\) receptor counterpart, and encodes for a protein of 363 amino acids with a molecular weight of 41 kDa. In humans, the AT\(_1\) receptor is widely expressed at relatively constant levels in adults and is localised in numerous tissues, including blood vessels, the heart, kidneys, adrenal glands, liver, and adipose tissue. In contrast, the AT\(_2\) receptor is mainly present during foetal development, and is believed to have an essential role in physiological development in general, in part through its action on vascular development. However, the density
of the AT2 receptor decreases rapidly after birth in most tissues.\(^1,2\) In adults, expression of the AT2 receptor under normal conditions is largely restricted to the adrenals, kidneys, uterus, ovary, heart, and specialised nuclei in the brain. Nevertheless, expression of the AT2 receptor is upregulated in various pathological conditions associated with tissue remodelling or inflammation, including hypertension, atherosclerosis, heart failure, myocardial infarction, tissue ischaemia, and in diabetes mellitus.\(^3-5\) Multiple hormonal and metabolic factors, as well as different cytokines, are involved in upregulation of the density of the AT2 receptor on cells. Conversely, downregulation of the AT2 receptor may be brought about by glucocorticoids and growth factors,\(^6\) and perhaps also by the AT1 receptor.

Both the AT1 and AT2 receptors are members of the G-protein-coupled receptor (GPCR) family and are believed to induce different signalling pathways and cellular responses. The AT1 receptor is implicated in most of the deleterious effects of Ang II in cardiovascular pathophysiology. Because of its potential countervailing effects to the AT2 receptor, activation of the AT2 receptor is believed to have beneficial cardiovascular effects. AT1 receptor antagonists appear to exert their effects on the one hand via blockade of activation of deleterious signalling pathways mediated by the AT1 receptor, and on the other hand, by stimulation of renin release and increased generation of Ang II which acts on unblocked AT2 receptors. It is, however, interesting to note that when one considers the two major agents currently most frequently used to inhibit the RAS in the treatment of hypertension and cardiovascular diseases, angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARB), both classes of drugs have different effects, but the final cardiovascular protective effects are similar. Indeed, ACE-Is reduce production of Ang II and thus the activation of both AT1 and AT2 receptors, whereas ARBs block binding of Ang II to the AT1 receptor but allow unopposed stimulation of the AT2 receptor. Yet, it is difficult to distinguish exactly which of the beneficial effects observed with ARBs arise from blockade of the AT1 receptor and which are due to activation of the AT2 receptor, or why ACE-Is have similar actions to ARBs despite their different sites of action on different steps of the RAS cascade.

In this review, we focus on the expression and the role of the AT1 receptor in pathological conditions, such as abnormal cell growth and apoptosis, hypertrophy and inflammation. The most recent advances in the understanding of AT2 receptor-mediated vascular signalling pathways are also reviewed.

**The role of the AT2 receptor in vasodilatation and BP regulation**

Ang II regulates many processes implicated in vascular pathophysiology, including cell growth/apoptosis of vascular cells, migration of vascular smooth muscle cells (VSMC), inflammatory responses, and extracellular matrix remodelling. Locally formed Ang II can activate cells regulating the expression of many substances, including growth factors, cytokines, chemokines, and adhesion molecules, which are involved in cell growth/apoptosis, fibrosis, and inflammation. Integrated vascular responses to Ang II are the result of combined AT1 and AT2 receptor-mediated actions. It is generally accepted that Ang II induces vasoconstriction, growth, migration, production of extracellular matrix components, and inflammation via the AT1 receptor, and promotes apoptosis, and inhibits proliferation and hypertrophy via the AT2 receptor.\(^6,7\) However, the finding that activation of the AT1 receptor may, in some tissues, result in parallel rather than opposite effects to AT2 receptor activation suggests that AT1 and AT2 receptors may share, at least in part, some common signalling pathways (figure 1).\(^8\)

![Figure 1](http://jra.sagepub.com)

**Figure 1**

Angiotensin II mediates its cellular effects through two receptorsubtypes, angiotensin II type 1 receptor (AT1) and angiotensin II type 2 receptor (AT2). The AT1, has been implicated in deleterious effects of angiotensin II in cardiovascular disease, whereas the AT2 seems to have beneficial effects. NO = nitric oxide.
There is abundant support for the concept that the AT₁ receptor contributes to control of vascular tone by mediating vasodilatation and counterbalancing AT₂ receptor-mediated vasoconstrictor effects of Ang II. Growing evidence has suggested possible links between Ang II receptors and bradykinin B₂ receptors (B₂R) regarding nitric oxide (NO) production.¹⁻¹² In vascular endothelial cells, the production of NO by Ang II is caused by activation of the AT₂ receptor.¹³ AT₂ receptor stimulation by exogenous Ang II leads to an increase in cyclic guanosine 3',5'-monophosphate (cGMP) levels, through a mechanism involving B₂R and NO release.⁹⁻¹² In vitro studies of endothelial cells have demonstrated that intracellular acidosis, as a result of AT₂ receptor activation, stimulates bradykinin formation by activating kininogenases.¹⁴ Katada and Majima showed production of bradykinin after AT₂ receptor activation in rat mesenteric arteries, suggesting that B₂R mediates vasodilatation by endogenous bradykinin release upon AT₂ receptor stimulation.⁹ In AT₂ receptor-knockout mice, participation of B₂R in flow-induced dilation requires the presence of a functional AT₂ receptor.¹⁵ This raised the hypothesis that the AT₂ receptor and B₂R form heterodimers which can interact through receptor crosstalk. Recently, the AT₂ receptor and B₂R were shown to form a stable functional heterodimer which leads to increased NO and cGMP production.¹⁰ However, additional studies concluded that NO production following AT₂ receptor stimulation may also occur independently of B₂R, through direct NO synthase activation (figure 2).¹⁷

AT₁ receptor-dependent vasoconstriction leads to increased peripheral resistance and BP elevation. Activation of the AT₁ receptor by promoting vasodilatation should reduce BP. Indeed, AT₂ receptor-knockout mice, although only slightly hypertensive at baseline, exhibited pressor hypersensitivity to Ang II compared to wild-type controls.¹⁶⁻²⁰ In AT₁ receptor-null mice, Ang II infusion, as well as sodium deprivation, failed to increase bradykinin and NO production, suggesting that the vasodilatory effect induced by the AT₂ receptor is mediated by these autacoids.²¹ Conversely, transgenic mice overexpressing the AT₁ receptor in the vasculature failed to show a pressor response to Ang II infusion.¹¹ The role of the AT₁ receptor in mediating control of vasomotor tone has been investigated in experimental studies in various vascular territories, including the mesenteric and kidney circulations and the uterus.¹²⁻²⁰ These studies have indicated that the AT₁ receptor plays a protective counter-regulatory role against the pressor and antinatriuretic actions of Ang II. In the presence of AT₁ receptor blockade, therefore, overstimulation

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**Figure 2**

Interaction between the angiotensin II type 2 receptor (AT₂) and the kinin/bradykinin type 2 receptor (B₂R)/nitric oxide (NO) system. Activation of the AT₂ increases intracellular acidosis which induces the release of bradykinin and binding to the B₂R. The AT₂ can also activate endothelial nitric oxide synthase (eNOS) to induce the production of NO and thus trigger vasorelaxation and reduce blood pressure (BP). Stimulation of the angiotensin II type 1 receptor (AT₁) by angiotensin II activates phospholipase C (PLC) and via activation of protein kinase C (PKC) and release of Ca²⁺ leads to vasoconstriction and increased BP. CaM = calmodulin; DAG = diacylglycerol; IP₃ = inositol triphosphate; MAPK = mitogen-activated protein kinase; sGC = soluble guanylate cyclase.
of the AT₂ receptor is likely to have a beneficial effect in controlling BP in hypertension. This agrees with the suggestion that dysregulation of AT₁ receptor-mediated vascular tone plays a role in the pathogenesis of BP elevation. Indeed, in isolated rat mesenteric resistance arteries, AT₁ receptor-mediated vasorelaxation induced either by Ang II or by the AT₁ receptor agonist CGP42112 was preserved after long-term treatment with the ARB candesartan cilexetil. Savoia et al. have further elucidated this with the demonstration that mesenteric arteries from stroke-prone spontaneously hypertensive rats chronically exposed to AT₁ receptor blockade exhibit enhanced AT₁ receptor expression and associated AT₁ receptor-mediated vasodilatation. Perhaps more importantly, it was shown for the first time in humans that after long-term treatment with the ARB valsartan, the expression of AT₁ receptors is enhanced in small peripheral resistance arteries from hypertensive diabetic patients. Increased AT₁ receptor expression was associated with enhanced dose-dependent AT₁ receptor-mediated vasodilatation to Ang II and reduced BP in treated patients. These results are supported by a very recent study where it was demonstrated that low dose of Ang II (i.e. 50 ng/kg per min) significantly reduced BP in female rats and where mRNA expression of the AT₁ receptor and angiotensin-converting enzyme was increased compared to male rats. In contrast to the results in the female rats, the effect of the same dose of Ang II was negligible in male rats. These authors have suggested that this imbalance of vasodilator and vasoconstrictor components of the RAS may contribute to sex-specific differences in response to RAS activation.

The role of the AT₂ receptor on cardiovascular structure

In addition to its role in the regulation of BP, Ang II participates in the control of cell growth and apoptosis, and in pro-oxidant and pro-inflammatory processes. Indeed, Ang II has been shown in both human and animal models to be involved in the development of cardiomycocyte hypertrophy, and in cardiac fibrosis and modulation of cardiac fibroblast growth and collagen synthesis. In addition, Ang II activates vascular cell apoptosis, contributing to vascular remodelling and cardiomycocyte loss in ischaemia-reperfusion and myocardial infarction in humans and in animal models.

Growth, apoptosis and hypertrophy

It is well accepted now that AT₂ receptor expression decreases after birth but may increases again in density in tissues in some pathophysiological conditions. Stimulation of this increased AT₂ receptor expression appears to regulate neointimal formation, cell proliferation, and inflammation in vascular injury, myocardial infarction and ischaemic diseases. Results of clinical trials support the notion that AT₁ receptor stimulation may contribute to the inhibition of restenosis following balloon angioplasty. In the VALPREST and VALPACE trials, AT₁ receptor blockade with ARBs, which have actions as AT₂ receptor stimulators, prevented restenosis of coronary lesions following balloon angioplasty. In agreement, experimental studies have shown a beneficial role of the re-expression of the AT₂ receptor after vascular injury leading to inhibition of cell growth and promotion of apoptosis. For example, gene transfer studies showed that AT₂ receptor expression in cultured adult VSMC antagonises the growth-promoting effects of the AT₁ receptor and proliferation induced by growth factors such as platelet-derived growth factor. Gene transfer studies in vivo have demonstrated that AT₂ receptor expression attenuated neointimal accumulation in injured carotid arteries. Vascular injury studies with mice harbouring disruption of the AT₂ receptor showed exaggerated neointimal development compared with wild-type mice. However, Yamamoto et al. recently showed opposite results and demonstrated that the AT₂ receptor had no effect on neointimal cell growth or narrowing after wire injury of femoral arteries.

The AT₂ receptor has also been shown to mediate apoptosis in numerous cells types in vitro and in vivo, including VSMC. Several years ago, Yamada et al. showed that the AT₂ receptor mediates apoptosis in PC12W cells. Since then, available data from literature are confusing. Stimulation of both the AT₁ and AT₂ receptors by Ang II has been shown to induce apoptosis in aortic VSMC, cardiomyocytes, glomerular epithelial cells, and renal proximal tubular cells. In an in vivo study in which rats were infused for one week with Ang II and treated simultaneously with either the ARB losartan or the AT₁ receptor blocker PD123319, activation of AT₁ receptors resulted in systolic BP increase, blood vessel growth and associated VSMC apoptosis, which may modulate the degree of growth. Expression of bax and active forms of caspase 3 was increased in the Ang II + PD123319 group, whereas expression of bcl-2 was not significantly different in any group. The expression of AT₁ and AT₂ receptor mRNA was downregulated by losartan and PD123319, respectively. Thus, when AT₁ receptors or AT₂ receptors are stimulated in vivo, apoptosis is enhanced in the media of blood vessels. In the case of AT₂ receptor stimulation, this may occur secondary to vascular growth and fine-tune...
the latter. Both bax and caspase 3 participate in the pathways of apoptosis triggered by in vivo AT1 receptor stimulation. Whether apoptosis induced in VSMC by the AT1 receptor in contrast to that induced by the AT2 receptor is primary or secondary remains unclear. In agreement with these findings, blockade of the AT1 receptor with irbesartan and of the AT2 receptor with PD123319 prevented Ang II-induced apoptosis in cultured cardiomyocytes.47 However, in spontaneously hypertensive rats subjected to chronic AT1 receptor blockade with losartan, activation of the AT2 receptor induces vascular cell apoptosis and thus participates in the early phase of vascular remodelling.50 More recently, reduced rate of apoptosis in AT2 receptor-deficient mice compared to wild-type mice was demonstrated in a model of cuff-induced vascular injury.51 However, the role of the AT1 receptor in apoptosis seems to be dependent on the cell type and phenotype.51-53 Indeed, blockade of the AT2 receptor did not abolish the pro-apoptotic effects of Ang II on endothelial cells, suggesting that Ang II-induced apoptosis of endothelial cells is not exclusively mediated by the AT1 receptor but depends on a complex interplay between both the AT1 receptor and the AT2 receptor.54

Cellular growth and apoptosis are also two major components of cardiac remodelling. Cardiac remodelling is the architectural change of the heart under certain physiological or pathological circumstances, such as organ development, exercise, ageing, hypertension and myocardial infarction. In addition, the process of cardiac remodelling usually includes remodelling of the extracellular matrix and microvascular network. Cardiac hypertrophy may be considered a specific form of cardiac remodelling characterised by an increase in the volume of myocytes triggered by pathological stimuli. The cardiac effects of the AT1 receptor are well established, but those of the AT2 receptor remain controversial.31,55,56 Pathological hypertrophy and failure of the human heart resulted in a decrease in AT1 receptor expression and an increase or no change in AT2 receptor expression. In isolated rat cardiac myocytes, overexpression of the AT2 receptor with different ratios of the AT1 receptor and the AT2 receptor did not prevent AT1 receptor-mediated myocyte hypertrophy.57 However, others have shown in neonatal rat cardiac myocytes that AT2 receptor stimulation inhibited the growth of cardiomyocytes and fibroblasts by countering AT1 receptor signalling.31,58 Studies in mice with genetic deletion of the AT1 receptor and pressure overload-induced hypertrophy or in models of Ang II-induced cardiac hypertrophy have demonstrated either that the AT1 receptor was required for cardiac hypertrophic growth or that the receptor had no effect on myocyte hypertrophy.39,59,60 Further studies have shown that neither AT1 receptor deletion nor overexpression affected cardiac hypertrophy in transgenic mice.60,64 In contrast, studies using AT2 receptor gene transfer in spontaneously hypertensive rats have demonstrated a decrease in cardiac hypertrophy and a reduction in myocardial fibrosis.62,63 Aortic banding of transgenic AT2 receptor mice resulted in reduction of left ventricular hypertrophy as demonstrated by decreased cardiomyocyte diameter and collagen content.64

Inflammatory processes
Ang II is a potent pro-inflammatory agent.65,66 Ang II may modulate some responses of immune and inflammatory cells, such as chemotaxis, proliferation, and the differentiation of monocytes into macrophages. The presence of an inflammatory response in the arterial wall has been described in vascular disease, including atherosclerosis and hypertension.3,65-67 Endothelial dysfunction is characterised by increasing adheresiveness of circulating monocytes to the endothelium and expression of adhesion molecules as observed in response to Ang II. Hypertensive patients present elevated serum levels of adhesion molecules, and patients with coronary artery disease have high levels of L-selectin on leukocytes.68,69 The role of the AT1 receptor in the process of vascular inflammation is unclear. C-reactive protein, which is an inflammatory marker and independent predictor of incident hypertension, causes a sustained increase in BP in mice and is associated with a reduction in vascular AT1 receptor expression.70 To date, most of the pro-inflammatory effects of Ang II have been attributed to the AT1 receptor, but the AT2 receptor has a potential anti-inflammatory role. Indeed, in the context of atherosclerosis, Sales et al. have shown that expression of the AT2 receptor was increased in ApoE-knockout mice after eight weeks of high cholesterol diet. Using a model of ApoE/AT2 receptor double-knockout mice, they found that loss of the AT2 receptor resulted in elevated macrophages, VSMC, and collagen content in atherosclerotic lesions.71 Iwai et al. confirmed the role of the AT2 receptor in atherosclerotic lesions, and showed that the anti-inflammatory and antiproliferative actions of the AT2 receptor may be mediated by inhibition of NADPH oxidase-dependent production of reactive oxygen species.72 However, the role of the AT2 receptor in hypercholesterolaemia-induced atherosclerosis remains unclear. Indeed, Daugherty et al. were unable to demonstrate any effect of the AT2 receptor deficiency on the size of atherosclerotic lesions in low-density lipoprotein receptor-knockout mice."
Ischaemic processes
Ang II has also been implicated in ischaemic conditions such as stroke, or responses to ischaemia such as angiogenesis. Several clinical studies have shown that blockade of the RAS in the brain prevented stroke.74,75 Accumulating evidence from basic research has suggested a possible role of the RAS in ischaemic brain damage. Blockade of AT1 receptors by an ARB reduced the ischaemic area after middle cerebral arterial occlusion in genetically hypertensive rats.76 All the components of the RAS system, from (pro)renin to angiotensin receptors, are expressed in the brain.77 Focal cerebral ischaemia induced by middle cerebral artery occlusion was exaggerated in AT1 receptor-deficient mice.78 These results suggest that the AT1 receptor plays an important role in cerebral ischaemia, and plays an opposing action towards AT1 receptor-mediated effects. These antagonistic actions of AT1 receptor stimulation are mediated, at least in part, by modulation of blood flow and oxidative stress in the ischaemic region. More recently, Zhou et al. demonstrated that in a model of genetic hypertension increased stimulation of the AT1 receptor resulted in enhanced cerebrovascular vasoconstriction, remodelling and inflammation, whereas sustained AT1 receptor inhibition normalised cerebrovascular compliance and prevented inflammation, which suggests a complementary role of AT1 receptor stimulation.79 Iwanami et al. have also provided evidence that the AT2 receptor is an important component to consider regarding cellular therapy in stroke disease. They injected into the brain of mice, after ischaemia-reperfusion injury, bone marrow stromal cells (BMSC), which can differentiate into cells with some characteristics of neurons and astrocytes and protect neurons by secretion of growth factors and cytokines. They demonstrated that deletion of the AT2 receptor attenuates protective effects of BMSC, suggesting that stimulation of AT2 receptor signalling in BMSC plays a pivotal role in the contribution of BMSC treatment to brain protection after focal brain ischaemia-reperfusion injury.79

Angiogenesis is a tightly regulated process associated with other ischaemic conditions and includes movement of endothelial cells out of existing vessels, migration towards angiogenic stimuli, proliferation and formation of new endothelial tubes.80 Silvestre et al. have reported that the AT2 receptor exerted anti-angiogenic effects associated with activation of apoptosis in AT1 receptor-deficient and wild-type mice with surgically-induced hind-limb ischaemia. They speculated that the AT2 receptor may control vessel growth associated with tissue ischaemia through the activation of apoptosis.81 Hypoxia-induced angiogenesis mainly involves an increase in vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase protein content.80 New lines of evidence indicate that the angiogenic properties of Ang II may be due to an AT1 receptor-mediated increase in VEGF expression and upregulation of VEGF receptors (KDR).82,83 AT2 receptor stimulation counteracted VEGF-induced endothelial cell migration and in vitro tube formation pathway in endothelial cells.84 Using the AT2 receptor agonist CGP42112A, these authors demonstrated that the antimitogenic effects of Ang II on endothelial cells were exclusively mediated by the AT2 receptor.

Signalling pathways induced via the AT2 receptor
Whereas AT1 receptor-mediated signal transduction pathways have been well delineated, AT2 receptor-mediated actions and signalling pathways remain poorly understood.18,85-87 However, the discovery of new partners and processes of homo- or hetero-dimerisation of the two Ang II receptor subtypes may provide new research avenues for the understanding of AT2 receptor signalling. In contrast to AT1 receptors, AT2 receptors are not internalised upon binding of Ang II. Three major pathways have been described for AT2 receptor signalling following G-protein activation: (a) stimulation of protein phosphatases causing protein dephosphorylation; (b) activation of NO/cGMP pathway; and (c) stimulation of phospholipase A2 and release of arachidonic acid.88 The first mechanism and the discovery of new AT2 receptor partners will be the focus of the review as the second mechanism has already been discussed, and the third mechanism, which has been described in cardiomyocytes, proximal tubular epithelial cells, neurons and more recently in BMSC, is beyond the scope of the present review (figure 3).89,92

Activation of three specific serine/threonine phosphatases by the AT2 receptor: protein phosphatase 2A; MAP kinase phosphatase; and SH2 domain-containing tyrosine phosphatase (SHP-1), has been observed in different cell types. Growth factors, including Ang II via AT2 receptors, mediate their growth actions through tyrosine kinase receptors and several kinase-driven phosphorylation steps. Activation of the AT2 receptor counteracts these growth-promoting actions by dephosphorylation through activation of the phosphatases indicated above. A first study has suggested that dephosphorylation could be involved in AT1 receptor-induced vasodilatory responses via attenuation of RhoA/Rho kinase activation and myosin light chain phosphorylation in A10 VSMC and in mesenteric arteries from stroke-prone spontaneously hypertensive rats.26 Activation of SHP-1 after AT2 receptor stimulation...
leads to inactivation of casein kinase II and the activation of Ste20-related kinase, and subsequently to the inhibition of RhoA in VSMC, effects independent from the NO/cGMP pathway.95

In addition to the inhibitory effects on growth, dephosphorylation also seems to play an important role in the induction of apoptosis. Activation of the AT₂ receptor had an inhibitory effect on AT₁ receptor-mediated ERK1/2 phosphorylation. The authors demonstrated that SHP-1 is activated specifically via the AT₂ receptor and induces dephosphorylation of ERK1/2. Preventing SHP-1 activation abrogates AT₂ receptor stimulation-induced ERK1/2 inhibition and the pro-apoptotic effect of AT₂ receptors in rat foetal VSMC. ERK1/2 inhibition by the AT₂ receptor was also shown to be mediated via MAP kinase phosphatase and protein phosphatase 2A. Pro-apoptotic effects of AT₂ receptors are also mediated via the activation of p38MAPK and cell death-promoting proteases.5

SHP-1 plays a central role in AT₂ receptor signalling cascades leading to inhibition of AT₁ receptor-induced PYK2 and Jun kinase stimulation, transactivation of epidermal growth factor (EGFR) tyrosine kinase, and insulin-induced phosphotyrosinolinositol 3-kinase and Akt activation.97-99 The AT₂ receptor negatively crosstalks with receptor tyrosine kinases such as basic fibroblast growth factor, EGFR, and insulin receptors by targeting a very early step of receptor tyrosine kinase activation, i.e. autophosphorylation of the receptor.99-101 In VSMC from AT₂ receptor-transgenic mice, inhibition of EGFR transactivation induced by AT₂ receptor stimulation was found to involve rapid activation of tyrosine phosphatase SHP-1 and its increased association with EGFR (figure 4).99 Several studies have reported crosstalk between the AT₂ receptor and the AT₁ receptor, a mechanism by which the AT₂ receptor may act as an AT₁ receptor antagonist.33 Multiple reports indicate that vascular functions of the AT₂ receptor are unmasked when AT₁ receptors are inhibited.27,102,103 Data from Ang II receptor-knockout mice confirm that there is physiological crosstalk, at the level of vascular tone and apoptosis, between AT₂ receptors and AT₁ receptors.18,94 The AT₂ receptor inhibited AT₁ receptor-mediated phospholipase D activation through a NO/cGMP-dependent mechanism most likely via phosphorylation of RhoA, leading to inhibition of VSMC contraction.104 Ang II receptors are also reported to form homo- or hetero-dimers, and

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**Figure 3**

Major pathways described for angiotensin II type 2 receptor (AT₂) signalling following G-protein activation: (a) stimulation of protein phosphatases causing protein dephosphorylation; (b) activation of nitric oxide (NO)/cyclic guanosine 3’,5’-monophosphate (cGMP) pathway; and (c) stimulation of phospholipase A₂ (PLA₂) and release of arachidonic acid (AA). MKP-1 = MAP kinase phosphatase; PP2A = protein phosphatase 2A; SHP-1 = SH2 domain-containing tyrosine phosphatase.
undergo complex associations with other GPCR. AT$_2$ receptor homodimers enhance apoptosis signalling. Heterodimerisation of the AT$_1$ receptor with the AT$_2$ receptor was detected in transfected cells expressing AT$_1$ receptors and AT$_2$ receptors, on foetal fibroblasts, and in myometrial biopsies. AT$_2$ receptors antagonised activation of AT$_1$ receptors by direct binding of both receptors (figure 5). The B$_2$R also associates with the AT$_2$ receptor and enhances NO production.

**Figure 4**
Stimulation of the angiotensin II type 2 receptor (AT$_2$) activates the phosphatase SH2 domain-containing tyrosine phosphatase (SHP-1) and leads to the activation of p38 mitogen-activated protein kinase (p38MAPK), nuclear factor (NF)-xB and inhibition of c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase 1/2 (ERK1/2) and RhoA through the activation of Ste20-related kinase (SLK). SHP-1 also inhibits the association of the angiotensin II type 1 receptor (AT$_1$) and epidermal growth factor receptor (EGFR), and facilitates the association of the AT$_2$ and the EGFR. CK2 = casein kinase II.

**Figure 5**
Angiotensin II interactions (adapted from Mogi et al. 86). Angiotensin receptors are G-protein-coupled receptors and may form dimers as part of their normal function. The angiotensin II type 1 receptor (AT$_1$) and the angiotensin II type 2 receptor (AT$_2$) form homo- or hetero-dimers. Dimerisation of the AT$_1$ with the AT$_2$ inhibits cell proliferation and growth effects of the latter, whereas homodimerisation of the AT$_2$ or the AT$_2$ enhances cellular effects of these receptors. Dimerisation of the AT$_2$ and the bradykinin type 2 receptor (B$_2$R) enhances nitric oxide (NO) production and subsequently vasodilatation.
Ang II modulates inflammatory processes by inducing cytokine release and pro-inflammatory transcription factors such as nuclear factor-kB (NF-kB). NF-kB in turn regulates the expression of adhesion molecules and cytokines in several cell types. It appears that most of the pro-inflammatory effects of Ang II are mediated by activation of the AT$_1$ receptor. In VSMC, both the AT$_1$ receptor and the AT$_2$ receptor mediated Ang II-induced NF-kB DNA binding, inhibition of NF-kB degradation and transcription of a NF-kB reporter gene. Through the activation of AT$_1$ receptors, NF-kB upregulated the expression of interleukin-6, vascular cell adhesion molecule-1 and monocyte chemotactic protein-1, whereas AT$_2$ receptor-mediated activation of NF-kB increased the expression of RANTES.

In vivo, Ang II infusion increased renal NF-kB activity, which was partially diminished by treatment with AT$_1$ receptor and AT$_2$ receptor antagonists. In rats with unilateral ureteral obstruction, both antagonists also decreased NF-kB activation in the obstructed kidney.

Several studies have revealed that GPCR can mediate their intracellular effects through signalling pathways that are independent of G-proteins. The two isotypes of angiotensin receptors can interact with accessory proteins called GPCR-interacting proteins. These proteins associate mainly with the carboxyl terminus of GPCR. AT$_2$ receptor-interacting proteins may play key roles in AT$_2$ receptor signalling. Two major GPCR-interacting proteins have been described to interact with the AT$_2$ receptor, AT$_2$ receptor-interacting protein (ATIP) and AT$_2$ receptor-binding protein of 50 kDa (ATBP50). In Chinese hamster ovary cells expressing the human AT$_2$ receptor, ATIP inhibited growth factor-induced ERK1/2 activation and DNA synthesis, and attenuated insulin receptor autophosphorylation similarly to the AT$_2$ receptor. ATIP appears to act as a novel early component of the growth inhibitory signalling cascade of AT$_2$ receptors. In contrast, ATBP50 may act as a membrane-associated Golgi protein regulating the delivery of AT$_2$ receptors to the cell surface. Knocking down ATBP50 using siRNA reduced the cell surface expression of the AT$_2$ receptor by translocation of the receptor from the Golgi apparatus and attenuated its antiproliferative effects. Finally, the AT$_1$ receptor has also been shown to interact with a transcription factor, promyelocytic zinc finger protein (PLZF), which is significantly expressed in the heart. After Ang II stimulation, PLZF is activated, translocated from the cytosol to the plasma membrane, and colocalises with the AT$_1$ receptor, resulting in receptor endocytosis. PLZF then translocates to the nucleus and activates the transcription of target genes. In cardiomyocytes, PLZF has been shown to upregulate protein synthesis and induce cardiac hypertrophy. This suggests that the AT$_2$ receptor may induce cardiac hypertrophy. However, this contrasts with the concept that AT$_2$ receptor activation directly opposes the effects mediated by the AT$_1$ receptor that enhance cardiac hypertrophy.

**Implications for therapy and conclusions**

*In vitro*, AT$_2$ receptor stimulation inhibits cardiac and vascular smooth muscle growth and proliferation, stimulates apoptosis, and promotes extra-cellular matrix synthesis. *In vivo*, the situation may be more complex. If chronic AT$_2$ receptor stimulation leads to cardiovascular hypertrophy as suggested by some, then long-term chronic AT$_1$ receptor blockade is potentially associated with AT$_2$ receptor stimulation by the endogenous RAS, and treatments leading to AT$_2$ receptor stimulation may require more in-depth and long-term evaluation.

In hypertension, AT$_2$ receptors are upregulated and participate in vasodilatation after *in vivo* chronic AT$_1$ receptor blockade only in the vasculature of hypertensive animals and in humans at high cardiovascular risk such as hypertensive diabetic individuals, particularly after treatment with ARBs. Moreover, in normotensive and hypertensive rats, there is evidence that pharmacological stimulation of AT$_2$ receptors contributes to BP reduction in the presence of AT$_1$ receptor blockade. The increased stimulation of AT$_2$ receptors that occurs in the presence of AT$_1$ receptor blockade is generally believed to contribute to the benefits of ARBs, not just through control of BP but also through the antihypertrophic and antifibrotic effects of AT$_1$ receptor stimulation. Ang II may also be produced according to some studies by alternative pathways not blocked by ACE-I therapy, although the physiological and clinical importance of this remains unclear. For these reasons, it has been suggested that ARBs might offer greater benefits than ACE-Is in patients with cardiovascular disease. However, clinical trials have failed to support the idea that ARBs are superior to ACE-Is in chronic heart failure or in patients at high cardiovascular risk. Nonetheless, it is increasingly appreciated that the effects of the AT$_2$ receptor are dependent on the pathophysiological condition being considered. There are species-dependent and vessel type-dependent differences in vascular responses to AT$_2$ receptor stimulation, which may explain the controversial results reported in the literature. It is therefore difficult to predict the effects in humans of long-term over-stimulation of AT$_2$ receptors resulting from ARB therapy. In hypertensive diabetic patients, ARB
therapy for one year demonstrated beneficial effects on BP lowering and vascular structural changes, associated with increased expression of AT2 receptors on resistance arteries. Finally, both in vivo and in vitro studies have documented sustained vasodilator and hypotensive actions of continuous AT2 receptor agonist administration, with no desensitisation of the vascular responses elicited. These findings predict that AT2 receptor stimulation should be a beneficial addition to AT1 receptor blockade. A major problem at this time is the lack of selective AT2 receptor agonists. The only such compound available is CGP42112A, a peptidic partial agonist, which at high concentrations acts as an AT2 receptor antagonist, and also interacts with the AT1 receptor. However, results from animal experimental studies have failed so far to provide clear answers regarding benefits of stimulation of AT2 receptors with CGP42112A.

In conclusion, experimental findings reviewed here are so far somewhat contradictory and point to the need for a more in-depth understanding of the role of the AT2 receptor in the cardiovascular system. The past few years have witnessed major advances in our understanding of the function of the AT2 receptor, which appears to mediate important cardiovascular actions of Ang II. However, the precise intracellular signalling mechanisms whereby this Ang II receptor subtype exerts its actions need further clarification. Although the AT2 receptor seems to have countervailing actions to the AT1 receptor regarding vasomotor effects, the situation is less clear for other pathophysiological conditions described in this review. Answers might come from the discovery of new signalling partners of the AT2 receptor, and from a better understanding of the complex interactions between the two angiotensin receptor subtypes.

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