Angiotensin II AT_2 subtype receptors: an emerging target for cardiovascular therapy

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This article reviews the impressive amount of knowledge accumulated in the last few years on the angiotensin II AT_2 subtype receptor.

Although still elusive, a large body of experimental evidence strongly suggests that it may play an important role in the adaptive changes of the cardiovascular structures in response to pathological conditions such as myocardial infarction, congestive heart failure or hypertension. The most intriguing aspects of the biology of this receptor, however, appear to be: 1) the regulation of its transcription, which plays an important role in the expression of the protein in adults or in injured tissues; 2) its interaction or cross-talk with the predominant angiotensin II receptor, the AT_1 subtype, or with the receptors of other growth factors or cytokines; and 3) its connections with the bradykinin/nitric oxide pathways. These aspects may be relevant for the therapeutical use of drugs which antagonize the renin-angiotensin system, such as angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists, as well as for new therapeutic approaches to the treatment of cardiovascular diseases.

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In the last two decades, membrane receptors coupled with intracellular signaling pathways have progressively become a primary target for designing new drugs provided with a selective pharmacological mechanism that interferes with processes involved in the pathophysiology of cardiovascular diseases. In this view, the discovery of a novel class of drugs which selectively interferes with the binding of angiotensin II to its specific AT_1 subtype receptor[1], the so-called -sartan family compounds, has represented an important breakthrough for the treatment of cardiovascular disease as well as an interesting tool for the investigation of the renin-angiotensin system, and of its receptor system. The renin-angiotensin system, in fact, through the action of its effector peptide angiotensin II, plays an important role in cardiovascular, electrolyte, and fluid homeostasis[2], and has significant effects on cardiovascular structure and function which contribute to the development of hypertension, congestive heart failure, vascular diseases, and renal failure[3].

Known biological effects of angiotensin II are largely mediated through the AT_1 subtype, a seven-domain, transmembrane G-coupled receptor[4]. However, multiple lines of evidence now indicate that there are several receptor subtypes, among which at least two distinct receptor subtypes (designated as subtypes 1 and 2) were defined on the basis of different structure, pharmacological and biochemical properties[5,6]. While most of the known effects of angiotensin II are attributable to its binding with the AT_1 subtype receptor[4] and to the subsequent activation of the intracellular cascade (Fig. 1), less is known about the AT_2 receptor subtype.

Experimental evidence accumulated so far suggests, however, that this receptor acts as an antagonistic system with respect to the AT_1 receptor. In fact, the AT_2 receptor has been related to antigrowth and antihypertrophic effects, vasorelaxation, antioxidant action, and may prompt cellular apoptosis[7].

In this article we will review some of the properties of AT_2 receptors which have revealed so far through extensive experimental research. We will also try to share with the readers the opinion that agonism of this receptor (directly mediated or indirectly achieved through pharmacological blockade of the AT_1 receptor subtype) may represent an interesting therapeutic target.

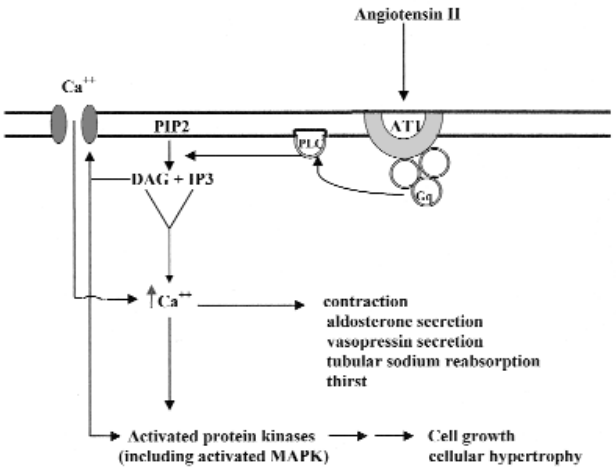

Figure 1. *Schematic representation of the AT_1 subtype receptor, and major biological functions. DAG = diacylglycerol; IP = inositol phosphate; MAPK = mitogen-activated protein kinase; PLC = phospholipase C.*

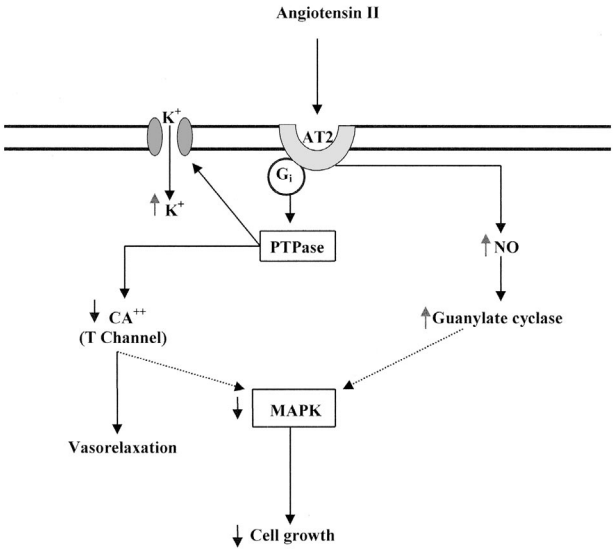

Figure 2. *Postulated most common intracellular pathways of the AT_2 subtype receptor, biological functions and influence on ion channels. MAPK = mitogen-activated protein kinase; NO = nitric oxide; PTPase = protein tyrosine phosphatase.*

In particular, we shall analyze the structure and signaling mechanism of the AT_2 receptor, its transcriptional regulation, its vascular and myocardial effects, and its interference with the bradykinin/nitric oxide systems.

Structure, intracellular signaling pathway, and regulation of AT_2 subtype receptor

A number of studies indicate that the AT_2 receptor is a seven-domain transmembrane receptor just like the AT_1 receptor subtype[8-11]. However, its avidity to bind angiotensin II is markedly lower than the AT_1 subtype. Although the two receptors display an elevated structural homology, they are coded by genes residing in different chromosomes, and they appear to have quite different signaling pathways, which obviously may account for the mutually antagonistic functions[4,12-14]. In fact, the transduction pathway of the signal of the AT_2 receptor has not been yet fully defined and may vary according to cell lines. Most evidence indicates that the AT_2 receptor is generally a G-protein-coupled receptor, and its intracellular third loop domain is strictly linked to Gi (inhibitory)[10] (Fig. 2). This specific interaction with the Gi may explain the observation that the growth inhibitory effect of the AT_2 receptor is at least partially mediated by the activation of various phosphatases, mostly the protein tyrosine phosphatase (PTPase)[8], which causes inactivation of the AT_1 receptor or growth factor-mediated stimulation of mitogen-activated protein kinase (MAPK)[15-20]. Two important MAPK, p42 and p44, are also known as extracellular signal-regulated kinase (ERK), a fundamental step in the cellular growth process. ERK inactivation secondary to PTPase activation through AT_2 receptors has been reported in neuronal cells and in the cardiac tissue of the AT_2 transgenic mouse[21]. On the other hand, the observation that in a pheochromocytoma cell line (PC12W) pretreatment with antisense nucleotide of a phosphatase inhibits the proapoptotic action mediated by AT_2 receptors further supports the role of AT_2-linked phosphatases[18,19].

Other fundamental functional differences between AT_2 and AT_1 receptors are represented by the capacity of AT_2 receptors to promote potassium channel opening[22] (a property which may favor cellular stability thus contributing to an antiarrhythmic effect postulated for losartan), and decreased current amplitude of T-type calcium channels[9] (in contrast to AT_1 receptors). However, the most intriguing difference remains the antagonistic effect on cellular growth, that is, AT_1 promotes cell growth and AT_2 mediates growth inhibition, a unique property for seven-transmembrane, G-protein-coupled receptors, since all other similar receptors related to other classes of growth factors mediate cellular growth.

A controversial aspect of the biology of AT_2 receptors is represented by the regulation of its transcription, and thus its expression, in adult tissues. In fact, AT_2 receptor mRNA is poorly represented in vascular tissues of rats and mice during early embryonic development[15,23], it increases during the more advanced stages of fetal development, but it declines very rapidly after birth to extremely low levels in some tissues of adult mammalians[15,23,24]. Using a fibroblast cell line to examine the regulation of the promoter activity of the AT_2 receptor gene, it has been shown that the expression of AT_2 gene is modulated by the growth state of the cell, and, in turn, it regulates the growth process of the cells[25]. In particular, in this cell culture model AT_2 receptor expression is low in the growing state and becomes higher in the confluent state[26,27]. The transcriptional regulation of the gene is linked to a specific putative negative regulatory

region located between positions -453 and -225, and is controlled by factors such as interferon regulatory factor (IRF)-1 (the same factor which activates the expression of inducible nitric oxide synthase and interleukin-1β-converting enzyme), IRF-2[25], and other growth factors such as serum, fibroblast growth factor, phorbolester (inhibitory effect) or insulin (stimulatory effect)[28].

With regard to the regulation of the expression in vascular tissues, the results of the studies in vascular smooth muscle cells are controversial: in particular, the expression in adult aorta is negligible[15]; insulin, insulin-like growth factor and serum depletion may have a stimulatory effect, while in contrast vasoactive substances linked to protein kinase C-calcium pathways such as norepinephrine and angiotensin II, downregulate AT_2 mRNA in cultured rat neonatal myocytes[29-31].

In this latter regard, we have recently shown that angiotensin II downregulates AT_2 mRNA expression via the AT_1 receptor binding in cultured rat aortic endothelial cells, transfected with the AT_2 receptor gene promoter region cloned into a CAT-reporter vector[32]. Transfected cells were studied following angiotensin-converting enzyme (ACE) inhibition to prevent the endogenous formation of angiotensin II. Cells were subsequently stimulated with either angiotensin II alone or in combination with the AT_1 receptor antagonist, DuP753. AT_2 receptor mRNA was assessed by RNase protection assay during the same pharmacological challenges. Stimulation with angiotensin II caused an increase in the AT_2 gene promoter activity, whereas mRNA expression was reduced. The concomitant treatment with the angiotensin II antagonist DuP753 and angiotensin II was associated with a further increase in the promoter activity, which had doubled. In addition DuP753 prevented mRNA reduction, and actually it produced a 2-fold increase in AT_2 receptor mRNA accumulation.

These data indicate that angiotensin II increases AT_2 receptor promoter activity and decreases AT_2 receptor mRNA accumulation in endothelial cells. The AT_1 subtype receptor is involved in the modulation of both these effects of angiotensin II. These findings suggest that an increase in the expression of AT_2 receptors may occur during treatment with AT_1 receptor antagonists, and indicate the existence of a cross-talk between AT_1 and AT_2 receptors.

In addition, previous data from our group suggest that a functional interaction between the two principal angiotensin II receptor subtypes (AT_1 and AT_2) operates also *in vivo*. In fact, we showed that in salt-restricted, nephrectomized rats, DuP753 caused a marked reduction in AT_1 subtype mRNA and a concomitant increase in AT_2 mRNA in the adrenal cortex[33]. In contrast, AT_1 but not AT_2 receptor subtype plays a role in the regulation of aldosterone synthase in the adrenal cortex[34]. At the cardiac level, we also showed that AT_1 and AT_2 receptors regulate atrial natriuretic peptide (ANP) in a synergic fashion, and that the agonistic effect of AT_2 receptors on ANP mRNA is unmasked only when the AT_1 receptors are blocked[35], thus confirming the tonic functional interaction between the two receptors at the cardiac level.

This cross-talk mechanism (Fig. 3) operating between angiotensin receptor subtypes may be of great interest in the comprehension of the pathophysiology of the renin-angiotensin system, and possibly for development of new therapeutic strategies to interact with the renin-angiotensin system.

In fact, while the potential significance of the shunting of angiotensin II towards AT_2 receptors to exploit their beneficial effects (antigrowth, vasodilation, etc.) (Fig. 4) during AT_1 blockade is minimized by their poor presence in adult cardiovascular tissues, the observation that AT_1 blockade enhances AT_2 receptor expression may, at least theoretically, highlight their biological significance and clinical relevance, as well as provide -sartan compounds with a new, specific pharmacologic effect. We are currently working in order to define exactly the AT_1-related transduction pathway that tonically inhibits AT_2 receptor expression by promoting mRNA degradation. Elucidation of this mechanism may lead to the identification of new pharmacological tools for interaction with the renin-angiotensin system. Moreover, larger selectivity or more tight binding to AT_1 subtypes of certain -sartan compounds may imply increased angiotensin II availability for coupling the AT_2 receptor.

With regard to the interaction of angiotensin receptors with tyrosine kinase receptors, Inagami et al.[36] have shown

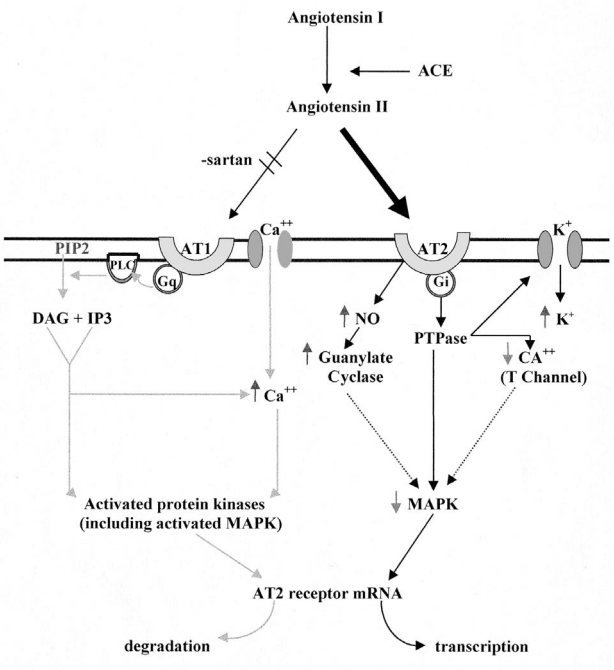

Figure 3. *Hypothetical cross-talk mechanism operating between AT_1 and AT_2 subtype receptors. AT_1 more avidly binds angiotensin II and exerts a tonic negative influence on AT_2 expression, by favoring AT_2 mRNA degradation. Blockade of AT_1 receptors may, on one side, prevent this down-regulation; on the other side, it promotes an up-regulation of the AT_2 promoter via a positive feedback mediated through the angiotensin II AT_2 complex. ACE = angiotensin-converting enzyme. Other abbreviations as in figures 1 and 2.*

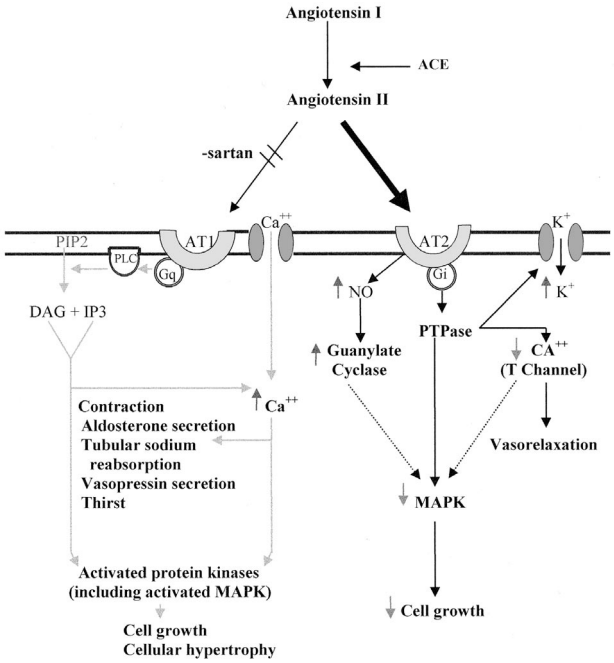

Figure 4. *Biological shunting of angiotensin II receptors during AT_1 blockade by sartan compounds. The balance shift towards the AT_2 receptors may induce opposite effects on cell growth. Abbreviations as in figures 1-3.*

that AT_1 receptor activates MAPK and p42/44 ERK, mediated by RAS, Raf-1, MAPK. In contrast, the AT_2 receptor counteracts the AT_1-mediated tyrosine kinase activation by stimulating several tyrosine phosphatases and serine-treonine phosphatases. This effect suppresses the cell growth process stimulated by various growth factors.

In this regard, to examine the effect of AT_1 and AT_2 receptors on cell proliferation we also investigated the interaction between angiotensin II receptors and tyrosine kinase receptors, such as the epidermal growth factor receptor. Preliminary data obtained by our group demonstrated that in NIH3T3 and R3T3 fibroblasts the transient expression of AT_1 and/or AT_2 receptors was able to activate the MAPK pathway in an angiotensin II dose-dependent manner. However, the AT_2 receptor was less effective than AT_1 to activate this pathway[37]. In AT_1 receptor transfected cells the activation of MAPK by the epidermal growth factor was enhanced in the presence of angiotensin II in a synergic fashion. In contrast, in AT_2 receptor transfected cells co-treatment with angiotensin II and epidermal growth factor showed an inhibitory effect on MAPK activation. In cells expressing both angiotensin II receptor subtypes the inhibitory effect of angiotensin II by the AT_2 receptor was enhanced by AT_1 receptor blockade and partially antagonized by vanadate, suggesting an involvement of PTPase after AT_2 receptor activation. These findings support a positive interaction between the AT_1 receptor and epidermal growth factor receptors whereas agonism of AT_2 receptors resulted in a decreased activation of MAPK leading to an inhibition of cell growth.

Cardiovascular effects of AT_2 receptors

Several studies have analyzed the role of AT_2 receptors in the cardiovascular system. Most of these studies consistently demonstrate an association between these receptors and growth inhibition in various cell types such as smooth muscle cells, endothelial cells, cardiomyocytes and cardiac fibroblasts[15,17,38-41]. In particular, using the model of the AT_2 receptor gene knockout mouse, the expression of components of the contractile apparatus such as h-caldesmon or calponin is delayed, suggesting that also cell differentiation is affected by the AT_2 receptor gene[23]. Other studies have described a proapoptotic effect of the AT_2 receptor in vascular smooth muscle cells[42], neonatal cardiomyocytes[18], endothelial cells[43], neuronal cells[44] and fibroblasts[19,41]. These interactions with apoptosis[45] may suggest a contributory role of AT_2 receptors in the remodeling processes, and imply that AT_2 receptors may be involved in the control of blood vessel structure by regulating the growth stimulatory effect of different growth factors and by modulating programmed cell death.

In turn, there is growing evidence that the expression of AT_2 receptors is increased in injured tissues, wounds and repair processes, and that, in particular, this stimulation of AT_2 expression occurs in cardiovascular tissues following lesions. For instance, AT_1 and AT_2 gene expression is markedly increased in experimental myocardial infarction 1 and 24 hours after coronary ligation[46], and AT_2 receptor expression is further upregulated following myocardial infarction in both the infarcted and non-infarcted portions[47]. Also, in the hypertrophic cardiac muscle the AT_2/AT_1 ratio is increased[48], and in the cardiomyopathy hamster, AT_2 receptor expression is increased in the fibroblasts of the fibrous region[49]; furthermore, in the failing human heart, especially in the areas which correspond to the highest fibroblast proliferation and collagen deposition the AT_2/AT_1 ratio is higher than in the normal heart[50], and AT_2 binding sites are increased especially in the areas showing the highest fibroblast proliferation and collagen deposition[51]. In the cardiomyopathic hamster model, the increase in AT_2 receptors plays an important anti-AT_1 receptor action on the progression of interstitial fibrosis during cardiac remodeling, by inhibiting both fibrillar collagen metabolism and growth of cardiac fibroblasts[49]. Obviously, these data concur to support a contributory role of the AT_2 receptor in the cardiac remodeling processes, and suggest that it opposes the role of AT_1 receptors, with the obvious implication that antagonism of AT_1 receptors, with the consequent shift of angiotensin II to bind AT_2 receptors, may favor both effects at the same time.

The data obtained from vascular tissues support the antiproliferative action of the AT_2 receptor. In fact, in adult vasculature injured by the balloon technique or by inflammation, the AT_2 receptor gene is reexpressed[15]. In addition, in adult rat aortic smooth muscle cells trans-

fected with the AT_2 expression vector, angiotensin II significantly increased the cell number, and this effect was abolished by treatment with an AT_1 receptor antagonist[52]. In contrast, in the cells expressing both receptors angiotensin II had little or no effect on the cell number, and the treatment with the AT_2 receptor antagonist unmasked the growth effect of angiotensin II mediated by the AT_1 antagonist[52].

Classical experiments by Stoll et al.[39], conducted in coronary endothelial cells, consistently demonstrated the antiproliferative effect of AT_1 and AT_2 receptor antagonists on the thymidine incorporation, i.e. an index of cell growth.

Finally, the vascular antiproliferative effect of the AT_2 receptor has been demonstrated in mesangial cells[53], and in the rat carotid artery injury model[15]. In this latter study the neointima of the vessels transfected with the AT_2 receptor transgene showed a 70% decrease compared to the vessels of untransfected rats. This protective effect was selectively prevented by the AT_2 blocking agent PD 123319. It is also important to point out that in the PC12W cells, a line of neuronal cells derived from pheochromocytoma, angiotensin AT_2 receptors have been shown to inhibit proliferation and to promote cell differentiation[54], suggesting a fundamental regulatory role of these hormonal receptors in the growth, development and remodeling tissue processes.

Beyond its inhibitory effects on hypertrophy and fibrosis related to angiotensin II, AT_2 receptors most likely mediate vasorelaxation[55,56] and may be involved in the blood pressure lowering effect of AT_1 antagonists. Recent data from our group[57] have shown that in salt-restricted rats, with powerful activation of the renin-angiotensin system, AT_2 blockade by the PD 123319 compound offsets the blood pressure reduction induced by losartan, thus suggesting that unopposed stimulation of AT_2 receptors may contribute to the blood pressure lowering effect of AT_1 blockade in this model. Consistent with this hypothesis are the observations that in AT_2 knockout mice blood pressure is significantly higher than in the wild -type control[58], and that AT_2 receptor blockade may augment the pressor effect of angiotensin II in the rat[56]. More recently Barber et al.[59] have reported consistent data in hypertensive rats showing that AT_2 receptors contribute also in this model to the antihypertensive effect of AT_1 receptor blockade.

Although the nature of the hypotensive effect of AT_2 receptors remains unclear, data indeed suggest that it may be related to vasodilation. In fact, in rat glomerular afferent arterioles, AT_2 receptors mediate vasodilation, and this effect is blocked by either disruption of endothelium or blockade of the cytochrome P 450 pathway[60]. This effect may be attributed to the interaction with epoxyeicosatrieinoic acid or the nitric oxide pathway. In this latter regard, studies by Siragy and Carey[61,62] in sodium-depleted animals have shown that renal nitric oxide and cyclic GMP production is increased by the enhanced activity of the renin-angiotensin system via the AT_2 receptor subtype, whereas the AT_1 receptor mediates the renal production of PGE_2[61]. Consistently, AT_2 receptor stimulation promotes nitric oxide release in dog coronary arteries[63].

The mechanism by which the angiotensin II-AT_2 receptor complex stimulates nitric oxide has not been fully elucidated. However, there is evidence strongly suggesting that AT_2 receptor-induced cyclic GMP production is mediated by the accumulation of bradykinin, since the effects of angiotensin II and losartan on aortic cyclic GMP content are blocked by the AT_2 antagonist receptor, as well as by the bradykinin B2 receptor antagonist icatibant or by L-nitro-arginine-methylester (nitric oxide synthase inhibitor)[64] (Fig. 5). This finding had been reported also in the bovine aortic endothelial cells. The specific mechanism by which AT_2 receptor stimulation leads to bradykinin accumulation has recently been elucidated by Tsutsumi et al.[65]. These authors have elegantly shown that AT_2 receptor stimulation is associated with changes in the Na^+/H^+ exchange activity, leading to the acidification of the intracellular environment, and consequently to the stimulation of enzymes such as kininogenase. This results in increased bradykinin formation. In this view, some emerging and interesting functional similarities between AT_1 receptor antagonists and ACE-inhibitors should be pointed out. On the other hand, the apparent difference in the clinical detection of cough could be ascribed either to the im-

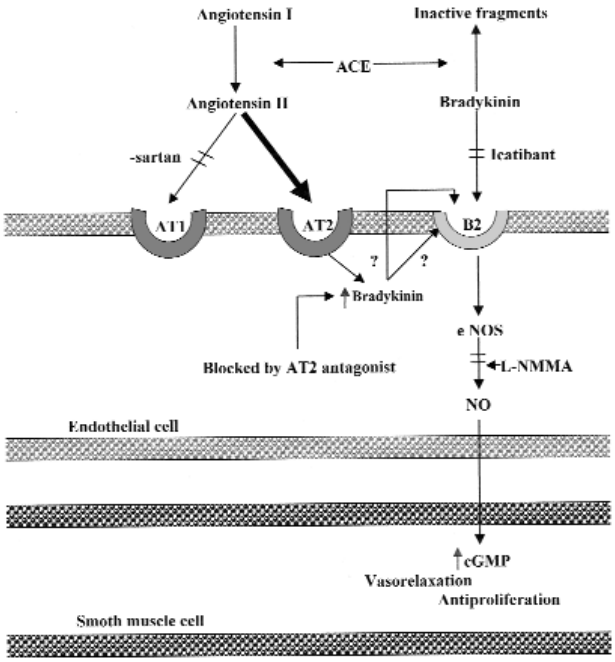

Figure 5. *Proposed interactions among the AT_2 receptors, the bradykinin and nitric oxide systems. ACE = angiotensin-converting enzyme; cGMP = cyclic guanosine monophosphate; eNOS = endothelial nitric oxide synthase; L-NMMA = N^G-monomethyl-L-arginine.*

portance of other autacoids such as substance P[66,67] in the genesis of cough or to the prevailing distribution of ACE in the lungs. The involvement of the kinin system in the effect of AT_2 receptors may in turn be related to the interaction between cytokines and AT_2 receptor up-regulation as observed in the inflammation model of the cuff-wrapped femoral artery in the rat[38].

Finally, it should be mentioned that AT_2 receptors may have an important role in the development of congestive heart failure. In fact, experimental data obtained by Liu et al.[68] in a model of myocardial infarction-induced heart failure showed that the administration of an AT_1 antagonist limited the increase in left ventricular volumes, reduced collagen deposition in the interstitium, and cardiomyocyte size. However, all these beneficial effects were prevented by an AT_2 receptor antagonist, thus demonstrating that agonism of AT_2 receptor by residual, unbound angiotensin II plays a relevant role in the effect of AT_1 blockade in this model. This feature may specifically characterize AT_1 antagonists in the treatment of cardiac diseases, and further differentiate these drugs from ACE-inhibitors. Actually, in this view the potential benefit of the combination of ACE-inhibitors and AT_1 antagonists represented by the more effective blockade of the renin-angiotensin system, may be partially offset by losing the agonism of AT_2 receptors by residual angiotensin II. Further studies will be obviously required to define this aspect and to prove the clinical effectiveness and safety of a combination therapy.

In conclusion, a large body of experimental evidence indicates that AT_2 receptors may play an important role in the pathophysiology of the renin-angiotensin system. The exact significance of this angiotensin II receptor subtype in humans has not as yet been defined. However, systematic investigation of the biology of the angiotensin receptor apparatus may lead to the discovery of new, fundamental biological interactions, that may result of great relevance for designing new therapeutic approaches to cardiovascular diseases.

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