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REVIEW

Angiotensin AT_2 receptors: cardiovascular hope or hype?

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Abbreviations: AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type 2 receptor; ACE, angiotensin-converting enzyme; BP, blood pressure; NO, nitric oxide; RAS, renin–angiotensin system; SHR, spontaneously hypertensive rats; VSMC, vascular smooth muscle cells; WKY, Wistar–Kyoto

It is widely accepted that the angiotensin II type 1 receptor (AT_1R) accounts for the majority of cardiovascular effects evoked by angiotensin II (Ang II), such as contraction/pressor activity and growth-promoting effects leading to cardiac and vascular hypertrophy. However, there has been an increasing body of evidence that indicates that the angiotensin II type 2 receptor (AT_2R) may exert pharmacological actions *per se* as well as play a role in pathophysiological processes. In particular, it has been suggested that the AT_2R may exert beneficial vasodilator and antigrowth effects, as well as contribute to the efficacy of AT_1R antagonists (see reviews by Matsubara, 1998; Horiuchi *et al*., 1999a; de Gasparo & Siragy, 1999; Unger, 1999; de Gasparo *et al*., 2000; Gallinat *et al*., 2000; Henrion *et al*., 2001).

Therefore, for the purposes of the current review, we have updated the status of such work, particularly in light of some recent data suggesting that the AT_2R, in fact, causes opposite effects, for example, cardiac growth-promoting effects (Senbonmatsu *et al*., 2000; Ichihara *et al*., 2001). In addition, we have critically reviewed whether or not experimental outcomes are consistent with the hypothesis that AT_2R may contribute to the therapeutic effects of AT_1R antagonists (de Gasparo *et al*., 2000; Carey *et al*., 2001a; Siragy, 2002).

Distribution of AT_2 receptors

Ang II mediates its biological actions by binding to distinct membrane-bound receptors and consequently activating multiple intracellular pathways. Two major Ang II receptor subtypes have been identified and cloned as AT_1R and AT_2R (de Gasparo *et al*., 2000). Ang II receptors have been localised throughout the vasculature, heart, kidneys, adrenals, nervous and endocrine systems. However, there is different anatomical distribution and expression of the AT_1R/AT_2R as well as differences in signalling pathways and function.

In foetal tissue, AT_2R is the predominant subtype expressed, although this situation is rapidly reversed after birth with the AT_1R becoming the dominant subtype in the adult (Matsubara, 1998; Horiuchi *et al*., 1999a; de Gasparo *et al*., 2000). While there is a relatively lower expression of the AT_2R in adult tissue, AT_2R predominates at particular sites including uterus, ovary, adrenal medulla as well as in distinct areas of the brain (Zhuo *et al*., 1995; de Gasparo *et al*., 2000; Roulston *et al*., 2003). The distribution of AT_2R in tissues relevant to the cardiovascular system is briefly considered below.

Kidney

AT_2Rs are detected in adult kidney, although extent and location varies considerably depending on techniques used. Autoradiographic studies using nonselective AT ligands with selective AT_1R and AT_2R displacing agents (Zhuo *et al*., 1995) were less able to detect AT_2R levels than AT_2R identified by selective AT_2R autoradiography using CGP42112 (Cao *et al*., 2000). Likewise, there are distinctions between AT_2R mRNA and immunohistochemical studies (Ozono *et al*., 1997; Miyata *et al*., 1999). Generally, AT_2R mRNA and protein were distributed throughout tubular and vascular segments of the renal cortex and medulla, although results were more equivocal for the glomerulus (Ozono *et al*., 1997; Miyata *et al*., 1999; Cao *et al*., 2000). AT_2Rs are also developmentally regulated with greater AT_2R expression observed in foetal kidney (Ciuffo *et al*., 1993; Shanmugam *et al*., 1995; Ozono *et al*., 1997).

One aspect of renal AT_2R function that has received attention is its role in pressure natriuresis. AT_2R may play a role in pressure natriuresis, thereby opposing the antinatriuretic effects of AT_1R activation, since the AT_2R antagonist PD123319 decreased urinary sodium excretion in renal hypertensive rats while valsartan exerted opposite effects (Siragy & Carey, 1999). This natriuretic effect of AT_2R was confirmed in AT_2R knockout mice in which pressure natriuresis was inhibited (Siragy *et al*., 1999a; Gross *et al*., 2000). However, the exact nature of AT_2R involvement in this field of research is somewhat unclear since others reported that AT_2R stimulation attenuated pressure natriuresis (Lo *et al*., 1995; Liu *et al*., 1999).

Sodium depletion is reported to upregulate renal AT_2R (Ozono *et al*., 1997), whereas AT_2R was downregulated only in the ischaemic kidney from 2-kidney, 1-clip rats (Wang *et al*., 1999). These contrasting effects are likely to be model-specific since both situations result in a heightened RAS; furthermore, Ang II infusion *per se* did not alter renal AT_2R expression

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(Wang *et al*., 1999). The AT_2R was also decreased in kidneys of SHR-SP compared with WKY rats, and growth-factor-dependent induction of AT_2R occurred in cultured mesangial cells from WKY rats but not from SHR-SP (Goto *et al*., 2002). In addition, there was a marked increase in AT_2R expression in rats with renal failure (Bautista *et al*., 2001).

Vascular

The common misconception that AT_2R do not exist in appreciable amounts in vasculature is slowly changing. AT_2Rs are actually located in many different vessel types, albeit at low (but functional) levels. Indeed, early reports of AT_2R comprising approximately 30–40% of AT receptors in rat aorta (Chang & Lotti, 1991; Viswanathan *et al*., 1991) were largely ignored until a functional vasodilator role of AT_2R began to emerge. Subsequently, AT_2Rs have been detected in vessels such as mesenteric (Matrougui *et al*., 1999, 2000; Touyz *et al*., 1999) and uterine (Cox & Cohen, 1996; Burrell & Lumbers, 1997; McMullen *et al*., 1999) arteries. AT_2R in vasculature is also developmentally regulated (Viswanathan *et al*., 1991; Nakajima *et al*., 1995), whereas the AT_1R is expressed at a relatively constant level throughout life (de Gasparo *et al*., 2000).

AT_2R mRNA expression and Ang II receptor autoradiography have also provided evidence for AT_2R in kidney vasculature (Zhuo *et al*., 1995, 1996; Matsubara, 1998; Miyata *et al*., 1999). Indeed, AT_2R predominate in the adventitia of the human renal artery and arcuate and interlobar arteries (Goldfarb *et al*., 1994; Zhuo *et al*., 1996), or in vascular smooth muscle cells of such vessels (Grone *et al*., 1992), although others did not detect AT_2R in human kidney (Sechi *et al*., 1992a). AT_2Rs are also present in endothelial cells and vascular smooth muscle cells in small resistance arteries obtained from rats (Nora *et al*., 1998; Matrougui *et al*., 1999), and AT_2Rs have recently been detected in mouse coronary arteries (Akishita *et al*., 2000a; Wu *et al*., 2002).

Various pathologies can affect AT_2R levels in vasculature. AT_2Rs are increased in skin during wound healing (Kimura *et al*., 1992; Viswanathan & Saavedra, 1992). Balloon injury to rat carotid arteries resulted in detectable AT_2R mRNA in vessel wall, which was otherwise below the limit of detection in uninjured vessels (Nakajima *et al*., 1995). Likewise, an inflammatory cuff model caused re-expression of AT_2R in media/neointima of mouse femoral artery (Akishita *et al*., 2000a). The AT_2R can also be regulated in the vasculature by Ang II itself in a heterogeneous manner, since chronic infusions of this peptide have been reported to decrease AT_2R expression in sheep uterine arteries (McMullen *et al*., 2001), but increase AT_2R expression in rat mesenteric arteries (Bonnet *et al*., 2001). In addition, there is an increase in AT_1R expression in VSMC from AT_2R knockout mice (Tanaka *et al*., 1999), whereas overexpression of AT_2R in vasculature of mice does not alter the level of expression of the AT_1R (Tsutsumi *et al*., 1999). There is greater expression of vascular AT_2R in young SHR (Touyz *et al*., 1999) and adult SHR (Otsuka *et al*., 1998) compared with WKY rats.

Heart

Both receptor subtypes exist in the heart although, in most animal studies, the AT_2R is the minority subtype (Chang & Lotti, 1991; Baker *et al*., 1992; Sechi *et al*., 1992b; Suzuki *et al*., 1993; Wang *et al*., 1998; Busche *et al*., 2000). AT_2Rs are expressed at low levels in adult rat cardiomyocytes (Busche *et al*., 2000) but are increased in both absolute levels and relative to AT_1R in hypertrophied rat and failing hamster hearts (Lopez *et al*., 1994; Ohkubo *et al*., 1997; Bartunek *et al*., 1999). AT_2R was increased in SHR heart compared with WKY in one study (Makino *et al*., 1999) but not in another by the same group (Makino *et al*., 1997). AT_2R increased within 1 day after myocardial infarction (Nio *et al*., 1995). Moreover, studies using single-cell reverse transcriptase–polymerase chain reaction have shown that the proportion of rat cardiomyocytes expressing AT_2R increased from a basal state of 10% to approximately 50% 1 week after myocardial infarction (Busche *et al*., 2000). By contrast, ischaemia and reperfusion decreased AT_2R mRNA and protein acutely in isolated working rat heart (Xu *et al*., 2000).

Although animal studies indicate that the AT_1R is the major binding site in adult hearts, the AT_2R gains particular prominence in human heart. In both normal noninfarcted or hypertrophied human hearts, there is a predominance of AT_2R binding sites in the myocardium (Brink *et al*., 1996; Matsubara, 1998; Wharton *et al*., 1998; de Gasparo *et al*., 2000). Even in studies that indicate that the AT_2R is not the major subtype, there were approximately equal proportions of both AT_2R and AT_1R in nonfailing human hearts (Tsutsumi *et al*., 1998). In most clinical reports, AT_1R density tends to decrease with cardiac dysfunction (Regitz-Zagrosek *et al*., 1995; Rogg *et al*., 1996; Asano *et al*., 1997; Haywood *et al*., 1997; Tsutsumi *et al*., 1998; Goette *et al*., 2000), whereas AT_2R density may be decreased (Regitz-Zagrosek *et al*., 1995; Matsumoto *et al*., 2000), increased (Rogg *et al*., 1996; Tsutsumi *et al*., 1998; Goette *et al*., 2000) or unchanged (Asano *et al*., 1997; Haywood *et al*., 1997) with increasing cardiac dysfunction. Discrepancies between studies are hardly surprising given the different types and severity of heart failure examined, together with a range of detection methods to identify AT_2R including ligand binding, autoradiography, mRNA expression and immunohistochemistry. Indeed, these data generally favour an increase in the ratio of AT_2R/AT_1R in human heart. Of those studies that have examined cellular localisation, the AT_2R was mainly localised, using autoradiography, on fibroblasts at sites of fibrosis (Brink *et al*., 1996; Tsutsumi *et al*., 1998; Wharton *et al*., 1998). However, in immunohistochemical studies using less-diseased cardiac tissue, AT_2R was confined to myocytes, not fibroblasts, in atrial tissue obtained from patients undergoing coronary artery bypass graft surgery (Matsumoto *et al*., 2000) or in myocardium of 4-week-old rats (Wang *et al*., 1998). Experimental data in failing myopathic hamster heart are consistent with AT_2R upregulation in fibrotic regions (Ohkubo *et al*., 1997).

AT_2R signalling

Kinase/phosphatase crosstalk

Numerous studies have revealed that the signal transduction mechanisms associated with AT_2R activation are appreciably different to those linked with AT_1R coupling. Moreover, it is becoming increasingly accepted that activation of AT_2R in various cell lines results in the stimulation of protein

phosphatases, which directly inhibit the protein kinase pathways (and hence growth-promoting function) associated with AT_1R (Horiuchi *et al*., 1999a).

In PC12W cells expressing only AT_2R, Ang II rapidly induces activation of protein tyrosine phosphatase (PTPase), which then causes dephosphorylation (and hence inactivation) of tyrosine residues; an effect that is abolished by general PTPase inhibitors such as the vanadate compounds (Bottari *et al*., 1992; Brechler *et al*., 1994). These findings have been extended to various other cell lines, including N1E-115 neuroblastoma cells (Nahmias *et al*., 1995), nondifferentiated NG108-15 cells (Buisson *et al*., 1995) and R3T3 fibroblasts (Tsuzuki *et al*., 1996a, b).

More recently, attempts have been made to elucidate the specific PTPases involved in AT_2R activation. In PC12W cells (Yamada *et al*., 1996; Horiuchi *et al*., 1997) and R3T3 cells (Yamada *et al*., 1996), for example, pretreatment with antisense oligonucleotide of mitogen-activated protein kinase phosphatase-1 (MKP-1) inhibited the proapoptotic effect mediated by the AT_2R. Furthermore, in cultured rat vascular smooth muscle cells (VSMC), Ang II was shown to stimulate mRNA expression and protein synthesis of a PTPase with selective activity for MAP kinase (Duff *et al*., 1993). A similar increase in MKP-1 mRNA levels, following AT_2R activation, has also been reported in adult rat ventricular myocytes (Fischer *et al*., 1998). Consistent with these findings, AT_2R overexpression in VSMC (Nakajima *et al*., 1995) demonstrated an inhibition of AT_1R-mediated MAP kinase activity (extracellular-regulated kinases (ERK) 1 and 2), which presumably involves activation of a particular MKP-1. Collectively, these results strongly suggest that MKP-1 is one of the phosphatases involved in AT_2R signal transduction.

SHP-1 is a soluble PTPase that has been implicated in the termination of signalling by cytokines and growth factor receptors, and there is now evidence to suggest that it may also serve as an early transducer in AT_2R signalling (Bedecs *et al*., 1997; Lehtonen *et al*., 1999; Cui *et al*., 2001; Shibasaki *et al*., 2001). In PC12W cells (Lehtonen *et al*., 1999) and rat foetal VSMC (Cui *et al*., 2001), a functional link has been established between AT_2R, SHP-1 tyrosine phosphatase activation and apoptosis, whereby transfection of an inactive SHP-1 mutant into cells not only prevented SHP-1 activation, but also inhibited AT_2R-mediated apoptosis (Lehtonen *et al*., 1999; Cui *et al*., 2001). Dephosphorylation of phosphothreonine by serine/threonine phosphatase (PP2A) can also inactivate MAP kinase (Gallinat *et al*., 2000). Indeed, studies in neurons cultured from neonatal rat hypothalamus and brain stem indicate that AT_2R stimulation activates PP2A (Huang *et al*., 1995), thereby inhibiting AT_1R-mediated MAP kinase activation (Huang *et al*., 1996) and inducing apoptosis (Shenoy *et al*., 1999). However, in some circumstances, AT_2R stimulation transiently increased ERK phosphorylation prior to inhibition of MAP kinases within the process of cell differentiation (Stroth *et al*., 2000).

It is worth noting that, in addition to modulating the more extensively studied ERK pathway (Nakajima *et al*., 1995; Huang *et al*., 1996; Yamada *et al*., 1996, 1998; Bedecs *et al*., 1997; Horiuchi *et al*., 1997), AT_2Rs are capable of inducing the dephosphorylation of other protein kinsases. Janus kinases and signal transducers and activators of transcription (STAT) represent important signalling pathways through which Ang II (*via* the AT_1R), and other growth factors, stimulate VSMC proliferation (Marrero *et al*., 1995; de Gasparo *et al*., 2000). In AT_2R cDNA-transfected rat VSMC, R3T3 fibroblasts and mouse foetal VSMC (which express AT_2R naturally), stimulation of AT_2 receptors has been shown to reduce AT_1 receptor-mediated tyrosine phosphorylation of STAT1, STAT2 and STAT3 (Horiuchi *et al*., 1999b), and also inhibits effects of the growth factors, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), on STAT1 activity (Horiuchi *et al*., 1999b). Thus, it appears that Ang II can impair the activity of various growth factor signalling pathways through the activation of AT_2R.

Collectively, the studies performed to date demonstrate that AT_2R stimulation can activate tyrosine and serine/threonine phosphatases, depending on the cell line in question. These phosphatases serve to reverse, or at least counter-regulate, the cell proliferative- and growth-promoting effects mediated by the various protein kinases in response to AT_1R activation.

While AT_2R-mediated ERK inactivation has been clearly established in cell culture, the question remains as to whether or not this modulatory influence extends to a physiological setting. Interestingly, Masaki *et al* (1998) reported that transgenic mice overexpressing cardiac AT_2R exhibit reduced cardiac ERK activity, relative to their wild-type litter mates. Conversely, foetal VSMC from AT_2R-null mice demonstrate a generally enhanced growth phenotype, as well as increased basal- and serum-induced ERK phosphorylation levels (Akishita *et al*., 1999). On the basis of these genetic manipulation studies, at least, it appears that ERK inactivation by the AT_2R may play a physiological role *in vivo*, in relation to cardiac and vascular growth.

NO/cyclic GMP pathway

A number of studies have demonstrated a link between AT_2R activation and alterations in cellular cyclic GMP levels. Ang II elicits an increase in cyclic GMP levels, in cultured bovine aortic endothelial cells (Wiemer *et al*., 1993; Saito *et al*., 1996), *via* an AT_2R-mediated, NO (and presumably soluble guanylyl cyclase)-dependent pathway (Wiemer *et al*., 1993). A stimulatory effect of Ang II on cellular NO and/or cyclic GMP levels has also been reported in other aortic endothelial cell preparations (Pueyo *et al*., 1998) and cultured N1E-115 neuroblastoma cells (Zarahn *et al*., 1992; Chaki & Inagami, 1993). Interestingly, Ang II-induced activation of the cellular NO-cyclic GMP pathway may be mediated partially (Zarahn *et al*., 1992), or in some cases, exclusively (Caputo *et al*., 1995; Saito *et al*., 1996; Pueyo *et al*., 1998) by AT_1R.

Ang II is also reported to stimulate NO release directly in isolated blood vessels (Seyedi *et al*., 1995; Thorup *et al*., 1998; Thorup *et al*., 1999); a component of which is mediated by AT_2R. In dog coronary microvessels and large coronary arteries, the Ang II-induced increase in nitrite levels was abolished by both AT_1R and AT_2R antagonists (losartan and PD 123319, respectively), as well as by the nonselective Ang II receptor antagonist, saralasin (Seyedi *et al*., 1995). In isolated perfused rat renal arteries, losartan significantly reduced Ang II-stimulated NO release, as measured by a NO-sensitive microelectrode, without abolishing the response altogether (Seyedi *et al*., 1995; Thorup *et al*., 1998, 1999), and it was concluded that the residual, losartan-insensitive increase in NO production may be mediated by an AT_2R mechanism. Collectively, the results of studies performed to date in both

cell culture and isolated vascular preparations, suggest that AT_1R and AT_2R may not always act in direct opposition to each other, at least at the level of cyclic GMP production.

Role of bradykinin

A study performed by Siragy *et al*. (1996) in conscious, uninephrectomised dogs initiated the concept that AT_2R may stimulate the release of endogenous bradykinin, in addition to NO *in vivo*. In that study, a non-AT_1R was identified as mediating renal bradykinin and cyclic GMP production in response to endogenous RAS activation (Siragy *et al*., 1996). With the use of the same renal microdialysis technique, bradykinin has indeed been shown to stimulate NO release *via* the activation of B_2 receptors (Siragy *et al*., 1997). Subsequent studies have revealed that both endogenous Ang II (following dietary Na^+ restriction) and exogenously infused Ang II stimulate an increase in cyclic GMP content in renal interstitial fluid of conscious rats; an effect that is abolished by AT_2R blockade (Siragy & Carey, 1996) or AT_2R antisense oligonucleotide (Moore *et al*., 2001), as well as NOS inhibition (Siragy & Carey, 1997, 1999; Siragy *et al*., 2000) and bradykinin B_2 receptor blockade (Siragy & Carey, 1999; Siragy *et al*., 2000; 2001). In a renal wrap model of hypertension, Ang II infusion elicited an AT_2R-mediated increase in bradykinin levels (Siragy & Carey, 1999), thereby confirming a direct link between AT_2R activation and subsequent bradykinin synthesis/release.

Genetic studies involving either the targeted deletion of the AT_2R gene (Siragy *et al*., 1999a) or AT_2 receptor over expression in VSMC of transgenic mice (Tsutsumi *et al*., 1999) have provided further support for a link between AT_2R-mediated vasodepression and associated bradykinin and/or NO production. Specifically, AT_2R-null mice exhibit markedly reduced basal- and Ang II-induced cyclic GMP and bradykinin levels in renal interstitial fluid, and are hypersensitive to the pressor and antidiuretic effects of Ang II, relative to their wild-type litter mates (Siragy *et al*., 1999a). It has been suggested that the exaggerated vascular reactivity to Ang II in AT_2R-null mice is at least partially due to an increase in vascular AT_1R expression (Tanaka *et al*., 1999); however, it is unlikely that this would account for the observed deficiency of the bradykinin–NO–cyclic GMP vasodilator cascade. On the other hand, AT_2R overexpression in VSMC in mice unmasked an Ang II-induced increase in aortic cyclic GMP content (which was reversed by cotreatment with either AT_2 or B_2 receptor antagonists, or NOS inhibition), and was associated with complete abolishment of the pressor response to Ang II *in vivo*; an effect that was also reversed by these same inhibitors (Tsutsumi *et al*., 1999). In addition, in stroke-prone spontaneously hypertensive rats, acute Ang II infusion produced a significant increase in the cyclic GMP content of aortic explants *via* a mechanism that involves AT_2R and endothelial-derived bradykinin and NO (Gohlke *et al*., 1998). While the precise nature of the AT_2R/bradykinin interaction is not fully understood, it has been proposed that Ang II, in reducing intracellular pH levels in endothelial cells, may in turn activate acid-optimum kininogenases to cleave bradykinin from intracellularly stored kininogens (Wiemer *et al*., 1993; Tsutsumi *et al*., 1999). However, there are also recent data indicating that AT_2R stimulation increases cyclic GMP independently of bradykinin B_2 receptors since cyclic GMP levels were in fact enhanced in PC12W cells in the presence of B_2 receptor blockade (Zhao *et al*., 2003); consistent with findings we have also observed in rat uterine arteries (Hannan *et al*., 2003b).

Thus, the weight of evidence presented to date indicates that the AT_2Rs are located within the cardiovascular system (heart, kidney, vasculature–albeit at lower levels of expression than AT_1Rs) with appropriate signal transduction pathways for potentially important functional effects (e.g. direct vasodilator pathways and indirect anti-AT_1R trophic effects).

AT_2R-mediated relaxation/vasodilatation

There is an increasing amount of literature that has demonstrated AT_2R-mediated relaxation directly in a range of isolated arteries including rabbit renal arterioles (Arima *et al*., 1997; Endo *et al*., 1997, 1998), rabbit cerebral arteries (Haberl, 1994), and rat mesenteric arteries (Matrougui *et al*., 1999, 2000; Dimitropoulou *et al*., 2001; Katada & Majima, 2002; Widdop *et al*., 2002). In the presence of an AT_1R antagonist, Ang II caused an approximate 30% increase in the diameter of preconstricted, microperfused rabbit afferent (Arima *et al*., 1997; Endo *et al*., 1998) and efferent (Endo *et al*., 1997) arterioles in a PD123319-sensitive manner. An AT_2 vasodilator effect was confirmed using the selective AT_2R agonist, CGP42112, in the absence of AT_1R blockade (Arima *et al*., 1997). Moreover, it has been suggested that, in the rabbit afferent arteriole, AT_2R-mediated, endothelium-dependent relaxation occurs *via* a cytochrome *P*-450-dependent, NO-independent pathway, which may involve the production of epoxyeicosatrienoic acid and subsequent opening of large conductance, Ca^{2+}-activated K^+ channels (Arima *et al*., 1997). Interestingly, impaired renal AT_2R vasodilator function is associated with exaggerated Ang II vasoconstrictor responses in the afferent arterioles of prehypertensive SHR rats (Endo *et al*., 1998). Collectively, these studies demonstrate a potentially important role of AT_2R in the regulation of glomerular haemodynamics. Moreover, recent studies from our laboratories indicate the complex nature of the renal effects of AT_2R, at least in the rabbit, since AT_2R activation counteracted both AT_1R-mediated vasoconstriction in the cortex and, unexpectedly, also AT_1R-mediated vasodilation in the medulla (Duke *et al*., 2003).

Ang II has also been shown to stimulate flow-induced dilatation of perfused rat mesenteric arteries *in situ* (Matrougui *et al*., 1999, 2000), whereby AT_2R blockade produced a decrease in diameter of arterial branches submitted to pressure and flow; an effect that was prevented by NOS inhibition and endothelial disruption (Matrougui *et al*., 1999). This suggests that endogenous Ang II may activate endothelial AT_2R-mediated NO release, thereby contributing to flow-induced dilatation. Indeed, Matrougui *et al*. (1999) reported that AT_2Rs mediate between 20 and 39% of dilatation in response to shear stress. Flow-mediated dilatation of perfused rat mesenteric arteries in the presence of AT_1R antagonism is also inhibited significantly by a bradykinin B_2 receptor antagonist, as well as by PD123319 (Katada & Majima, 2002). Interestingly, in that study, arteries isolated from kininogen-deficient Brown Norway Katholiek rats displayed markedly impaired AT_2R-mediated vasodilator responses, relative to their wild-type counterparts (Katada & Majima, 2002), suggesting an important role of endogenous bradykinin synthesis in the mechanisms underlying acute AT_2R vasodilatation. However,

Dimitropoulou *et al*. (2001) reported, on the basis of functional, whole-cell and single-channel patch-clamp studies, that Ang II relaxes rat mesenteric microvessels *via* stimulation of AT_2R, with the subsequent opening of large-conductance, calcium- and voltage-activated K^+ (BK_{Ca}) channels, leading to membrane repolarisation and vasodilation. It was proposed that arachidonic acid metabolites may serve as intermediate messengers in this novel, endothelium-independent AT_2-BK_{Ca} channel pathway.

Thus, acute AT_2R-mediated vasodilator responses may be endothelium-dependent (Arima *et al*., 1997; Matrougui *et al*., 1999) or -independent (Dimitropoulou *et al*., 2001), according to the techniques employed, and appear to involve a range of signalling pathways, including NO (Matrougui *et al*., 1999) and bradykinin production (Katada & Majima, 2002), activation of cytochrome *P*-450 epoxygenase pathways (Arima *et al*., 1997) and modulation of K^+ channel activity (Arima *et al*., 1997; Dimitropoulou *et al*., 2001).

In other circumstances, direct AT_2R-mediated relaxation was not found (Zwart *et al*., 1998), but instead the AT_2R antagonist PD123319 enhanced AT_1R-mediated contraction in uterine arteries (Zwart *et al*., 1998; McMullen *et al*., 1999; St-Louis *et al*., 2001; Hannan *et al*., 2003a), which implied a vasodilator action of AT_2R, most likely involving NO and bradykinin (Hannan *et al*., 2003a). The sensitivity of the experimental preparation is an important consideration since conventional wire myographs are less likely to detect AT_2R-mediated relaxation (Zwart *et al*., 1998) compared with cannulated blood vessel preparations (Matrougui *et al*., 1999, 2000; Dimitropoulou *et al*., 2001; Katada & Majima, 2002; Widdop *et al*., 2002). In one study, when complete concentration–response curves were constructed for AT_2R activation in isolated mesenteric arteries, the relaxation produced was concentration-dependent but relatively small ($\sim$25%) compared with maximum acetylcholine-mediated relaxation (Widdop *et al*., 2002). However, others reported full AT_2R-mediated relaxation using the same preparation (Dimitropoulou *et al*., 2001). While the discrepancy between the two studies may relate to the use of different precontraction agents, even submaximal changes in resistance-like vessels could potentially result in marked haemodynamic effects. Moreover, this PD123319-sensitive AT_2R-mediated vasodilatation, in contrast to AT_1R-mediated vasoconstriction, was a highly reproducible phenomenon; maintained even in the presence of chronic AT_1R blockade, when circulating Ang II levels are elevated (Widdop *et al*., 2002). This preservation of AT_2R function is critical when considering its potential physiological role in the antihypertensive effects of AT_1R antagonists (see later).

In vivo evidence for AT_2R-mediated vasodilatation is less exhaustive and has largely come from blood pressure (BP) measurements using two separate approaches: either indirectly based on enhanced Ang II-mediated vasoconstriction in the presence of AT_2R blockade or infusing Ang II in the presence of AT_1R blockade in order to stimulate AT_2R (Scheuer & Perrone, 1993; Munzenmaier & Greene, 1996; Gohlke *et al*., 1998). While these latter studies provided functional evidence of AT_2R activation, there were no uniform BP-lowering effects, most likely because any potential BP reductions may have been masked by direct vasoconstriction caused by infusion of a large dose of Ang II alone (Gohlke *et al*., 1998). Instead, Barber *et al*. (1999) selectively stimulated AT_2R using the agonist CGP42112 and found that it caused a depressor response in conscious SHR in the presence of AT_1 receptor blockade; an effect which was subsequently confirmed by Carey *et al*. (2001a).

More recently, direct haemodynamic measurements have confirmed the inferences made from BP measurements. In particular, AT_2R stimulation (Ang II infused in the presence of AT_1R blockade) caused coronary vasodilatation, but not renal vasodilatation, in anaesthetised rats previously given a myocardial infarction (Schuijt *et al*., 2001), although no vasodilatation was evident in noninfarcted anaesthetised rats (Schuijt *et al*., 1999). In addition, Lambers *et al*. (2000) reported that AT_1R blockade unmasked Ang II-mediated increases in uterine blood flow, which were reversed by PD123319 and NOS inhibition. Similarly, Ang II-induced renal vasodilatation was unmasked by AT_1R blockade and this effect was enhanced with renal failure (Bautista *et al*., 2001). More recently, the AT_2R depressor response previously observed in SHR (Barber *et al*., 1999) has been examined in haemodynamically instrumented conscious rats. Against a background of AT_1R blockade, the AT_2R agonist, CGP42112, caused generalised vasodilatation in the renal, mesenteric and hindquarters circulations of SHR but not WKY rats (Li & Widdop, 2003). Consistent with a vasodilator role for the AT_2R, it was also established in the same study that the AT_2R antagonist PD123319 itself exerted haemodynamic effects consisting of modest pressor activity and renal and mesenteric vasoconstriction. Thus, these data suggest that the AT_2R tonically modulated vascular tone in the renal and mesenteric circulations, at least in conscious SHR (Li & Widdop, 2003), which agrees with a recent study in which antisense oligodeoxynucleotide directed against the AT_2R mRNA infused into the renal interstitium in rats increased blood pressure (Moore *et al*., 2001). In this context, a functional role of AT_2R may also be apparent in human forearm vasculature. In healthy male volunteers, Ang II evoked dose-dependent vasoconstriction in the forearm circulation (Phoon & Howes, 2001), whereas this effect was converted into dose-dependent vasodilatation in elderly female patients who were on 3-week candesartan therapy (Phoon & Howes, 2002). In the latter study, PD123319 elevated baseline forearm vascular resistance suggesting a tonic AT_2R vasodilator influence.

In terms of the *chronic* pharmacodynamic effects of selective AT_2R stimulation, the combination of Ang II infusion and AT_1R blockade failed to decrease BP (Li *et al*., 1998; Cao *et al*., 1999; Diep *et al*., 1999; Tea *et al*., 2000), most likely because of large pressor doses of Ang II invariably being employed. However, in mice overexpressing AT_2R in vasculature, an Ang II infusion over 14 days, in the presence of an AT_1R antagonist, caused a prolonged depressor effect in these transgenic mice (Tsutsumi *et al*., 1999). Moreover, Carey and colleagues demonstrated that either CGP42112 or Ang II combined with valsartan progressively decreased systolic BP (SBP) in normotensive rats over 8–9 days to a greater extent than AT_1R blockade alone. Furthermore, a 4-day infusion of CGP42112 alone actually lowered SBP (Carey *et al*., 2001b), which is consistent with a persistent vasodilator action of AT_2R without desensitisation, even in the presence of AT_1R blockade (Widdop *et al*., 2002).

Therefore, it may be speculated that AT_2R-mediated haemodynamic effects (Tsutsumi *et al*., 1999; Bautista *et al*., 2001; Schuijt *et al*., 2001; Li & Widdop, 2003) were more

apparent in pathological states in which there is often an upregulation of AT_2R. For example, AT_2R-mediated vasodilatation occurred in SHR but not in WKY rats (Barber *et al*., 1999; Li & Widdop, 2003), although this was not the case using the isolated mesenteric artery (Matrougui *et al*., 2000). However, the conscious animal data are consistent with analogous *in vivo* protocols in which AT_2R-mediated increases in vascular cGMP production occurred in SHRSP (Gohlke *et al*., 1998) but not in WKY rats (Pees *et al*., 2003).

Structural effects mediated by AT_2R

In vitro

Distinct from the plethora of acute pharmacodynamic effects of AT_2R already described, there is an increasing body of evidence that AT_2R may tonically modulate cardiovascular structure, although the reported changes are, in some case, contradictory.

Given the signalling profiles of AT_2R, much research has focused on its role in growth and remodelling. A variety of *in vitro*/*in situ* studies have found that AT_2R activation exerts antigrowth effects, largely based on potentiated growth in the presence of the AT_2R antagonist PD123319. Using cultured rat coronary endothelial cells, Stoll *et al*. (1995) reported that Ang II did not induce proliferation unless AT_2R were blocked, whereas Ang II alone caused AT_1R-mediated growth of VSMC. This study nicely illustrated the fact that net growth depends on the expression of AT_2R on appropriate cellular targets, since only the endothelial cells expressed both AT_1R and AT_2R. Consistent with those data, in VSMC transected with AT_2R, PD123319 unmasked Ang II-mediated proliferation (Nakajima *et al*., 1995). Experiments performed using cardiomyocytes (Booz & Baker, 1996; van Kesteren *et al*., 1997), cardiac fibroblasts (Ohkubo *et al*., 1997; Ozawa *et al*., 1996; Tsuzuki *et al*., 1996b; van Kesteren *et al*., 1997) and isolated perfused hypertrophic hearts (Bartunek *et al*., 1999), all reported an increase in AngII-induced growth with AT_2R blockade.

Cellular differentiation is also closely linked to the antiproliferative and regenerative effects of AT_2R stimulation. Early studies indicated that AT_2R activation inhibited proliferation and promoted differentiation in PC12W and NG108-15 cells (Laflamme *et al*., 1996; Meffert *et al*., 1996), as well as axonal regeneration *in vitro* and *in vivo* (Lucius *et al*., 1998). Apoptosis is also considered to play an important role in normal development as well as in response to pathological changes, such that the process of cardiovascular remodelling is determined by the balance between cell growth and proliferation *versus* apoptosis. AT_2R evokes proapoptotic effects in a number of cell types *in vitro* including PC12W, fibroblasts and VSMC (Yamada *et al*., 1996; 1998; Tsuzuki *et al*., 1996a; Dimmeler *et al*., 1997), while AT2R-mediated growth inhibition in endothelial cells involves remodelling of the extracellular matrix components (Fischer *et al*., 2001).

In vivo

In the *in vivo* setting, there are a number of different experimental models that mirror the *in vitro*, growth-potentiating effect of PD123319. These include PD123319-induced enhanced angiogenesis (Munzenmaier & Greene, 1996), increased media thickness (Ceiler *et al*., 1998; Diep *et al*., 1999), increased neointima (Akishita *et al*., 2000a), increased cardiac and renal fibrosis (Ohkubo *et al*., 1997; Morrissey & Klahr, 1999) as well as enhanced atherosclerotic development (Daugherty *et al*., 2001). However, in other studies, PD123319 did not alter LV or vascular hypertrophy (Makino *et al*., 1997; Ohkubo *et al*., 1997; Li *et al*., 1998; Tea *et al*., 2000; Varagic *et al*., 2001).

Furthermore, there are also reports that oppose the conventional view that AT_2R is linked with antigrowth since PD123319 actually blocked vascular hypertrophy in SHR or Ang II-mediated vascular hypertrophy and fibrosis (Levy *et al*., 1996; Sabri *et al*., 1997; Otsuka *et al*., 1998; Cao *et al*., 1999). Moreover, Cao *et al*. (2002) recently suggested that PD 123319 was reno-protective in subtotally nephrectomised rats, since it reduced proteinuria and inflammatory markers of renal injury, albeit in a similar fashion to AT_1R blockade. Unusually, AT_2R stimulation also promoted cellular proliferation in normal kidney, but was associated with apoptosis (Cao *et al*., 2000). Similarly, Ang II acting at both AT_1R and AT_2R has been shown to stimulate NFκB, which is a known proinflammatory mediator (Ruiz-Ortega *et al*., 2000; 2001; Wolf *et al*., 2002).

On the other hand, selective AT_2R stimulation using CGP42112 (Janiak *et al*., 1992) or vascular AT_2R over expression (Nakajima *et al*., 1995) inhibited neointimal growth. These data, together with previous *in vitro* studies using CGP42112 (Stoll *et al*., 1995; Ozawa *et al*., 1996; Dimmeler *et al*., 1997), further support an antigrowth role for AT_2R.

Mice

Discrepancies noted between some of the fore-mentioned rat studies may relate to the variety of experimental models that have been used, notwithstanding differences in drug doses, length of treatment, etc. Conceivably, targeted deletion of the AT_2R would help resolve these issues. Table 1 lists the basal effects of the AT_2R in cardiac and vascular tissue, as deduced from either targeted deletion or cardiac overexpression of AT_2R. In keeping with the antigrowth role of AT_2R, a number of studies have found that vascular pathologies have been exacerbated in the AT_2R knock out animals with aortic banding or femoral cuffs, although there was no effect on cardiac hypertrophy (Table 1). These changes included enhanced perivascular fibrosis of coronary arteries, vascular thickening, neointimal growth and apoptosis. By contrast, Inagami and colleagues, using a different AT_2R knockout strain, reported that AT_2R stimulation was actually responsible for pressure-overload cardiac hypertrophy and fibrosis caused by aortic banding or Ang II infusion (i.e. absent in AT_2R knockout mice) (Senbonmatsu *et al*., 2000; Ichihara *et al*., 2001). These controversial data (Schneider & Lorell, 2001) are consistent with a number of previously mentioned studies in rats suggesting prohypertrophic/proliferative actions of AT_2R (Levy *et al*., 1996; Sabri *et al*., 1997; Cao *et al*., 1999; 2000; 2002), although recent studies using this same AT2 knockout strain found no differences from wild-type controls with respect to basal cardiac structure and function (Xu *et al*., 2002).

Clearly, there are discrepancies between studies that may relate to the development of the AT_2R knock outs

Table 1 Cardiovascular structural effects in mice that have been attributed to the AT_2R[a]

Study[b]	*Experimental intervention*	*Strain*	*Cardiac hypertrophy*	*Perivascular fibrosis*	*Interstitial fibrosis*	*Vascular remodelling*
Akishita *et al*. (2000b)	Aortic banding	FVB/N	→	−ve	—	−ve
Wu *et al*. (2002)	Aortic banding	FVB/N	→	−ve	—	−ve
Senbonmatsu *et al*. (2000)	Aortic banding	C57BL/6	+ve	—	+ve	—
Ichihara *et al*. (2001)	Ang II infusion	C57BL/6	+ve	+ve	+ve	—
Sugino *et al*. (2001)#	Ang II infusion	C57BL/6	→	—	—	—
Kurisu *et al*. (2003)#	Ang II infusion	C57BL/6	→	−ve	−ve	—
Brede *et al*. (2001)	untreated	FVB/N	—	—	—	−ve
Yang *et al*. (2002)#	MI (4 weeks)	C57BL/6	→ (↑ ED wall thickness)	—	—	—
Xu *et al*. (2002)	MI (24 weeks)	C57BL/6	→	—	→	—
Ichihara *et al*. (2002)	MI (1 week)	C57BL/6	+ve	—	+ve	—
Oishi *et al*. (2003)	MI (2 weeks)	FVB/N	−ve	→ (subthreshold stimuli)	→ (subthreshold stimuli)	—
(Ma *et al*. (1998)	Uretal ligation	C57BL/6	—	−ve (renal)	−ve (renal)	—
Akishita *et al*. (2000a)	Femoral artery cuff	FVB/N	—	—	—	−ve
Suzuki *et al*. (2002)	Femoral artery cuff		—	—	—	−ve
Wu *et al*. (2001)	Femoral artery cuff	FVB/N	—	—	—	−ve

[a]Changes in structural indices refer to effects of AT_2R on the particular experimental intervention, and not the effect of experimental intervention *per se*.
[b]The majority of studies have used AT_2R knockout mice, except those using mice with cardiac AT_2R overexpression#
→, no effect; −ve, inhibitory effect; +ve, excitatory effect; −, not determined.

independently by two groups (Hein *et al*., 1995; Ichiki *et al*., 1995). As pointed out, these strains differ slightly with respect to basal BP, pressor sensitivity to exogenous Ang II and genetic background (Hein *et al*., 1995; Ichiki *et al*., 1995; Schneider & Lorell, 2001), and even with regard to the structural indices measured (Inagami & Senbonmatsu, 2001). One also has to be cognisant of the potential for compensatory changes that contribute to phenotype. Indeed, the relatively small increase in basal BP in the AT_2R knock out, despite reductions in vasodilator (bradykinin, cGMP) signalling pathways (Siragy *et al*., 1999a), was explained on the basis that there was an upregulation of AT_1R-mediated vasodilator prostanoids that offset any substantial hypertension in the AT_2R knockout model (Siragy *et al*., 1999b).

Thus, there are reported differences between the AT_2R knockout strains with respect to cardiac hypertrophy when applying conventional loads using either Ang II or aortic banding (Table 1). AT_2R exerted either no change or caused cardiac hypertrophy in response to these stimuli, although the magnitude of cardiac hypertrophy induced by aortic banding in the corresponding wild types differed substantially between studies (Akishita *et al*., 2000b; Senbonmatsu *et al*., 2000; Schneider & Lorell, 2001). Nevertheless, the lack of evidence for AT_2R antigrowth in the heart *per se* (Opie & Sack, 2001) is consistent with recent studies in which AT_2R was overexpressed in cardiomyocytes but did not alter cardiac mass (Masaki *et al*., 1998; Sugino *et al*., 2001; Kurisu *et al*., 2003), although Yang *et al*. (2002) reported greater end-diastolic wall thickness and higher ejection fraction at baseline in these transgenic mice than in wild-type controls.

In addition, the divergent effects of the AT_2R knockout strains on cardiac fibrosis may relate to the measurement of different fibrotic indices between studies (Table 1) (Inagami & Senbonmatsu, 2001). However, one would have to reconcile contrasting AT_2R effects on cardiac interstitial *versus* coronary perivascular fibrosis in those studies, whereas this is not the case in mice overexpressing cardiac AT_2R. In this model, there was a significant reduction in the degree of Ang II-induced cardiac interstitial and perivascular fibrosis observed (Kurisu *et al*., 2003), implying that AT_2R negatively regulates cardiac fibrosis, in line with the majority of data obtained from rats. Moreover, this AT_2R antifibrotic effect was mediated *via* a kinin/NO-dependent mechanism (Kurisu *et al*., 2003). Curiously, in the AT_2R knockout strain which exhibited no cardiac fibrosis (i.e. profibrotic AT_2R phenotype: Senbonmatsu *et al*., 2000; Ichihara *et al*., 2001), Ma *et al*. (1998) reported enhanced renal fibrosis (i.e. antifibrotic AT_2R phenotype), implicating tissue-specific bidirectional fibrotic changes.

Myocardial infarction (MI) has also been produced in mice, but again with conflicting results that may relate to the different times examined after MI and/or strains (Table 1). In the AT_2R knockout strain which could not evoke a prohypertrophic/fibrotic response (Senbonmatsu *et al*., 2000; Ichihara *et al*., 2001), there was increased rupture immediately following MI although survival rate was not different from controls 6 weeks after MI (Ichihara *et al*., 2002). By contrast, in the AT_2R knockout strain which exhibited enhanced perivascular fibrosis (Akishita *et al*., 2000b), the survival rate was lower than controls 2 weeks after MI but without any difference in the incidence of rupture (Oishi *et al*., 2003). Moreover, the MI-induced left ventricular enlargement and fibrosis seen in wild types was attenuated in one study (Ichihara *et al*., 2002) but enhanced in another (Oishi *et al*., 2003), in line with the contrasting pre-existing phenotypes. However, others have reported no differences in MI remodelling after 24 weeks (Xu *et al*., 2002). In addition, in mice with cardiac AT_2R overexpression, left ventricular function was enhanced compared with wild types, as assessed by magnetic resonance imaging techniques (Yang *et al*., 2002). Moreover, this left ventricular remodelling was preserved when measured invasively and noninvasively 28 days after myocardial infarction (Yang *et al*., 2002).

Collectively, in the context of growth modulatory effects of AT_2R, there is good evidence for AT_2R to inhibit basal growth with respect to neointimal/peripheral vessel injury, albeit in a limited number of studies. There is also reasonable consensus that AT_2R does not alter cardiac hypertrophy appreciably, but

does regress cardiac fibrosis in both rats and mice (Table 1). However, the contradictory findings derived from seemingly similar AT_2R knockout strains emphasise the need for pharmacological studies in wild-type mice to clarify the role of AT_2R in this species.

The ability of AT_2R to inhibit perivascular/interstitial fibrosis and neointimal growth, while not altering cardiac mass, probably relates to the level of AT_2R expression in these tissues. AT_2R are constitutively expressed on cultured fibroblasts (Dudley *et al*., 1991; Dudley & Summerfelt, 1993) and are present at perivascular and vascular sites (Nora *et al*., 1998; Akishita *et al*., 2000b; Suzuki *et al*., 2002). AT_2R are less abundant in cardiac myocytes compared with cardiac fibroblasts, and are further upregulated in fibroblasts under conditions of cardiac load/pathology (Ohkubo *et al*., 1997; Tsutsumi *et al*., 1998; Wharton *et al*., 1998). Moreover, AT_2R activation at these sites has been linked with reduced collagen synthesis and inhibition of growth of cardiac fibroblasts and mitogen signals (Ohkubo *et al*., 1997; Tsutsumi *et al*., 1998). On the other hand, Mifune *et al*. (2000) reported that AT_2R activation caused collagen production in cultured vascular smooth muscle cells transfected with AT_2R, and this fact has been used as an argument to support a profibrotic role for AT_2R (Inagami & Senbonmatsu, 2001). However, there were heterogeneous effects reported from the same study, since AT_2R activation in fact inhibited collagen production in cultured fibroblasts (Mifune *et al*., 2000), which is the effector cell more likely to be involved in the fibrotic process.

Role of AT_2R in cardiovascular action of AT_1R blockade

Evidence has been presented here and elsewhere (Matsubara, 1998; Horiuchi *et al*. , 1999a) describing opposing actions of AT_1R (predominantly excitatory) and AT_2R (predominantly inhibitory). AT_1R blockade has emerged as an effective treatment for hypertension and heart failure, in much the same manner as ACE inhibitors. However, these RAS inhibitors have divergent effects with respect to plasma levels of Ang II. Unlike ACE inhibitors, AT_1R blockade increases circulating levels of Ang II that could in theory act on the unopposed AT_2R. In this way, the ability of Ang II to stimulate AT_2R in the presence of blockade of (excitatory) AT_1R could provide additional complementary therapeutic benefit. Indeed, this point is increasingly being used as a marketing ploy to distinguish AT_1R blockers from ACE inhibitors; therefore, it is timely to review this hypothesis.

An early study performed using anaesthetised SHR suggested that AT_2R may be involved in the acute natriuretic and diuretic effects of losartan because PD123319 and Hoe 140 attenuated these effects although they did not affect the losartan-induced fall in BP (Munoz-Garcia *et al*., 1995). More conclusive evidence for an AT_2R contribution to the effect of an AT_1R antagonist was provided in the seminal study by Liu *et al*. (1997), who used the conventional rat coronary artery ligation model of heart failure. These authors found that chronic treatment with the AT_1R antagonist, L-158809, improved left ventricular ejection fraction and caused regression of cardiac hypertrophy and fibrosis. These effects were partly attributed to unopposed AT_2R activation since the AT_2R antagonist PD123319 reversed the L-158809-induced changes in ejection fraction, LV volumes and myocyte cross-sectional area. In addition, the L-158809-induced reduction in cardiac fibrosis tended to be reversed, although the latter effect was not significant. Moreover, the involvement of AT_2R activation during AT_1R blockade also involved subsequent bradykinin production (Liu *et al*., 1997), which is consistent with the vasodilator signalling pathways already described.

Thereafter, a number of acute and short-term studies have examined the AT_2R contribution to AT_1R blockade by examining the potential reversal, by PD123319, of the effects of 'sartan' compounds. Jalowy *et al*. (1998) established that the size of the myocardial infarct in pigs caused by ischaemia/reperfusion over several hours was reduced by the AT_1R antagonist candesartan. Remarkably, a 30-min pretreatment with either AT_2R or bradykinin B_2 receptor antagonists (PD123319 or Hoe 140) reversed the beneficial effect of AT_1R blockade, which was consistent with earlier data from anaesthetised SHR (Munoz-Garcia *et al*., 1995). In addition, acute studies from the laboratories of Siragy and Carey (1996, 1997) have documented that, in conscious rats, Ang II infusions or sodium depletion caused an increase in renal interstitial fluid levels of cGMP that was blocked by PD123319. Furthermore, valsartan reduced SBP in sodium-depleted rats and renal-dependent hypertensive rats, and this effect of valsartan was blocked by a 30-min coinfusion of the AT_2R antagonist PD123319 (Siragy & Carey, 1999; Siragy *et al*., 2000), although this 'PD123319 reversal' has not always been seen in studies by the same group (Siragy *et al*., 2002).

In some respects, it is quite remarkable that PD could reverse the effects of AT_1R blockade in the fore-mentioned acute studies because it was shown some years ago that PD123319 actually displaced the metabolite of losartan from protein binding sites in a nonspecific manner. This served to increase the degree of AT_1R blockade, which was assessed as inhibition of Ang II-mediated vasoconstriction (Widdop *et al*., 1992). These findings were subsequently confirmed by others (Wong *et al*., 1992). However, this interaction was only examined acutely over 24 h, and so the extent of this potentially confounding issue during chronic treatment conditions is not known.

Several studies of a more *chronic* nature in rats have also assessed changes in cardiac and vascular structure as well as BP (Table 2). In addition to the reported AT_2R involvement in the effects of chronic AT_1R blockade in heart failure (Liu *et al*., 1997), it was claimed that PD 123319, given as a daily, 70-min infusion for 1 week, reversed the losartan-induced reduction in SBP in sodium-depleted rats, as measured noninvasively by tail cuff method (Gigante *et al*., 1998). However, we could not confirm these findings in analogous experiments in which direct arterial BP measurements were made (Jones *et al*., 1999). In other studies, AT_2R blockade exerted negligible effects on the antihypertensive effect of AT_1R blockade in SHR, but PD123319 did in fact reverse valsartan-induced reductions in vascular smooth muscle growth and vascular mass. Moreover, this vascular remodelling involved AT_2R-mediated smooth muscle cell apoptosis (Tea *et al*., 2000). By contrast, Varagic *et al*. (2001) found that PD123319 treatment caused a small reversal of losartan-induced antihypertensive effect in SHR. In addition, despite a lack of effect on left ventricular mass, AT_2R blockade fully reversed candesartan-induced reductions in cardiac fibrosis, assessed by biochemical assay (Varagic *et al*., 2001). Thus, partial (Liu *et al*., 1997) or full (Varagic *et al*.,

Table 2 Contribution of the AT_2R to effects of chronic AT_1R blockade[a]

Study	*Strain*	*Experimental design*	*Blood pressure*	*Cardiac hypertrophy*	*Cardiac fibrosis*	*Vascular remodelling*
Liu *et al*. (1997)	Lewis rat	MI, 'PD reversal'	No	Yes	Yes (trend only)	—
Gigante *et al*. (1998)	Wistar rat	Low salt diet, 'PD reversal'	Yes	—	—	—
Jones *et al*. (1999)	WKY rat	Low salt diet, 'PD reversal'	No	No	—	—
Tea *et al*. (2000)	SHR	'PD reversal'	No	—	—	Yes
Varagic *et al*. (2001)	SHR	'PD reversal'	Yes (small)	No	Yes	—
Collister *et al*. (2002)	Sprague–Dawley rat	'PD reversal'	No (further ↓BP)	—	—	—
Xu *et al*. (2002)	AT_2R KO mouse (C57BL/6)	MI, valsartan	No	Yes	Yes	—
Wu *et al*. (2002)	AT_2R KO mouse (FVB/N)	Aortic banding, valsartan	No (but sub-depressor valsartan)	No	Yes	Yes
Wu *et al*. (2001)	AT_2R KO mouse (FVB/N)	Femoral artery cuff, valsartan	No	—	—	Yes

[a]Assessed by the ability of PD123319 to reverse the effects of AT_1R blockade in rats ('PD reversal'), or an attenuated effect of AT_1R antagonist in AT_2R knockout mice.
Yes, AT_2R does contribute; no, AT_2R does not contribute; —, not determined.

2001) reversal by PD123319, of reduced cardiac fibrosis caused by AT_1R blockade, occurred after 2–3 months treatment with this AT_2R antagonist. In this context, chronic treatment with PD 123319 alone to myopathic hamsters enhanced cardiac interstitial fibrosis after 44, but not 20, weeks, implicating growth-modulatory effects only after prolonged treatment (Ohkubo *et al*., 1997). This finding may help explain the partial effect of PD123319 on reversing the antifibrotic effect of AT_1R blockade in the heart failure setting (Liu *et al*., 1997), notwithstanding the complex heterogeneous changes in AT_2R expression that may be model and/or tissue specific (see earlier).

Interestingly, in all the fore-mentioned studies examining an AT_1R/AT_2R interaction, SBP was measured using the noninvasive, but relatively stressful, tail cuff method. When BP was measured by radiotelemetry, combined AT_1R and AT_2R blockade actually further reduced BP relative to AT_1R blockade alone, albeit in normotensive rats measured only over a 10-day period (Collister *et al*., 2002), which could imply a nonspecific interaction (Widdop *et al*., 1992).

Thus, there are very few studies that have assessed the ability of PD 123319 to reverse the antihypertensive and remodelling effects of AT_1R antagonists under *chronic* treatment conditions, partly because of a lack of availability of the AT_2R antagonist. While there is some evidence for structural changes (Table 2), there appears to be, at best, only a minor role of AT_2R in the BP-lowering effects of AT_1R antagonists when given chronically (e.g. Varagic *et al*., 2001). Nevertheless, the lack of reversal, by PD123319, of chronic 'sartan'-induced haemodynamic changes, can still be reconciled with the well-described direct AT_2R-mediated vasodilator effect *in vitro* (see earlier) or *in vivo* (Barber *et al*., 1999; Carey *et al*., 2001a; Li & Widdop, 2003). AT_2R-mediated vasodilatation relies on directly stimulating vascular AT_2R (with Ang II or CGP42112). On the other hand, the determination of whether or not PD123319 can reverse 'sartan'-induced hypotension is often used as an indication of potential AT_2R vasodilator involvement in AT_1R blockade. However, any observed response will be the net effect of (opposing) interactions between pharmacodynamic (genuine 'PD reversal') and pharmacokinetic (nonspecific, enhanced AT_1R blockade (Widdop *et al*., 1992) events.

In mice, three studies published to date have examined the effects of valsartan in AT_2R knockout mice under three different pathological states (Table 2). In each case, the ability of valsartan to regress neointimal formation induced by femoral artery cuff (Wu *et al*., 2001), inhibit perivascular fibrosis and coronary artery thickening induced by aortic banding (Wu *et al*., 2002) or improve cardiac haemodynamics after MI (Xu *et al*., 2002) was attenuated in the mice lacking AT_2R compared with wild types. Interestingly, these studies were conducted using both the antigrowth (Wu *et al*., 2001, 2002) and prohypertrophic/fibrotic (Xu *et al*., 2002) AT_2R knockout phenotypes. Clearly, additional rat and mice studies are required to elucidate fully the role of AT_2R in the setting of *chronic* AT_1R blockade.

Thus, on close inspection, there are remarkably few chronic treatment studies on which to judge the somewhat seductive hypothesis, based mainly on acute studies, that AT_2R stimulation contributes to the cardiovascular effects of AT_1R antagonists. The striking AT_2R effects on BP, inferred from experiments in which the acute administration of PD 123319 reversed the acute antihypertensive effects of AT_1R antagonists, have not been seen in the limited number of chronic studies published using rats. On the other hand, there was evidence for regression of cardiac fibrosis and vascular remodelling evoked by AT_2R activation, which are of greater physiological importance during long-term antihypertensive therapy, although the AT_2R effects on cardiac hypertrophy were more equivocal (Table 2). Limited studies in mice also would point towards a role of AT_2R in the effects of AT_1R antagonists.

In the clinical setting, the most recent meta-analysis comparing AT_1R blockade with either placebo or ACE inhibition did not report a clear-cut superiority of AT_1R blockade in reducing all-cause mortality or hospitalisation rate in patients with heart failure (Jong *et al*., 2002) despite an earlier smaller analysis reporting a survival benefit with AT_1R

blockade (Sharma *et al*., 2000). As such, these clinical data may somewhat dampen the interest generated from experimental studies on the possible involvement of AT_2R stimulation in the therapeutic effects of AT_1R antagonists. However, several large trials, which will more than double the current patient population surveyed, are yet to conclude (Dickstein & Kjekshus, 1999; Pfeffer *et al*., 2000). Therefore, before we reject the hypothesis that AT_2R counter-regulates AT_1R function in the setting of heart failure (Opie & Sack, 2001), it is possible that there may be refinement of current evidence that does not readily distinguish AT_1R antagonists from ACE inhibitors, at least in the heart failure population.

In addition, there is evidence that indicates AT_1R antagonists are not all the same (Siragy, 2002). In this context, there is the intriguing possibility that AT_1R antagonists may differentially stimulate AT_2R-mediated effects *in vivo*. Recently Siragy *et al*. (2002) found that valsartan, but not losartan, caused prolonged elevations in cGMP levels in renal interstitial fluid of sodium-depleted rats. The fact that these effects were blocked by PD123319 would imply that the degree of AT_2R activation caused by different AT_1R antagonists may differ.

Of course, there is great difficulty in addressing any AT_1R/AT_2R interaction in the clinical situation. Nevertheless, this issue has been indirectly examined in recent preliminary studies using two different patient populations (Phoon & Howes, 2001, 2002). In one study in elderly female patients, Ang II caused dose-dependent forearm vasodilatation when tested during 3-week candesartan treatment; interestingly, a short-term infusion of PD 123319 in these patients elevated baseline forearm vascular resistance, suggesting that tonic AT_2R-mediated vasodilatation contributes to the haemodynamic profile of AT_1R blockade (Phoon & Howes, 2002). However, the AT_2R antagonist did not appear to block Ang II-induced vasodilatation, indicating perhaps non-AT_2R vasodilator mechanisms in response to exogenous Ang II were also involved (Phoon & Howes, 2002). Moreover, it was unclear if the potential AT_2R involvement was due to the antihypertensive therapy and/or a unique patient population since, in an earlier study in healthy male volunteers without AT_1R blockade, Ang II caused forearm vasoconstriction and PD123319 did not affect baseline forearm vascular resistance (Phoon & Howes, 2001).

Conclusions

AT_2R function is likely to be context-specific, as recently suggested (Schneider & Lorell, 2001). This likelihood is well exemplified by studies at a number of levels: (i) Growth modulatory effects of Ang II *in vitro* depend on the type of AT receptor on a given cell. AT_2R are natively expressed on cultured endothelial cells but not on cultured VSMC, such that antiproliferative actions of AT_2R offset AT_1R-mediated growth-promoting effects in endothelial cells but not VSMC (Nakajima *et al*., 1995; Stoll *et al*., 1995), (ii) Bidirectional changes in effector response can be elicited by AT_2R depending on the cell type. AT_2R evoked increased collagen production in VSMC and mesangial cells, but decreased collagen production in fibroblasts (Mifune *et al*., 2000). (iii) AT_2R are upregulated in cardiac hypertrophy and heart failure (Lopez *et al*., 1994; Tsutsumi *et al*., 1998; Wharton *et al*., 1998), which impacts on whether or not an AT_2R involvement is noted with respect to hypertrophy/growth and fibrosis (Liu *et al*., 1997; Ohkubo *et al*., 1997; Tsutsumi *et al*., 1998; Bartunek *et al*., 1999; Varagic *et al*., 2001). (iv) AT_2R exerts vasodilatation *per se* in hypertensive and failing states compared with appropriate controls (Barber *et al*., 1999; Schuijt *et al*., 2001; Li & Widdop, 2003). Likewise, an involvement of AT_2R in the acute (Siragy & Carey, 1999; Siragy *et al*., 2000) or chronic (Liu *et al*., 1997) therapeutic effects of AT_1R blockade has usually been observed in experimental models in which there is increased RAS activity and/or pathological states where there is more likely to be an upregulation of AT_2R.

Thus, it is apparent that there is marked tissue heterogeneity which is likely to reflect the balance of AT_1/AT_2 receptor expression in various tissues, which may be partly determined by the choice of experimental model. Indeed, the fact that there appears to be a greater diversity of AT_2R effects on cardiac hypertrophy (stimulatory, inhibitory or no effect) than on cardiac fibrosis (predominantly inhibitory), most likely reflects the greater AT_2R expression on cardiac fibroblasts (Ohkubo *et al*., 1997; Tsutsumi *et al*., 1998; Wharton *et al*., 1998). An alternative view that AT_2R causes stimulatory effects, while increasingly being reported (Senbonmatsu *et al*., 2000; Ichihara *et al*., 2001), requires further consideration in the context of pharmacological studies to match genetic manipulations.

In any case, the elucidation of the actions of AT_2R has gained prominence partly because of a postulated role in the therapeutic effects of AT_1R antagonists. Current experimental data, although still incomplete, supports a role for the AT_2R in contributing to the regression of structure caused by AT_1R blockade in a context-specific manner. However, while the most recent meta-analysis of human clinical trials comparing AT_1R antagonists with either placebo or ACE inhibition did not report a clear-cut superiority of AT_1R blockade in reducing all-cause mortality or hospitalisation rate in patients with heart failure (Jong *et al*., 2002), more subtle aspects of cardiovascular remodelling between these two classes of antihypertensives are yet to be investigated, as is any potential AT_2R involvement.

In addition, other factors need to be borne in mind when treating with an AT_1R antagonist. For example, AT_1R blockade and/or Ang II can themselves increase AT_2R expression in vasculature and endothelial cells in some (Gigante *et al*., 1997; De Paolis *et al*., 1999; Bonnet *et al*., 2001), but not all (Otsuka *et al*., 1998) studies. In this context, recent data also showed that an over expression of AT_2R in VSMC downregulates AT_1R expression as well as basal DNA synthesis and proliferation of VSMC from WKY rats (Jin *et al*., 2002) but not from SHR (Su *et al*., 2002). Therefore, therapeutic outcome may be influenced, not only by pathological status of AT_2R expression, but also by the autocrine/paracrine regulation of the cellular milieu in the target organs.

Future directions

Many of the actions attributed to AT_2R pathophysiology have been inferred from changes in function due to AT_2R blockade (either pharmacological using virtually one compound, or gene deletion methods) in either the presence or absence of AT_1R blockade. Thus, the development of other novel AT_2R

agonists and antagonists is required in order to limit our reliance on too few available AT_2R ligands.

Further studies examining the cardiovascular effects of chronic selective AT_2R stimulation *per se*, as well in combination with AT_1R antagonists, are imperative in order to elucidate further the pathophysiological role of the AT_2R in cardiovascular disease. The fact that there was a sustained, enhanced effect of combined AT_2R stimulation plus AT_1R blockade that was greater than AT_1R blockade alone (Carey *et al*., 2001a), together with the absence of functional AT_2R desensitisation (Widdop *et al*., 2002), suggests that directly targeting the AT_2R in cardiovascular disease will be a fruitful avenue for future research (Barber *et al*., 1999; Carey *et al*., 2001b; Siragy & Carey, 2001).

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