The angiotensin II type 2 receptor:
an enigma with multiple variations

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The angiotensin II type 2 (AT2) receptor has been an enigma since its discovery ten years ago. This receptor binds ANG II with a high affinity but is not responsible for mediating any of the classical physiological actions of this peptide, all of which involve the AT1 receptor. Furthermore, the AT2 receptor exhibits dramatic differences in biochemical and functional properties and in patterns of expression compared with the AT1 receptor. During the past decade, much information has been gathered about the AT2 receptor, and the steadily increasing number of publications indicates a growing interest in this new and independent area of research. A number of studies suggest a role of AT2 receptors in brain, renal, and cardiovascular functions and in the processes of apoptosis and tissue regeneration. Despite these advances, nothing stands out as the major singular function of these receptors. The study of AT2 receptors has reached a crossroads, and innovative approaches must be considered so that unifying mechanisms as to the function of these unique receptors can be put forward. In this review we will discuss the advances that have been made in understanding the biology of the AT2 receptor. Furthermore, we will consider how these discoveries, along with newer experimental approaches, may eventually lead to the elusive physiological and pathophysiological functions of these receptors.

seven transmembrane domain receptors; G protein; phosphatases; apoptosis; tissue regeneration

In 1989, two independent groups of investigators provided pharmacological evidence for the existence of two major subtypes of angiotensin II (ANG II) receptors (16, 159). These subtypes were subsequently named the ANG II type 1 (AT1) and ANG II type 2 (AT2) receptors. It became apparent over the next few years that the AT1 receptors were responsible for mediating all of the well-known stimulatory actions of ANG II on blood pressure, water, and sodium intake, renal sodium retention, secretion of vasopressin and aldosterone, and cell growth/proliferation (141). In addition, the AT2 receptor was cloned and sequenced, intracellular signaling pathways have been identified, and although the exact physiological functions are not established, it is now known that these receptors mediate anti-growth and apoptotic actions of ANG II. Furthermore, there is a growing body of evidence that stimulation of AT2 receptors may offset or oppose the AT1 receptor-mediated actions of ANG II on cell growth, blood pressure, and fluid intake. The aim of this review is to provide an analysis of the current understanding of AT2 receptors, from molecular characterization to putative (patho)physiological roles, ten years after their discovery.
Molecular Characterization

AT₂ receptors have been cloned and characterized in a variety of species, including humans (91, 147), rats, and mice. Expression cloning from a rat pheochromocytoma (PC12W) cell library (64) and a rat fetus library (99) resulted in identification of the rat AT₂ receptor cDNA, which consists of a 1089-bp open reading frame. The AT₂ receptor gene encodes a 363-amino acid protein corresponding to a molecular mass of 41 kDa. Although they exhibit a similar binding affinity for ANG II, AT₁ and AT₂ receptors share only a 32–34% identity at the amino acid level. Similar findings have been obtained using a mouse fetal cDNA library (106), revealing a 95% homology between the rat and mouse AT₂ receptor genes and also the presence of three exons, with the entire coding region being located at the third exon (54, 55). Hydropathy analysis indicated that the AT₂ receptor belongs to the seven transmembrane domain (7-TMD) receptor superfamily. In humans and mice, the AT₂ receptor is located on the X chromosome (region Xq24-q25 and region XA2-A4, respectively) (73), suggesting that these receptors may be involved in congenital X chromosome-linked diseases.

Comparison of the human AT₂ receptor cDNA sequence with the human AT₂ receptor genomic clone revealed that the former is composed of three exons and spans ≥5 kb. Whereas exons one and two encode for 5′-untranslated regions, exon three contains the complete open reading frame (90). The promoter region includes an interferon consensus site and a putative embryonic long terminal repeat binding protein site (90). Finally, by study of 5′-terminal deletion mutants, sequence elements located in intron 1 have been reported to be necessary for efficient transcription of the human AT₂ receptor (156).

Thus far, cloning studies have revealed only one AT₂ receptor. However, certain studies have suggested the presence of heterogeneous populations of AT₂ receptors. For example, in N1E-115 murine neuroblastoma cells, separation of solubilized membranes revealed two biochemically and pharmacologically distinct AT₂ receptor populations (118). Similar results have been obtained by Yee et al. (166), describing the existence of two pharmacologically, biochemically, and immunologically different subpopulations of AT₂ receptors. Furthermore, studies from Tsutsumi and Saavedra (144) have indicated that AT₂ receptors in different brain regions exhibited differential sensitivity to GTPγS, and these investigators suggested the presence of AT₂a and AT₂b receptors. However, the coding region of the AT₂ receptor is located on a single exon, and alternative splicing is therefore not possible. Thus these studies may well be describing another so-far-unknown angiotensin receptor and not an AT₂ receptor subtype.

Biochemical Characterization

AT₁ and AT₂ receptors are only 32–34% identical at the amino acid level but elicit a similar affinity for ANG II. The lowest homology between these receptors exists between their third intracellular loops (3rd ICL). The 3rd ICL is regarded as one of the major regions of the G protein-coupled receptor that is responsible for their signal transduction. Delivery of synthetic 3rd ICL peptides of the AT₂ receptor into vascular smooth muscle cells (VSMC) results in decreased DNA synthesis, diminished cell proliferation, and a reduction in mitogen-activated protein (MAP) kinase activity, similar to the effects of ANG II acting via the AT₂ receptor (37). Moreover, in neurons cultured from newborn rat hypothalamus and brain stem, intracellular injection of a 22-amino acid peptide (PEP-22) corresponding to the putative 3rd ICL of the AT₂ receptor elicited an increase in delayed rectifier K⁺ current (Kᵥ) similar to that obtained with ANG II acting via AT₂ receptors in these cells (67). Using a chimeric receptor in which the 3rd ICL of the AT₂ receptor was replaced with that of the AT₁ receptor, Dittus et al. (23) observed a loss in affinity for [125I-Sar²-Ile⁸]ANG II and [125I]-CGP 42112A. Collectively these data support the hypothesis that the 3rd ICL of the AT₂ receptor is as closely linked to intracellular signaling as to the determination of ligand-binding properties.

It has been hypothesized that certain amino acids that are conserved between the AT₁ and AT₂ receptors are important for ANG II binding to both receptor subtypes. When binding to its AT₁ receptor, ANG II must interact with the positively charged side chain of the lysine residue 199 that is situated in the fifth TMD. By replacing this conserved lysine residue at position 215 in the fifth TMD of the AT₂ receptor with different amino acids, Yee et al. (166) and Pulakat et al. (116) have shown that a positively charged amino acid at this particular position is required for ANG II binding. Mutation of tyrosine 108 in the extracellular loop 1 (ECL1) to an alanine in the AT₂ receptor resulted in wild type-like binding for ANG II, whereas substitution of either arginine 182 in ECL2 or aspartate 297 in ECL3 drastically impaired ANG II binding (39). Moreover, amino-terminal deletion of residues dramatically reduces the affinity of the AT₂ receptor toward ANG II, whereas the binding of selective AT₂ receptor ligands, such as PD-123319 and CGP-42112A, was not dependent on the amino terminus (167). These findings support the hypothesis that ANG II and selective AT₂ receptor ligands bind to different domains of the AT₂ receptor and that the binding mechanisms of ANG II to AT₁ and AT₂ receptors appear to be closely related. However, in a recent study performed by Miura and Karnik (96), the effects of various modifications of ANG II side chains have been evaluated, and the data suggest that ANG II binding to both receptor subtypes takes place through different types of epitope recognition. Whereas the AT₁ receptor seems to be “relaxed” and no single interaction appears to be critical for ligand binding, the AT₂ receptor is in a constrained conformation and is only activated when bound to ANG II.

Studies have indicated that His256 located in the sixth TMD of the AT₁ receptor is necessary for its
activation by ANG II. Mutation of the AT2 receptor by replacing the conserved amino acid His273 (149) resulted in a loss of binding affinity to $^{125}$I-Sar1-Ile8]ANG II and $^{125}$I-CGP 42112A. Studying the role of two other conserved residues in the sixth TMD of the AT2 receptor, Heerding et al. (40) observed that mutation of Trp269 did not affect ANG II binding, whereas it was impaired after Asp279 mutation. Thus these results indicate that certain amino acids are required for high-affinity binding of ANG II to the AT2 receptor.

The extracellular domains of the AT1 receptor contain three consensus N-glycosylation sites (21). However, the extracellular domains of the AT2 receptor contain five potential glycosylation sites (21). A low degree of AT2 receptor glycosylation account for the different molecular masses of this receptor in various tissues (120). The PC12W AT2 receptor contains three N-linked oligosaccharide side chains. However, these side chains do not have a major influence on the affinity of the receptor, nor are they essential for AT2 receptor expression (121).

AT1 receptor stimulation by ANG II is followed by a rapid desensitization, which is paralleled by a decrease in the number of cell surface receptors. In contrast to AT1 receptors, which are internalized into endosomes, AT2 receptors do not undergo endocytosis when bound by ANG II (42). Moreover, other studies performed by Csikos et al. (19) indicate the existence of a so-far-unknown endogenous factor that, similar to the AT2 receptor ligands, seems to prevent AT2 receptor degradation.

Many biochemical data have been gathered in recent years and have helped to characterize the AT2 receptor and provide comparisons with the AT1 receptor. However, at this time the three-dimensional structure of the AT2 receptor has only been determined by mathematical approaches. Determination of the crystalline structure of the AT2 receptor would provide important information concerning structural properties, agonist binding, and effector coupling. Unfortunately, these studies will probably be quite difficult to perform because obtaining single crystals of G protein-coupled receptors has traditionally been a complicated process.

Pharmacological Characterization

Although AT1 and AT2 receptors display a low degree of homology, ANG II exhibits a similar affinity at both receptor subtypes. Discrimination of these subtypes became possible with the development of highly selective receptor antagonists (141). AT1 receptors exhibit a low affinity to tetrahydromidazolepyridines, such as PD-123177 and PD-123319, and a high affinity for a class of compounds called "sartans," which from the chemical point of view belong to the biphenylimidazoles. On the other hand, AT2 receptors show a very low affinity for biphenylimidazoles and a high affinity for tetrahydromidazolepyridines (10). Moreover, compared with AT1 receptors, AT2 receptors also display a high affinity for the peptide CGP-42112A, which is a partial agonist (10).

The four putative extracellular domains of the AT1 receptor each contain a cysteine residue, which together presumably form two disulfide bonds that stabilize the tertiary structure of the receptor. On the other hand, AT2 receptors have eight additional cysteine residues within their extracellular domains, which might be the reason that both receptor subtypes can be distinguished by reducing agents such as dithiothreitol (DTT). Although the binding affinity of AT2 receptors to ANG II is enhanced by DTT, this reducing agent decreases the affinity of the AT1 receptors (15, 16, 133).

ONTOKENY AND EXPRESSION OF AT2 RECEPTORS

Before the discovery of the ANG II receptor subtypes, ligand binding and autoradiographic analyses had revealed that the anatomic localization of specific receptors for ANG II in mammals was consistent with the role of this peptide in cardiovascular control, fluid balance, hormone secretion, and cell growth. After the discovery of ANG II receptor subtypes, it became clear that the AT1 receptors were responsible for mediating these well-known physiological actions of ANG II, and that the distribution of AT1 receptors matched that of the "classical" ANG II receptor. It also became apparent that the expression pattern of AT2 receptors in mammals was fairly unique and on the whole distinct from the AT1 receptor. Nowhere was this difference more apparent than in developing tissues. AT1 receptors are abundant in a number of adult tissues but are expressed only to a minor extent in embryonic/neonatal tissues. In general terms, AT2 receptors are localized in numerous embryonic and neonatal tissues, but their expression declines rapidly after birth and is then restricted to certain organs and brain areas (31, 34, 35, 61, 62, 95, 143). For example, in the adult rat, AT2 receptors are localized in high quantities in peripheral organs such as the kidney, uterus, and adrenal medulla (9, 93, 110, 122, 123). In adult rat brain, nuclei that contain high densities of AT2 receptors include the inferior olives, locus coeruleus, ventral septum, lateral septum, mediodorsal thalamic nuclei, medial amygdala, red nucleus, and medial geniculate nucleus (76). More recent studies indicate that the pattern of expression of AT2 receptors, at least in the brain, is more complex than the above generalization. It appears that certain brain areas express AT2 receptors during embryonic life, other areas express these sites only after birth, and in some areas the AT2 receptor persists until maturity (111). Certainly, the presence of high levels of AT2 receptors within the neonatal brain supports the suggestions that this receptor has a role in development.

Once again, it should be noted that the expression pattern of AT1 and AT2 receptors in adult tissues is quite different, and this distinction is very apparent in the brain. In adult rat brain, the AT1 receptors are localized in areas such as the subfornical organ, paraventricular nucleus, dorsal vagal complex, and area postrema, consistent with their role in cardiovascular control, fluid balance, and hormone secretion. The localization of AT2 receptors, outlined above, is limited
to areas that are involved in sensory, motor, and visual control, although there is no physiological evidence of a role for AT₂ receptors in these processes.

The lack of coexpression of AT₁ and AT₂ receptors in many tissues is interesting when the physiological evidence that has begun to accumulate is considered, which suggests that these receptors have offsetting effects. For example, studies have indicated that the AT₁ receptor-mediated actions of centrally injected ANG II on water intake and vasopressin release are enhanced by blockade of AT₂ receptors (45). This anomaly might be explained by interactions between neuronal circuits that are controlled by AT₁ and AT₂ receptors.

In summary, there is now a body of evidence concerning the localization of AT₂ (and AT₁) receptors in mammalian tissues, and some studies have detailed the pattern of expression of AT₂ receptors during development. This knowledge will be invaluable to our understanding of the physiological and pathological roles of the AT₂ receptor.

REGULATION OF AT₂ RECEPTOR EXPRESSION

An understanding of the factors that regulate the expression of AT₂ receptors is important for at least two reasons. First, from the previous section it is apparent that AT₂ receptors exhibit a rather unique pattern of expression in mammalian tissues, especially during ontogenic development. Thus it is important to understand what influences the expression of AT₂ receptors during ontogeny. Second, one of the factors that governs the responsiveness of a particular hormone/neurotransmitter is the level of expression of its receptor. Therefore, to fully understand and unmask the physiological actions of ANG II that are mediated by the AT₂ receptor, it is necessary to understand the determinants of the AT₂ receptor expression. From the following paragraphs, it will be apparent that a number of factors, both extracellular and intracellular, contribute to the regulation of expression of AT₂ receptors.

The majority of studies performed on AT₂ receptor regulation have been in vitro cell culture experiments. Along these lines, it became clear early on that the cell culture conditions can affect the expression of AT₂ receptors. For example, in the fibroblast cell line R3T3, which is known to express AT₂ but not AT₁ receptors, the number of AT₂ binding sites depends greatly on the cell density (25). Whereas the expression of AT₂ receptors is very low in actively growing R3T3 cells, their expression is substantially increased when the cells become confluent. This regulation of AT₂ receptors seems to occur at multiple levels, involving translational and/or posttranslational as well as transcriptional control (14). Furthermore, promoter/luciferase reporter deletion analysis of the AT₂ receptor in R3T3 cells revealed that growth-dependent AT₂ receptor expression is influenced by the transcription factors interferon regulatory factor-1 and -2 (50). The expression of AT₂ receptors within cultured cells is also regulated by the presence of serum in the growth media. For example, the addition of serum to quiescent and growth-inhibited R3T3 cells markedly decreases AT₂ receptor expression in these cells (25). In contrast, the presence of serum is necessary for full expression of AT₂ receptors in primary neuronal cultures from neonatal rat hypothalamus and brain stem (53).

The fact that serum dramatically influences the expression of AT₂ receptors in cultured cells led to the idea that growth factors may have a role in the regulation of these receptors, and this is indeed the case. For example, addition of growth factors such as bovine fibroblast factor, insulin-like growth factor I, and transforming growth factor-β to quiescent and contact-inhibited R3T3 cells decreases the expression of the AT₂ receptors (25). Similar results have been obtained in PC12W cells. Serum, growth factors, and the glucocorticoid dexamethasone cause a marked decrease in AT₂ receptor expression, mainly through a reduction in gene transcription (69). It should also be pointed out that the effects of growth factors or serum on AT₂ receptor expression in PC12W and R3T3 cells are dependent on the respective cell passage (77), indicating another layer of complexity in the regulation of AT₂ receptor expression in these cells. In primary neuronal cultures (53), short-term incubations with nerve growth factor (NGF) resulted in increased AT₂ receptor mRNA levels, whereas long-term incubations with this factor evoked the opposite effect. Treatment of these cultured neurons with insulin led to a reduction in steady-state levels of AT₂ receptor mRNA (53), whereas AT₂ receptor expression can be induced by insulin and insulin-like growth factors in VSMC (65). In serum-deprived adult VSMC, the presence of these factors was essential to observe expression of AT₂ receptors. These apparently contradictory observations suggest that the effects of insulin on the AT₂ receptor gene expression are cell type specific. The finding that the effects of certain agents on the AT₂ receptor expression are not necessarily identical in different cell types emphasizes the importance of thoroughly discriminating data gathered in each situation.

Although it is clear that growth factors have a major influence on the expression of AT₂ receptors in cultured cells, it is apparent that other extracellular factors can influence the expression of these sites. Treatment of R3T3 fibroblasts with ANG II or the AT₂ receptor agonist CGP-42112A increases the number of AT₂ receptors in these cells in a time- and concentration-dependent manner (25). A similar upregulation of AT₂ receptors by ANG II has been noted in rat cerebral cortical neuronal cultures (127). In PC12W cells, the muscarinic receptor agonist carbachol elicits an increase in AT₂ receptor mRNA levels (128), an effect abolished by the nitric oxide inhibitor nitro-L-arginine methyl ester (l-NAME).

Thus it is established that a number of extracellular factors, and in particular growth factors, can influence the expression of AT₂ receptors in cultured cells. It is also apparent that the expression of these receptors may be influenced by intracellular factors. As demonstrated by Murasawa et al. (103), CAMP downregulates the AT₂ receptor in PC12W cells by destabilizing AT₂
receptor mRNA and not through inhibition of AT_2 receptor gene transcription. Factors that are known to alter cAMP levels within a given cell may, therefore, influence AT_2 receptor-mediated effects by reducing the expression of this receptor. Intracellular cation levels also seem to have an impact on AT_2 receptor expression. In PC12W cells, changes in intracellular sodium levels regulate AT_2 receptor expression at the transcriptional level, whereas a potassium channel blocker-dependent upregulation appeared to be, at least in part, posttranslational (139).

In vivo studies on the regulation of AT_2 receptors have been more limited, although recent studies have begun to indicate a role for certain steroid hormones in the regulation of these sites. For example, estrogen increases the levels of AT_2 receptors in human myometrium (87), whereas aldosterone decreases the expression of AT_2 receptors in the adrenal medulla (153). However, other studies have failed to demonstrate any regulatory action of mineralocorticoids and glucocorticoids on brain AT_2 receptors (124, 125).

In summary, both in vitro and in vivo studies have demonstrated regulatory factors, both extracellular and intracellular, that alter the expression of AT_2 receptors in various tissues and cells. These regulatory influences are summarized in diagrammatic form in Fig. 1. However, both systems of study have their own drawbacks. Cell culture experiments have the problems that are inherent in in vitro experimentation, and studies on regulation in vivo are hampered by the presence of multiple influences, making it difficult to assess the role of one particular regulator. An approach that combines both in vivo and in vitro studies will probably yield the most useful data.

**AT_2 RECEPTOR-MODULATED SIGNALING PATHWAYS**

From previous sections it is clear that the molecular, biochemical, and pharmacological properties of AT_2 receptors and patterns of expression differ greatly from those of AT_1 receptors. It was quickly recognized that the intracellular signaling pathways that couple to these receptor subtypes are also very different. Initial studies indicated that AT_2 receptors do not transduce via any of the traditional signaling pathways that are modulated by activation of AT_1 receptors (increased phosphoinositol hydrolysis; inhibition of adenylate cyclase). In fact, there was no consistent evidence that AT_2 receptors signaled via any of the well-known intracellular pathways, giving credence to the suggestion that it might be a clearance receptor for ANG II (24). However, during the past ten years, a number of studies have shed light on the intracellular signaling pathways that are modulated after activation of AT_2 receptors. Similar to the AT_1 receptor, the AT_2 receptor can modulate a number of different signaling mechanisms, depending on the cell/tissue type. In the following paragraphs we will review these signaling pathways, and in a later section we will attempt to relate these intracellular mechanisms to certain cellular functions.

**G proteins**

As stated earlier, the AT_2 receptor is predicted to be a seven-TMD receptor on the basis of structural analysis (54, 55). The first indications of G protein-mediated signaling through this receptor came from electrophysiological studies. In one study it was determined that the AT_2 receptor-mediated stimulation of K_v current in neurons cultured from rat hypothalamus and brain stem occurred through a pertussis toxin (PTX)-sensitive G protein, probably G_i (66). A subsequent study in NG108–15 neuroblastoma × glioma cells demonstrated that the AT_2 receptor-mediated inhibition of T-type Ca^{2+} current involved a G protein (12). Biochemical studies have also indicated that the AT_2 receptor couples through G protein-mediated mechanisms. For instance, the AT_2 receptor-mediated stimulation of serine/threonine phosphatase 2A (PP-2A) activity and subsequent decrease in extracellular signal-regulated kinase (Erk1/Erk2) MAP kinase activities in cultured neurons involves a PTX-sensitive G protein (51, 52). Furthermore, the AT_2 receptor-mediated induction of MAP kinase phosphatase 1 (MKP-1) and decrease in Erk1/Erk2 activities in PC12W cells are mediated
through GI (48). Finally, a role for GI protein in transducing AT₂ receptor-mediated signals was confirmed through studies indicating that the AT₂ receptor associates with GIα2 and GIα3 in rat fetal tissue (171).

In summary, the available evidence suggests that the AT₂ receptor couples to intracellular signaling pathways through the PTX-sensitive G protein GI. However, some studies have indicated that AT₂ receptors can couple via a G protein-independent mechanism, especially in the stimulation of protein tyrosine phosphatases (see Protein Phosphatases).

Lipid-Signaling Pathways

Accumulating evidence indicates that stimulation of AT₂ receptors by ANG II leads to a modulation of certain lipid-signaling pathways. In neonatal rat cardiac myocytes (82), rabbit proximal tubule epithelia (60), and cultured neurons from newborn rat brain (172), short-term (1- to 10-min) stimulation with ANG II elicits an increase in phospholipase A₂ (PLA₂) activity and arachidonic acid (AA) release. The effects of ANG II on PLA₂/AA in cultured neurons were abolished by PTX, indicating the involvement of a PTX-sensitive G protein (172). In PC12W cells, longer-term (1- to 24-h) activation of AT₂ receptors elicits an increase in synthesis of the sphingolipid ceramide (28, 75). Thus activation of AT₂ receptors can lead to a mobilization of different lipid-signaling molecules, depending on the cell type. As will be seen in a later section, these short- and long-term modulatory effects on lipid messengers are related to certain physiological actions of the AT₂ receptor.

Protein Phosphatases

A number of studies have indicated that activation of AT₂ receptors leads to a stimulation of protein phosphatases. The earliest indication of such a relationship came from studies in PC12W cells, which indicated that ANG II elicits a rapid dephosphorylation of tyrosine residues of 60- to 150-kDa proteins. This effect was abolished by inhibition of phosphotyrosine phosphatase (PTPase), indicating a role for this class of enzymes (8, 11). Similar rapid and transient increases in PTPase activity after AT₂ receptor activation have been observed in R3T3 fibroblasts (146, 148) and NIE-115 cells (6, 104). In the latter case, ANG II was shown to stimulate the activity of SHP-1, a soluble PTPase. An interesting point is that these rapid effects of ANG II on PTPase activity in PC12W and NIE-115 cells seem to be G protein independent, because they are not affected by PTX or GTPγS or GDPβS (6, 11). It is also interesting that ANG II elicits a long-term induction of the PTPase MKP-1 in PC12W cells, an effect mediated by GI (46). A similar induction of MKP-1 is seen after AT₂ receptor activation in adult rat ventricular myocytes (27), but not in NIE-115 cells (6). Thus it appears that stimulation of AT₂ receptors elicits an increase in PTPase activity via G protein-dependent and -independent mechanisms.

Evidence also exists that AT₂ receptors couple to a stimulation of serine/threonine phosphatases, especially in central nervous system tissue. Studies from primary cultured neurons of newborn rat hypothalamus and brain stem indicate that stimulation of AT₂ receptors elicits a time- and concentration-dependent increase in PP-2A activity via a PTX-sensitive pathway (51). Indirect evidence that AT₂ receptors signal via activation of serine/threonine phosphatases has come from a number of different studies. For example, the AT₂ receptor-mediated increase in neuronal Kv current is abolished by inhibition of PP-2A (66). In other studies it has been determined that ANG II elicits an AT₂ receptor-mediated upregulation of AT₂ receptor mRNA in cerebral cortical neuronal cultures, an effect that is mediated through activation of serine/threonine phosphatases (127). Finally, AT₂ receptor-mediated increase in apoptosis of hypothalamus/brain stem cultured neurons is mediated by PP-2A (126).

In summary, the available evidence indicates that ANG II can elicit an AT₂ receptor-mediated stimulation/induction of PTPases or a stimulation of PP-2A, depending on the cell/tissue type. This is summarized in diagrammatic form in Fig. 2. As will be seen in a later section...

Fig. 2. ANG II-induced stimulation of phosphatases. MKP-1, mitogen-activated protein (MAP) kinase phosphatase-1; PP-2A, serine-threonine phosphatase 2A; SHP-1, a soluble phosphotyrosine phosphatase (PTPase) activity.
section, these effects of ANG II on PTPases and PP-2A are related to physiological functions.

Protein Kinases

A number of studies have investigated the effects of AT2 receptor stimulation on Erk1 and Erk2 MAP kinase activities. One rationale for these studies was that ANG II inhibits the growth of a number of cell types via AT2 receptors (see AT2 receptor-mediated physiological effects) and that growth regulatory pathways often involve activation of Erk1/Erk2. A further rationale was that PP-2A and MKP-1, both of which are activated through AT2 receptors, are powerful inhibitors of Erk1/Erk2 (100, 160). Studies in PC12W cells and R3T3 fibroblasts indicate that ANG II acts at AT2 receptors to inhibit Erk1 and Erk2 MAP kinase activities via a G protein-dependent mechanism (49, 165). In neurons cultured from newborn rat hypothalamus and brain stem, stimulation of AT2 receptors results in a time-dependent decrease in Erk1 and Erk2 activities that is mediated through a PTX-sensitive G protein and through activation of PP-2A (52). Studies from N1E-115 cells also demonstrated an AT2 receptor-mediated decrease in Erk MAP kinase activities (6). However, in N1E-115 cells, this effect of ANG II does not involve a G protein, PP-2A, or MKP-1, but is mediated through activation of SHP-1 PTPase (6). From these studies it is clear that activation of AT2 receptors has an inhibitory effect on Erk MAP kinase activities, albeit via dissimilar mechanisms in different cell types. This inhibitory effect of AT2 receptor activation on these MAP kinases is consistent with the role of this receptor in growth inhibition (135), apoptosis, and differentiation.

Recent studies indicate that Erk MAP kinases are not the only protein kinases that are modulated by AT2 receptor activation. Janus kinases and signal transducers and activators of transcription (JAK/STAT) are key elements of the signaling pathways through which ANG II (via AT1 receptors), growth factors, and cytokines stimulate growth of VSMC. In fact, it has been shown that ANG II elicits an AT1 receptor-mediated phosphorylation of JAK, with subsequent phosphorylation of STAT (88). It has been demonstrated that stimulation of AT2 receptors in adult VSMC (transfected with the AT2 receptor cDNA) inhibits the AT1 receptor-mediated tyrosine phosphorylation of STAT1, STAT2, and STAT3 without affecting JAK activity (48). AT2 receptor activation also inhibited the tyrosine phosphorylation of STAT1 induced by interferon-γ and epidermal growth factor in these cells, and similar results were obtained in fetal VSMC, which express endogenous AT2 receptors (48). The inhibitory effects of AT2 receptor activation on STAT phosphorylation appear to be due to the well-documented inhibition of Erk MAP kinase activation. In summary, it appears that ANG II can inhibit the activity of multiple growth factor signaling pathways through activation of AT2 receptors.

Stimulation of AT2 receptors in certain cell types leads to changes in cellular cGMP levels and nitric oxide (NO) production. The changes in cGMP levels have been shown to occur through different mechanisms and to be in opposite directions, depending on the particular cell type involved. In PC12W and rat adrenal glomerulosa cells, ANG II elicits an AT2 receptor-mediated decrease in basal and atrial natriuretic peptide-stimulated particulate guanylyl cyclase activity, causing a fall in cGMP levels (8, 11). This effect of ANG II is due to activation of a PTPase (8). In neurons cultured from newborn rat hypothalamus and brain stem, ANG II elicits an AT2 receptor-mediated decrease in cellular cGMP levels. In contrast to the effects in PC12W cells, this action of ANG II appears to involve stimulation of a phosphodiesterase enzyme and is not an effect on guanylyl cyclase (137). Studies from N1E-115 cells demonstrate that ANG II increases cellular cGMP levels via a mechanism that involves both AT1 and AT2 receptors and NO-mediated stimulation of soluble guanylyl cyclase (170). The connection between AT2 receptors and cGMP/NO has also been made through in vivo studies in renal and cardiovascular tissues. In conscious rats, intravenous infusion of ANG II elicits an increase in the level of cGMP in the renal interstitial fluid, an effect that is mediated through increased production of NO in the kidneys, and presumably subsequent activation of soluble guanylyl cyclase (130). Inhibition of cardiac AT2 receptors in hypertrophied heart from adult rats amplifies the left ventricular growth (AT1 receptor-mediated) response to ANG II via suppression of cGMP (5). Studies have also shown that ANG II increases aortic cGMP in adult spontaneously hypertensive stroke-prone rats through a mechanism that involves AT1 receptors and synthesis of NO (33). Finally, stimulation of AT2 receptors in proliferating rat neointima leads to a decreased tissue level of cGMP (97). From the above discussion it is apparent that the modulatory actions of ANG II via the AT2 receptor on cGMP/NO are quite variable and involve different mechanisms depending on the cell type.

Signaling Summary

In the previous sections we have summarized the information that is available concerning the intracellular signaling pathways that are modulated by ANG II via AT2 receptors. From these studies it is clear that AT2 receptors can couple to multiple signaling molecules in a fashion similar to many other hormone/neurotransmitter G protein-coupled receptors. There is some consistency among the various studies, in that it is now established that activation of AT2 receptors leads to a stimulation of tyrosine or serine/threonine phosphatases and subsequent inactivation of Erk MAP kinases. However, discrepancies exist between certain studies, e.g., role of G proteins, and increase and/or decrease in cGMP levels. Although these differences may be explained by the type of cell or tissue, or the use of tumor/transfected cell lines, it is apparent that it will
take much more work to fully establish the various intracellular pathways that are coupled to AT<sub>2</sub> receptors.

**AT<sub>2</sub> RECEPTOR-MEDIATED PHYSIOLOGICAL EFFECTS**

**Brain**

ANG II has a number of well-known effects mediated by the brain, including stimulation of drinking behavior, increases in blood pressure, modulation of baroreceptor function, and stimulation of vasopressin release. However, the majority of studies have indicated that they are AT<sub>1</sub> receptor-mediated effects and have failed to recognize any role of AT<sub>2</sub> receptors (44, 72, 117). One notable exception are studies showing that administration of the AT<sub>2</sub> receptor antagonist PD-123177 potentiates the stimulatory effects of ANG II on drinking behavior and vasopressin secretion (45). This is one example of a situation in which AT<sub>2</sub> receptors appear to oppose AT<sub>1</sub> receptor-mediated effects. Thus it is possible that some of the AT<sub>2</sub> receptor-mediated brain circuitry may function to counteract the stimulatory actions of ANG II on some of the physiological processes such as vasopressin secretion. However, as stated earlier, most of the AT<sub>2</sub> receptors in the adult brain are associated with areas involved in sensory control, and the physiological actions mediated through AT<sub>2</sub> receptors at these sites are as yet undefined. Despite a lack of knowledge concerning the physiological actions of ANG II via brain AT<sub>2</sub> receptors in vivo, numerous in situ and in vitro electrophysiological studies have indicated that ANG II can elicit AT<sub>2</sub> receptor-mediated changes in neuronal activity and membrane ionic currents.

In situ investigations have revealed that AT<sub>2</sub> receptor-mediated actions are site specific. Although AT<sub>2</sub> receptor stimulation in the locus coeruleus reduces excitatory postsynaptic potentials and glutamate-induced depolarizations (163), ANG II application onto inferior olivary neurons elicits excitatory effects (3).

Whole cell and single-channel voltage clamp experiments on primary cultured neurons from newborn rat hypothalamus and brain stem have resulted in much information concerning the neuronal currents/channels that are modulated by AT<sub>2</sub> receptor activation. Superfusion of cultured neurons with ANG II in the presence of an AT<sub>1</sub> receptor blocker elicits an increase in neuronal Kv and transient (A-type) K<sup>+</sup> currents (68, 172). Interestingly, these stimulatory actions of ANG II via AT<sub>2</sub> receptors on Kv and A-type K<sup>+</sup> currents are exactly opposite to the AT<sub>1</sub> receptor-mediated actions of ANG II on those currents (138, 152). In addition, a small population of neurons within the cultures used in these studies contain both AT<sub>1</sub> and AT<sub>2</sub> receptors, and ANG II has opposite (AT<sub>1</sub> and AT<sub>2</sub> receptor-mediated) effects on Kv and A-type K<sup>+</sup> currents in these cells (32). Consistent with the stimulatory effects of ANG II on neuronal Kv current, both ANG II and CGP-42112A activate a delayed rectifier K<sup>+</sup> channel in these neurons (89). Although AT<sub>2</sub> receptor activation does not affect voltage-dependent calcium currents in cultured neurons (68), it does result in inhibition of T-type Ca<sup>2+</sup> currents in NG108–15 cells (12).

The above studies in cultured neurons have also enabled the investigation of the intracellular signaling pathways that mediate the stimulatory effects of ANG II on Kv current. As stated in an earlier section, ANG II elicits an AT<sub>2</sub> receptor-mediated increase in PLA<sub>2</sub> activity, with subsequent generation of AA in cultured neurons. It has been determined that this is a key event in the AT<sub>2</sub> receptor-mediated stimulation of neuronal Kv current (172). Furthermore, this modulation of Kv current appears to be mediated through the 12-lipoxygenase (12-LO) metabolite of AA, 12-HETE, and AA itself. It has been suggested that these intracellular mediators affect channel activity through modulation of PP-2A (172). This putative pathway is summarized in Fig. 3.

In summary, the stimulatory effects of ANG II on Kv and A-type K<sup>+</sup> current in cultured neurons suggest that AT<sub>2</sub> receptor activation results in hyperpolarization or decreased neuronal excitability. Identification of the type(s) of neuron(s) that express(es) the AT<sub>2</sub> receptor will enable determination of whether AT<sub>2</sub> receptor-mediated changes in ionic currents and neuronal activity modify neurotransmitter release. Also, it will be interesting to see whether studies are able to establish

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**Fig. 3. Intracellular signaling pathways involved in AT<sub>2</sub> receptor-mediated stimulation of neuronal Kv and I<sub>A</sub>, 12-HETE, 12-hydroxyeicosatetraenoic acid; 12-HPETE, 12-hydroperoxyeicosatetraenoic acid; I<sub>A</sub>, A-type K<sup>+</sup>-current; Kv, voltage-dependent delayed rectifier K<sup>+</sup>-current; 12-LO, 12-lipoxygenase; PLA<sub>2</sub>, phospholipase A<sub>2</sub>.**
a connection between AT<sub>2</sub> receptor-mediated alterations of ionic currents and neuronal cell death after injury. Such a link is possible, because it has been determined that stimulation of outward K<sup>+</sup> current is one of the events that precedes neuronal apoptosis that is induced through a variety of stimulators (168, 169).

Cardiovascular System

It has been known for many years that ANG II contributes significantly to cardiovascular regulation and that the majority of its actions in this respect are mediated through AT<sub>1</sub> receptors. However, the expression of AT<sub>2</sub> receptors within cardiovascular tissues under certain physiological/pathophysiological conditions complicates matters and suggests that there is a far-from-complete understanding of the role of AT<sub>1</sub>/AT<sub>2</sub> receptors in cardiovascular regulation (47).

Heart. The available data concerning the expression of AT<sub>2</sub> receptors in the heart under (patho)physiological conditions are still controversial. However, an understanding of the underlying expression patterns is highly important because the relative proportion of ANG II receptor subtypes may influence processes such as myocardial hypertrophy and fibrosis.

Both AT<sub>1</sub> and AT<sub>2</sub> receptors are expressed in the fetal as well as in the adult heart of different species. Western blot analysis has demonstrated that the AT<sub>2</sub> receptor is present in neonatal rat cardiac myocytes but could not be detected in fibroblasts or aortic smooth muscle cells from young animals (155). Similar to other tissues, AT<sub>2</sub> receptor levels are significantly higher in the neonatal rat heart than in the young rat heart (155), an observation that has been made in other species as well. In sheep, for instance, cardiac AT<sub>1</sub> receptor gene expression remained relatively unchanged during fetal and newborn life, whereas AT<sub>2</sub> receptor mRNA levels were high during fetal life but rapidly decreased after birth (119).

In the failing ventricle of the human heart, the level of AT<sub>1</sub> receptor gene expression appears to decrease, whereas the level of AT<sub>2</sub> receptor expression is unaffected (38). A study performed by Wharton et al. (158) demonstrated that the density of AT<sub>2</sub> binding sites in endocardial, interstitial, and infarcted regions in the ventricles of patients with end-stage ischemic heart disease or dilated cardiomyopathy is significantly increased compared with the noninfarcted myocardium. Also, Gullestad et al. (36) reported that, after heart transplantation, both subtypes of ANG II receptors are downregulated, whereas an upregulation of AT<sub>2</sub> receptors has been demonstrated in hypertrophied and aging rat hearts (43). Finally, ventricular pressure overload induced by aortic banding in the rat does not exert any alterations in the AT<sub>1a</sub>-, AT<sub>1b</sub>-, and AT<sub>2</sub>-receptor mRNA levels (162).

In rats, it has been established that AT<sub>1</sub> and AT<sub>2</sub> receptors are upregulated after myocardial infarction. Nio et al. (107) and Zhu et al. (173) observed an enhanced AT<sub>1</sub>- and AT<sub>2</sub>-receptor gene expression with levels peaking at 7 days and 1 day after myocardial infarction, respectively. Together with other findings (see AT<sub>2</sub> RECEPTORS AND PATHOLOGICAL SITUATIONS), these observations point to a possible role of ANG II receptor subtypes in tissue remodeling and repair.

The question as to which specific cell type within the heart expresses the AT<sub>2</sub> receptor is also still a matter of intense debate. In neonatal rats, AT<sub>2</sub> receptor protein is detectable in cardiac myocytes but not in fibroblasts (155). Mechanical stretch of myocytes evokes increases in both AT<sub>1</sub>- and AT<sub>2</sub>-receptor mRNA levels (70), indicating that nonsecretory pathways activated by myocyte stretching are involved in ANG II receptor regulation. In chronic heart failure, AT<sub>2</sub> receptor upregulation occurs in fibroblasts and is associated with a downregulation of AT<sub>1</sub> receptors in atrial and left ventricular tissues (145). Thus the AT<sub>2</sub> receptor expression level seems to be determined by the extent of interstitial fibrosis.

In cardiomyopathic hamsters, Ohkubo et al. (112) not only demonstrated an AT<sub>2</sub> receptor reexpression by cardiac fibroblasts within the fibrous regions in failing hearts, but they also reported on an AT<sub>2</sub>-receptor-mediated inhibition of collagen metabolism and fibroblast growth. Finally, with the use of laser-assisted cell picking and subsequent single-cell PCR, Busche et al. (13) detected expression of AT<sub>2</sub> receptor mRNA in ~10% of adult rat cardiomyocytes before and 1 day after myocardial infarction, demonstrating that these cells are capable of expressing AT<sub>2</sub> receptors.

Thus, a definite physiological role has not been attributed to the AT<sub>2</sub> receptor in the heart. It has, however, been reported that an inhibition of cardiac AT<sub>2</sub> receptors in vivo results in amplification of the immediate left ventricular growth response to ANG II, which is associated with enhanced protein kinase C translocation and reduced left ventricular cGMP content (5). Although AT<sub>1</sub> receptor-induced RNA and protein synthesis involves increased c-myc expression in neonatal rat cardiac myocytes, the antiproliferative effects mediated by AT<sub>2</sub> receptors do not involve changes in c-myc levels (80).

Several studies have investigated the role of ANG II in the induction of apoptosis in the heart and have attributed the effects to AT<sub>1</sub> receptors. It has been reported that AT<sub>1</sub>-, but not AT<sub>2</sub>-receptor stimulation induces programmed cell death in cardiomyocytes (17, 63). However, findings by Busche et al. (13) showing that adult rat cardiomyocytes are able to express AT<sub>2</sub> receptors may have important implications regarding the role of AT<sub>2</sub> receptors and cardiomyocyte apoptosis.

Similar to AT<sub>1</sub> receptors, AT<sub>2</sub> receptors have been implicated in cardiovascular regulation. As demonstrated by Masaki et al. (92), cardiac-specific overexpression of the AT<sub>2</sub> receptor attenuates AT<sub>1</sub>-receptor-mediated pressor and chronotropic effects of ANG II. In the (mRen-2)27 transgenic rat, a hypertensive model that involves enhanced expression of the renin-angiotensin system (RAS), activation of AT<sub>2</sub> receptors offsets the sodium-induced pressor response (109). However, future studies will have to determine how and via which pathways the balance between both ANG II receptor subtypes affects cardiovascular regulation.
Blood vessels. It has been demonstrated that ANG II exerts opposite effects on the growth of certain cells within blood vessels by binding to either AT$_1$ or AT$_2$ receptors. Whereas ANG II induces cell proliferation by activating its AT$_1$ receptor, stimulation of the AT$_2$ receptor inhibits cell growth in different cell types (94, 102, 105, 135). The growth-suppressing effects that are mediated by AT$_2$ receptors do not involve repression of c-fos and c-jun gene expression (134) but are mediated through the inhibition of cyclin D1 expression and cyclin D1-associated kinase activity (79). After transfection of an AT$_2$ receptor expression vector into a balloon-injured carotid artery, Nakajima et al. (105) observed an AT$_2$ receptor-mediated reduction in neointima formation. Moreover, transfection of the AT$_2$ receptor into VSMC in vitro resulted in diminished cell proliferation and decreased MAP kinase activity.

In contrast to Wistar Kyoto rats (WKY) and adult spontaneously hypertensive rats (SHR), an increased expression of the AT$_2$ receptor has been demonstrated in vessels from young SHR (142). In these particular animals, both AT$_1$ and AT$_2$ receptors contribute to blood pressure regulation, because the ANG II-induced contractile responses are reduced by the AT$_2$ receptor antagonist PD-123319 and abolished by the AT$_1$ receptor antagonist losartan.

Kidney

Evidence has been provided that AT$_2$ receptors are expressed in the kidney. As demonstrated by Ozono et al. (115), substantial amounts of AT$_2$ receptor protein are present in fetal and newborn rat kidney, whereas only minor concentrations are observed in the adult organism. However, AT$_2$ receptors are reexpressed in the adult rat kidney in response to sodium depletion. In renal hypertensive rats, AT$_2$-Receptor expression is diminished in the ischemic kidney, whereas it is not affected in rats with ANG II-induced hypertension (155).

In the adult human kidney, the AT$_1$ receptor predominates in the glomeruli, juxtaglomerular apparatus, and proximal tubes, and the AT$_2$ receptor is the major ANG II receptor subtype in the adventitia of the arcuate and interlobular arteries and the renal capsule (174).

With regard to the physiological role of ANG II in the kidney, it has been described that AT$_2$ but not AT$_1$ receptors induce expression of the chemokine RANTES in cultured rat glomerular endothelial cells and in vivo (163). This observation suggests that AT$_2$ receptors may be involved in glomerular chemotaxis of monocytes/macrophages. Ma et al. (84) reported that the remodeling process within the renal interstitium involves ANG II actions at AT$_2$ receptors on collagen-producing cells. In the kidney of rats with unilateral ureteral obstruction, AT$_2$ receptor blockade suppresses apoptosis of tubular cells and p53 expression (98), confirming a role of these sites in apoptosis in the kidney as well as in other tissues/cells (see AT$_2$ RECEPTORS AND PATHOLOGICAL SITUATIONS).

As for other tissues, opposing actions of both ANG II receptor subtypes have been observed in the kidney. Whereas AT$_1$ receptors mediate the synthesis of prostaglandin E$_2$ (PGE$_2$), activation of the AT$_2$ receptor inhibits this ANG II-induced PGE$_2$ generation and mediates production of cGMP (129). Also, during sodium depletion in conscious rats, renal NO production is increased (130), and renal prostaglandin F$_2$-a formation is augmented through an AT$_2$ receptor-dependent mechanism (131). It has been shown by Munoz-Garcia et al. (101) that the effects of losartan on urine flow and sodium excretion can partially be attributed to the actions of ANG II at AT$_2$ receptors and to kinins and prostaglandins.

According to several studies, AT$_2$ receptors in the kidney play a role in controlling pressure natriuresis and blood pressure regulation. Studies have indicated that, although AT$_2$ receptors do not influence total renal blood flow, activation of these sites results in a blunting of pressure natriuresis in adult rat kidney (81). Furthermore, as described by Endo et al. (26), stimulation of AT$_2$ receptors modulates the AT$_1$ receptor-mediated vasoconstriction, and altered AT$_2$ receptor function may contribute to an exaggerated vasoconstrictor action to ANG II. With respect to the molecular mechanism that is involved, it has been reported by Arima et al. (4) that the AT$_2$ receptor-mediated vasodilatory actions of ANG II in the afferent arteriole involve a cytochrome P-450 pathway (4).

AT$_2$ Receptor Knockout Mice. AT$_2$ receptor knock-out mice have been established via two independent groups of investigators (41, 57). Although these gene knockouts did not prove lethal, the AT$_2$ receptor-mutant mice demonstrate significant physiological differences in their cardiovascular, renal, and central nervous systems compared with wild-type mice, particularly under certain stress conditions. We will discuss these differences further. In addition, the AT$_2$ receptor-deficient mice have provided more evidence that these receptors have a role in apoptosis, as will be discussed later.

One group of investigators (41) reported that the AT$_2$ receptor-mutant mice display a normal basal blood pressure, but the mice developed in the other study (57) exhibit a significantly higher basal blood pressure compared with the wild-type mice. Both groups reported that the AT$_2$ receptor-mutant mice exhibit an increased vasopressor response in response to intravenous injections of ANG II. The increased vascular reactivity in these knockout mice was at least partly due to increased expression of vascular AT$_1$ receptors (140). These significantly higher levels of aortic AT$_1$ receptor mRNA and protein suggest that AT$_2$ receptors counteract the AT$_1$ receptor-mediated vascular actions of ANG II through regulation of AT$_1$ receptor expression.

Ma et al. (84) used a model of unilateral ureteral obstruction in male wild-type and AT$_2$ receptor-deficient mice to investigate the role of ANG II in renal remodeling. Obstructed kidneys from the AT$_2$ receptor knockout mice showed severe interstitial fibrosis compared with those of control mice, both 5 and 14 days after obstruction. In addition, significantly fewer apop-
To investigate the potential influence of \( \text{AT}_2 \) receptor expression on the development of VSMC, Akishita et al. (1) studied cell cultures derived from wild-type and \( \text{AT}_2 \) receptor-deficient mice. Their results suggest that \( \text{AT}_2 \) receptor expression during the fetal vasculogenesis influences the growth phenotype of VSMC via modulation of the Erk cascade. In addition, the time-dependent expression of several VSMC differentiation markers differs in the aortas of wild-type and \( \text{AT}_2 \) receptor-deficient mice (165). At an early stage of differentiation there were no differences in the expression of \( \alpha \)-smooth muscle actin between wild-type and \( \text{AT}_2 \) receptor-mutant mice. In contrast, the mRNA levels for calponin, which is expressed in the late stage of VSMC differentiation, were significantly higher in aortas from the wild-type mice compared with the \( \text{AT}_2 \) receptor knockout mice aortas. Because these findings are in parallel with the pattern of \( \text{AT}_2 \) Receptor expression during development, they support the hypothesis that the \( \text{AT}_2 \) receptor is involved in vascular differentiation.

Although it has been determined that the pressor response elicited by infusion of ANG II is increased in \( \text{AT}_2 \) receptor knockout mice (41, 57), the underlying mechanism is still unknown. One possibility may be a significantly higher systemic vascular resistance in these mutant mice, as reported by Akishita et al. (2). Also, the vascular contractility of aortic rings in vitro was increased in \( \text{AT}_2 \) receptor knockout mice, indicating that \( \text{AT}_2 \) receptors are able to modulate vascular contractility (2).

Recent studies indicate that, in \( \text{AT}_2 \) receptor knockout mice, ANG II infusion for 7 days results in a sustained increase in systolic blood pressure and a reduction in urinary sodium excretion, whereas neither systolic blood pressure nor a reduction in urinary sodium excretion changed in wild-type mice (132). Moreover, \( \text{AT}_2 \) receptor-deficient mice displayed low basal levels of bradykinin and cGMP within the renal interstitial fluid compared with wild-type mice. These low levels could not be increased by ANG II infusion or dietary sodium restriction, implying that \( \text{AT}_2 \) receptors play a counterregulatory, protective role, mediated by bradykinin and NO, against the anti-natriuretic and pressor actions of ANG II.

Other studies on the \( \text{AT}_2 \) receptor knockout mice indicate a role of these receptors in certain brain-controlled behaviors and physiological actions. For example, \( \text{AT}_2 \) receptor-mutant mice exhibit attenuated exploratory behavior (41, 57). Furthermore, mice that lack the \( \text{AT}_2 \) receptor display an anxiety-like behavior that may involve the central noradrenergic system (113). It also appears that the \( \text{AT}_2 \) receptor has a role in the hyperthermia that is induced by either injection of the cytokine interleukin-1\( \beta \) (IL-1\( \beta \)) or by cage-switch stress. Compared with wild-type animals, the \( \text{AT}_2 \) receptor knockout mice exhibited a significantly less severe degree of hyperthermia after IL-1\( \beta \) injection and a significantly greater response to hyperthermia after cage-switch stress (157). These studies suggest that ANG II, via the \( \text{AT}_2 \) receptor, plays a role in stress-induced hyperthermia.

Compared with other gene knockouts, such as NGF receptor-mutant mice (18), which undergo early postnatal death, both \( \text{AT}_2 \) receptor- and \( \text{AT}_1 \) receptor-deficient mice (58) are born in expected numbers. This observation does not necessarily mean that \( \text{AT}_2 \) receptors have no role in embryonic development. However, it might support the hypothesis that \( \text{AT}_2 \) receptors exert important physiological functions that need to be compensated for by complementary hormone systems in \( \text{AT}_2 \) receptor-deficient animals (similar to \( \text{AT}_1 \) receptors with regard to, for example, blood pressure control). Hopefully, the \( \text{AT}_2 \) receptor-mutant mice will provide further insight into the role of these receptors, even if it might be difficult to discriminate \( \text{AT}_2 \) receptor-mediated effects from the overlying compensatory actions.

### \( \text{AT}_2 \) Receptors and Pathological Situations

During the past ten years, the association of the \( \text{AT}_2 \) receptor with certain pathological situations has been a recurring story. \( \text{AT}_2 \) receptor expression is dramatically altered in a number of tissues after experimental or disease-induced damage, and there is much evidence that \( \text{AT}_2 \) receptors contribute to the cellular processes that follow injury situations. The role of \( \text{AT}_2 \) receptors in pathological situations will be reviewed in the following paragraphs, with particular focus on their function in cell death through apoptosis and in tissue regeneration.

#### Apoptosis

Cell death by apoptosis is a prominent feature of normally developing tissue, as well as tissues that have undergone injury or damage (114). Among many other factors, the intricate balance between pro- and anti-apoptotic proteins determines whether a cell will survive or will be subjected to apoptosis. It has been widely accepted that extracellular signal-regulated kinases (Erk1/2) and NGFs promote cell survival, whereas cysteine proteases (caspases), ceramides, and stress kinases such as c-Jun NH\( \_2 \)-terminal kinases (JNK) facilitate cell death.

Enhanced \( \text{AT}_2 \) receptor levels have been observed in both the cortex and the hippocampus after global ischemia in the rat brain (85). These increases occurred during the reperfusion period, suggesting that \( \text{AT}_2 \) receptors are somehow connected to the molecular events that eventually lead to delayed neuronal cell death. Similar observations have been made in vitro,
where it has been shown that glutamate excitotoxicity results in increased levels of AT$_2$ receptor mRNA and protein in rat cortical neurons (86). The increase in AT$_2$ receptor mRNA levels was not only prevented by the N-methyl-o-aspartate receptor antagonist MK-801 but also by the NO synthase inhibitor L-NAME.

Based on the observation that increased AT$_2$ receptor expression correlates with extensive cell death in the developing fetus, studies have been performed to investigate a possible role of AT$_2$ receptors and apoptosis. It was reported for the first time in 1995 that AT$_2$ receptor stimulation in serum-deprived PC12W cells leads to induction of programmed cell death by interfering with NGF-induced signaling (165). AT$_2$-receptor activation inhibited the NGF-mediated MAP kinase activation and led to an increase in MKP-1 phosphatase-1 activity (49). This increased MKP-1 activity inactivated the anti-apoptosis protein Bcl-2 and upregulated the pro-apoptosis Bax protein (46), eventually resulting in programmed cell death. The same investigators have shown that interferon regulatory factor IRF-1 upregulated AT$_2$ receptors in the apoptotic cells, indicating that cytokines may play a role in ANG II-induced apoptosis (46, 50). Finally, three specific amino acids located in the 3rd ICL (lysine 240, asparagine 242, and serine 243) have been identified as the key residues for AT$_2$ receptor-mediated SHP-1 activation, ERK inhibition, and subsequent induction of apoptosis (74).

Another recently discovered AT$_2$ receptor-activated pathway involves ceramides. Ceramides are among the most hydrophobic second messengers within a cell and can induce cell death by activating stress kinases or caspases such as caspase 3 (CPP-32). In PC12W cells, ANG II elicits an AT$_2$ receptor-mediated increase in ceramide levels, and this results in cell death (30). In this study, which has been recently confirmed by others (75), it was observed that sphingomyelin levels were not affected by ANG II treatment. This suggests an AT$_2$ receptor-mediated activation of ceramide synthase rather than an activation of sphingomyelinase. These results are consistent with the findings of Dimmeler et al. (22), who reported that ANG II elicits an AT$_2$ receptor-mediated increase in apoptosis in human umbilical vein endothelial cells (HUVEC) via stimulation of CPP-32 activity. This observation is particularly interesting, because CPP-32 is a known substrate for ceramides. A recent study on neuronal cultures from newborn rat brain (126) demonstrated that ANG II alone was neither capable of inducing JNK activity nor apoptosis after AT$_2$ receptor stimulation. However, if cultures were exposed to ultraviolet radiation, a condition that stimulates JNK activity and induces moderate apoptosis, then ANG II treatment elicited an AT$_2$ receptor-mediated potentiation of apoptosis. In addition, this ANG II treatment reduces the levels of the DNA repair enzyme PARP (poly ADP ribose polymerase), which is a known target of the caspase CPP-32. The putative signaling pathways that are involved in AT$_2$ receptor-mediated apoptosis are presented as a diagram in Fig. 4.

Li et al. (78) examined the ANG II-induced apoptosis of skin fibroblasts cultured from AT$_2$ knockout and wild-type mice embryos. Whereas ANG II caused apoptosis of skin fibroblasts generated from wild-type animals, treatment with ANG II had no significant apoptotic effects on fibroblasts derived from AT$_2$ receptor gene-deleted mice. Once again, this suggests a contribution of the AT$_2$ receptor in promoting programmed cell death.

**Tissue Regeneration**

ANG II stimulates VSMC growth via the AT$_1$ receptor, and increased tissue ANG II levels have been

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Fig. 4. Apoptotic signaling pathways activated by AT$_2$ receptors. CPP-32, caspase-3; Erk1/2, p44/p42 MAP kinase; JNK, c-j un NH$_2$-terminal protein kinase; NGF, nerve growth factor.

**APOPTOSIS**
- DNA laddering
- TUNEL staining

**stimulate**

**inhibit**
detected during wound healing. An abdominal surgical incision evokes a marked and transient downregulation of AT$_1$ receptors in adult rat skin (71). Using a similar technique, Viswanathan and Saavedra (151) investigated the expression of ANG II receptor subtypes in the skin of 2-wk-old rats after wounding. Three days after wounding, these authors observed an increase in both AT$_1$ and AT$_2$ receptors in the dermis and in a localized band within the superficial dermis of the surrounding skin.

AT$_2$ receptors seem to play an important role for tissue repair, not only in the skin but also in the nervous system. In PC12W cells, which are used as an in vitro model of sympathetic neurons, AT$_2$ receptor stimulation induces neurite extension (94). These morphological changes are paralleled by alterations in the expression patterns of different cytoskeletal proteins, such as neurofilament-M subunit (29) and microtubule-associated proteins (136), pointing to an involvement of the AT$_2$ receptor in axonal regeneration. After an axonal injury, AT$_2$ receptor mRNA levels are increased. As demonstrated by Gallinat et al. (30), a lasting and pronounced increase in the AT$_2$ receptor gene expression after sciatic nerve transection occurs in both sciatic nerves as well as in dorsal root ganglia neurons. Together with the observation that Schwann cells express AT$_2$ receptors (7), this specific AT$_2$ receptor expression pattern after peripheral nerve injury points to a role of these cells in neuroregenerative events.

Recently, it has been demonstrated that AT$_2$ receptor stimulation directly contributes to the process of axonal regeneration in the CNS of adult rats. Intravascular application of ANG II-soaked collagen foams significantly improved the axonal regeneration of retinal ganglion cells after optic nerve crush (83). Co-treatment with the AT$_2$ receptor antagonist PD-123177, but not the AT$_1$ receptor antagonist losartan, entirely abolished the ANG II-induced axonal regeneration, providing for the first time evidence for receptor-specific neurotrophic actions of ANG II via the AT$_2$ receptor.

**FUTURE DIRECTIONS**

Since its discovery in 1989, the angiotensin AT$_2$ receptor has received an ever-increasing amount of attention from the research community. Initially, the interest from many investigators in this receptor was due to the fact that it represented another ANG II binding site. However, intensive research over the past decade has not only provided a number of fascinating insights into the AT$_2$ receptor itself but has also established AT$_2$ receptor research as an independent field of study. Sufficient progress has been made so that the AT$_2$ receptor and its physiological roles are now less of a puzzle. In the following paragraphs we have singled out some of the research areas in which future studies may well lead to a greater understanding of the roles and importance of AT$_2$ receptors.

AT$_2$ receptors have been connected to various physiological and pathophysiological processes, but in only a few instances has direct involvement of this receptor subtype been demonstrated. In general, it appears that AT$_2$ receptors play a role in the structural changes that follow tissue injury. For instance, AT$_2$ receptor expression in the heart is substantially increased after myocardial infarction, although an actual role of this receptor in the numerous events that are precipitated by such an injury is still undefined. Included in the events that occur after myocardial infarction are collagen deposition, fibrosis, and angiogenesis. It is clear that AT$_1$ receptors promote collagen synthesis and support neo-vascularization (102). Because AT$_2$ receptors have been shown to counteract many of the AT$_1$ receptor-mediated processes, it is conceivable that activation of the AT$_2$ receptor inhibits these AT$_1$ receptor-mediated effects. Furthermore, the intricate balance between AT$_1$- and AT$_2$ receptor-induced actions and expression of these receptor subtypes might determine the net effect of the RAS in a given situation.

It is also possible that AT$_2$ receptors are involved in the inflammatory events that follow injury. This is because second messengers such as ceramides, which are an important part of cytokine-induced signaling cascades, are also part of the signaling pathways stimulated by AT$_2$ receptors (28, 75). Moreover, AT$_2$ receptors are regulated by the cytokine interleukin-1ß (56), suggesting a role of these sites in the inflammatory process.

One of the best known facts about AT$_2$ receptors is that they exhibit distinct patterns of expression in vivo. For example, AT$_2$ receptors are expressed in high levels in many neonatal/fetal tissues and are overexpressed in certain tissues after injury situations. Numerous factors and cell culture conditions have been identified that influence the expression of the AT$_2$ receptor in vitro, but these studies have not been able to help us understand the expression patterns of these sites in vivo. When the putative role of AT$_2$ receptors is considered during tissue repair and remodeling, nerve regeneration, and apoptosis, the mechanisms/factors that are responsible for induction and suppression of AT$_2$ receptors in vivo may have a positive impact in our understanding of the physiological processes involved in development and the pathological mechanisms that are involved in certain diseases.

In 1995, the first orally active AT$_2$ receptor antagonist, losartan, was clinically introduced for the treatment of hypertension. Since then, several other compounds that selectively block the AT$_2$ receptor have been developed and approved by the Food and Drug Administration. By inhibiting the AT$_2$ receptor-mediated negative feedback of ANG II on renin release in the kidney, these drugs evoke an overstimulation of the uninhibited AT$_1$ receptor by enhancing ANG II levels in plasma. However, because the exact physiological roles of the AT$_2$ receptor are not well defined, it is uncertain as to whether this overstimulation of AT$_2$ receptors would produce beneficial or harmful side effects.

In summary, it is clear that the past ten years have seen a dramatic increase in our understanding of the enigmatic AT$_2$ receptor. So far, the vast majority of advances have been made using traditional physiological, biochemical, molecular, and genetic approaches.
This technique has been utilized with great success in infarction, or in development of the nervous system, in the events that follow nerve injury or myocardial hypertension and in lowering AT1 receptor levels to multifaceted AT2 receptor. The challenge during the next ten years is to gain a much better understanding of the physiological and pathophysiological roles of AT2 receptors, and employment of this and other novel approaches may help the research community to achieve these goals.

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