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Review

Beyond blood pressure: new roles for angiotensin II

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Abstract. Since the discovery 100 years ago by Tigerstedt and Bergman of renin, an acid protease generating angiotensin peptide, numerous discoveries have advanced our understanding of the renin-angiotensin system (RAS). The recent cloning of angiotensin receptors and the availability of specific receptor ligands have allowed characterization of angiotensin-receptor-mediated actions, and an increasing number of studies using biochemical, pharmacological and molecular biological methods has focused on the many different physiological actions of the RAS in various tissues. Angiotensin II, the main effector peptide of the RAS, exerts most of its known actions in blood pressure control and body fluid homeostasis via the AT_1 receptor. AT_2 receptors not only play a role in growth control and cell differentiation but have been implicated in apoptosis and tissue regeneration. This review focuses on the extrarenal functions of angiotensin, especially in neuronal cells and the nervous system, and on recent advances in angiotensin receptor research.

Key words. Angiotensin II; AT_1 receptors; AT_2 receptors; apoptosis; differentiation; regeneration; signaling pathways.

The renin-angiotensin system

The renin-angiotensin system (RAS) with its effector peptide angiotensin II (ANG II) represents one of the phylogenetically oldest hormone systems and has been a subject of intensive research for more than 100 years [1]. ANG II exerts a variety of actions on different target organs via specific receptors designated AT_1 and AT_2 [2]. The identification of these two main mammalian angiotensin receptors became possible in 1989 with the development of specific receptor ligands for the AT_1 receptor, such as losartan and valsartan, and the AT_2 receptor, such as CGP 42112, PD 123177 and PD 123319 [3].

Most of the known physiological effects of angiotensin, e.g. vasoconstriction, aldosterone release, renal sodium reabsorption as well as central osmoregulatory actions including the release of pituitary hormones into the circulation, have been attributed to the AT_1 receptor. These effects constitute the role of angiotensin peptides as neuromodulator/neurotransmitters in the brain. The AT_1 receptor has, moreover, been shown to mediate cell growth and/or proliferation in various cell types including vascular smooth muscle cells [4, 5], cardiomyocytes [6] and coronary endothelial cells [7]. The contribution of the AT_1 receptor to blood pressure regulation has been examined using AT_1 receptor knock-out mice [8] resulting in a reduction in systolic blood pressure.

The discovery that ANG II was not only a growth promoter (through AT_1 receptors) but also a growth

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inhibitor (mediated by AT_2 receptors) led to a deeper understanding of the RAS in cellular functions, e.g. cell differentiation, tissue regeneration and programmed cell death.

The enzymatic cascade of the RAS starts with the α_2 globulin angiotensinogen which is predominantly synthesized in the liver and constitutively released into the circulation. The aspartyl protease renin, mainly derived from the juxtaglomerular cells in the kidney, generates the biologically inactive angiotensin I (ANG I) in plasma and tissues which is finally converted to ANG II with the help of angiotensin-converting enzyme (ACE), cathepsin D or heart chymase (fig. 1). The octapeptide hormone ANG II exerts its actions via two different receptor subtypes which are characterized in detail below. Finally, activation of several aminopeptidases leads to degradation of angiotensin and to the generation of biologically active metabolites such as ANG III [2–8], ANG IV [3–8] and ANG 1–7 (fig. 1) and subsequently to inactive metabolites.

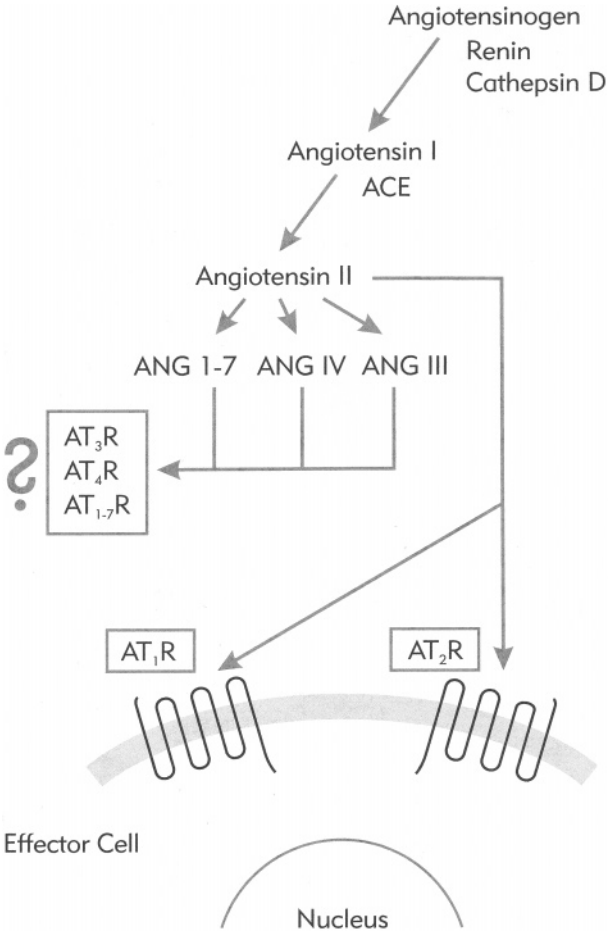

Figure 1. The renin-angiotensin system: pathways for the formation of the angiotensin peptides and their interaction with different angiotensin receptor subtypes.

Angiotensin receptors

Using peptidic and non-peptidic receptor antagonists, different cell surface angiotensin receptor subtypes have been identified, named AT_1 and AT_2. AT_1 receptors exhibit a high affinity for sartans, e.g. losartan, candesartan, telmisartan, eprosartan and valsartan ($K_i = 10$–50 nM) and a low affinity for CGP 42112A ($K_i > 0.5$ M) and PD 123177 ($K_i > 10$ M). On the other hand, binding sites displaying a high affinity for CGP 42112A ($K_i < 1$ nM) and PD 123177 ($K_i = 10$–100 nM) but a low affinity for sartans ($K_i > 1$ mM) are designated as AT_2 receptors [9]. Several groups have reported that higher concentrations of CGP 42112A exert agonistic effects, thus representing an AT_2 receptor agonist with a high affinity but low potency [7, 10].

Molecular cloning and binding studies have clearly identified two AT_1 receptor isoforms, designated AT_{1a} and AT_{1b} [11, 12]. These isoforms differ in tissue distribution and regulation [13]. Whereas the AT_{1a} receptor seems to be identical with the classical AT_1 receptor, the AT_{1b} receptor displays differences in terms of binding characteristics of selective receptor ligands: PD 123319 > losartan > CGP 42112A [14].

Biochemical data obtained from Siemens et al. [15] also indicate the existence of an additional AT_2 receptor. According to these authors, two different AT_2 receptor isoforms can be discriminated in the neuroblastoma cell line N1E-115 with the help of PD 123319. However, the molecular data point to the existence of a single AT_2 receptor, since its entire coding sequence is localized in one exon without interruption by an intron.

Atypical angiotensin receptors

The development of highly selective angiotensin receptor antagonists also led to the identification of binding sites which differ from those of AT_1 and AT_2 receptors. One example for such a binding site is the so-called AT_4 receptor which exhibits a high affinity for ANG IV (ANG 3–8; cf. fig. 1). Neither losartan nor CGP 42112A and PD 123177 are able to compete for this binding site when reasonable pharmacological concentrations are applied [16, 17].

In endothelial cells, ANG IV stimulates the expression of plasminogen activator inhibitors. Moreover, in cardiac fibroblasts of rabbits, the synthesis of nucleic acids is increased by ANG IV [18]. The AT_4 receptor is expressed in the brain of rabbits and rats where a stimulation of fos immunoreactivity has been detected after ANG IV treatment. This effect could not be suppressed by costimulation with the AT_2 receptor antagonist, PD 123177 [19].

Another atypical binding site for angiotensin has also been described in cell cultures containing 95% human

cardiac fibroblasts [20]. Angiotensin (1–7) > ANG II > ANG IV competitively bound to this site, while losartan and PD 123319 were not able to compete with these agonists. In these cells, angiotensin induced cell proliferation which was not abolished by pretreatment with either losartan or PD 123319. Finally, intracellular receptors have been described which are able to mediate angiotensin effects [21].

Tissue distribution of angiotensin receptors

The angiotensin receptor subtypes have been detected in various tissues [7, 22–30]. In rats, AT_{1a} and AT_{1b} receptors are expressed in the liver and the adrenal glands to almost the same extent. While the AT_{1a} receptor is dominant in vascular smooth muscle cells in the ovary, heart and hypothalamus, the AT_{1b} receptor dominates in tissues involved in central osmoregulation.

In the adult organism, both AT_1 and AT_2 receptor subtypes are expressed in a similar manner in the adrenal glands and the heart [31–45]. On the other hand, the AT_2 receptor is expressed to a greater extent in the uterus, in ovarian granulosa cells, and in distinct areas of the brain, but less in the vascular endothelium [7]. Moreover, the AT_2 receptor is predominantly expressed in fetal tissues, pointing to a role for this receptor subtype in developmental and differentiation processes [46, 47], a function entirely different from the known cardiovascular or volume control of ANG II mediated through the AT_1 receptor. After birth, however, the ratio of AT_1 to AT_2 receptor expression is reversed with the AT_1 receptor subtype dominating in the adult organism; thus, the AT_2 receptor appears to be suppressed in a number of tissues in adult life [for a review see ref. 2]. In the case of pathological incidents like skin lesions [48], post-infarct remodeling [49] or sciatic [50] or optic nerve transection [51], AT_2 receptor expression increases. This receptor has not only been associated with the control of cell growth [2] and—in nervous tissue—with differentiation [52, 53] as well as regeneration [50, 51] and wound repair [54], but has been, furthermore, implicated in glutamate-mediated neuronal cell injury [55, 56] and apoptosis [57, 58]. However, the physiological role of the AT_2 receptor is not yet completely understood and is still the subject of intensive research.

AT_1-receptor-mediated effects

Experimental studies in the last few years have strongly suggested that the RAS plays an important role in the development and maintenance of arterial hypertension. Via its AT_1 receptor, angiotensin not only exerts short-term actions to raise blood pressure, but, furthermore, evokes by its direct growth-stimulating actions long-term effects leading to renal and cardiovascular pathology, e.g. nephrosclerosis and left ventricular hypertrophy (LVH) and vascular media hypertrophy [for a review see ref. 2]. Therefore, antihypertensive therapy has to focus not only on the minimization of secondary events such as stroke, heart failure or renal disease but also on the avoidance and reduction of structural changes in end organs.

Inhibition of the RAS can be carried out at three different levels (cf. fig. 1):

1) Inhibition of renin: although renin acts very specifically on angiotensinogen to generate angiotensin peptides, renin inhibitors have not yet been developed for clinical use due to limited therapeutical potential.

2) Inhibition of ACE: apart from generating angiotensin, this enzyme participates in the metabolism of several other peptides, e.g. bradykinin.

3) Blockade of AT_1 receptors: the latest most specific approach to oppose angiotensin-mediated actions is the specific antagonization of AT_1 receptors, discussed in more detail below.

All components of the RAS are present in the brain, but they do not coincide in some regions; for example, renin is only reported in low amounts in the cerebral cortex and the basal ganglia, whereas angiotensin and angiotensinogen mRNA can be found in the basal ganglia, septum, amygdala, cortex, hypothalamus and other brain regions [59–62]. Due to the blood-brain barrier, only ANG II endogenously produced in the brain has access to brain ANG II receptors. Neurons express detectable amounts of AT_1 (e.g. hypothalamus, brain stem) and AT_2 receptors (e.g. thalamus, cerebellum, inferior olivary nucleus, retina [28, 51]), but in the circumventricular organs, which have no blood-brain barrier, the high expression of AT_1 receptors seems to be located mostly on glial cells [63].

AT_1 receptor antagonists

The first specific non-peptide AT_1 receptor antagonist clinically introduced as an antihypertensive agent in 1995 was losartan. Additional compounds of this class of drugs followed or will follow in the near future, such as valsartan, irbesartan, eprosartan, candesartan, telmisartan or tasosartan. These compounds are in most cases non-competitive receptor antagonists and do not exert intrinsic activities. They are orally active and exhibit a high selectivity for the AT_1 receptor in various tissues.

For reducing blood pressure, the efficacy of AT_1 receptor antagonists is comparable to that of first-line drugs such as thiazide diuretics, β-blockers, calcium channel blockers and α receptor antagonists. Patients treated

with AT_1 receptor antagonists show a responder rate of 50% or more on monotherapy, which can be further increased by combination with an additional compound of another class, especially diuretics.

The AT_1 receptor antagonists have, moreover, been shown to be well tolerated and, surprisingly, have not so far revealed any class-specific side-effects when used appropriately. Indeed, the number of patients showing unwanted effects or withdrawing from therapy in clinical trials was comparable to patients treated with placebo [64].

An important feature of this substance group is their organ-protective action resulting in a wide range of possible therapeutic advantages in the treatment of hypertension. A few examples are given below.

A tissue-protective action of AT_1 receptor blockade has e.g. been shown in salt-loaded stroke-prone spontaneously hypertensive rats (SHRSP), an animal model of malignant hypertension. In these rats, chronic AT_1 receptor blockade with losartan prevented stroke, malignant nephrosclerosis and cardiac infarction, and increased survival [65–67]. Long-term antihypertensive treatment of SHRSP with losartan can, furthermore, engender beneficial actions on cardiac function and metabolism [68]. Candesartan has been reported to reduce or prevent the development of intima lesions following vascular injury through inhibition of direct and indirect growth-promoting effects of angiotensin in vascular smooth muscle cells [69]. The production of peroxide in human macrophages—which plays a role in the advance of atherosclerosis—can also be decreased by AT_1 receptor blockade, pointing to a beneficial role of AT_1 receptor antagonists in tissue protection [70].

AT_2-receptor-mediated effects in cell differentiation and antiproliferation

In tissue development or remodeling, excessive growth induced by growth factors needs to be controlled by an antiproliferative cellular programme. The two angiotensin receptor subtypes, AT_1 and AT_2, mediate antagonizing effects in terms of growth modulation. AT_2 receptor stimulation inhibits the AT_1-mediated proliferation in various cell types including rat coronary endothelial cells and PC12W cells [7, 52]. Opposing effects of AT_1 and AT_2 receptors on microvascular growth and neointima formation have also been reported [71, 72], and studies from knock-out mice models [73, 74] demonstrated an involvement of AT_2 receptors in blood pressure regulation and behaviour. In adrenal adenomas, a reduction in AT_2 receptor mRNA was observed, whereas no consistent differences in AT_1 receptor mRNA were seen [75]. These results suggest a correlation of AT_2 receptors with adrenal tumorgenesis. Indirect evidence for a role of AT_2 receptors in differentiation and development has been obtained from studies determining the distribution of angiotensin receptor subtypes. During ontogenesis, there is abundant and transient expression of AT_2 receptors in several tissues including the brain [22–24, 28, 76]. In adult animals, the embryonic pattern of high AT_2 and low AT_1 receptor levels is reversed [47, 77]. Due to the transitory abundance of AT_2 receptors in the nervous system, their presence in neuronal cell lines [78, 79], their interaction with T-type calcium currents in non-differentiated NG108-15 cells [80, 81]—calcium playing a crucial role in neuronal differentiation [82]—the involvement of AT_2 receptors in neuronal development has been proposed.

Angiotensin receptor signaling pathways

Analysis of the amino acid sequences of the AT_1 and AT_2 receptors has revealed that they belong to the G-protein-coupled 7-transmembrane receptor family. However, the intracellular signaling pathways after receptor stimulation are quite different. The signaling pathways of the AT_1 receptor are now largely understood [83]. Binding of ANG II to the AT_1 receptor leads to the 'classical' G-protein-related cascades with phospholipase (PL)C, PLD and PLA_2 stimulation. PLC activation leads to the hydrolysis of phosphatydilinositol 4,5 bisphosphate (PIP2) to inositol triphosphate (IP_3) and diacylglycerol. IP_3 invokes a subsequent increase in intracellular calcium, causing protein kinase C (PKC) activity to increase [84, 85]. The cascade induced by PLD and PLA_2 leads to the formation of fatty acids, e.g. arachidonic acid, which is a precursor of leukotrienes and prostaglandins. Further research has discovered the contribution of additional signaling mechanisms involving ras/raf-mediated activation of mitogen-activated protein kinases (MAP kinases), the Janus kinase (JAK)/signal transducers and activators of transription (STAT) system, and c-Jun N-terminal kinase family (JNK) activation via tyrosine and serine/threonine phosphorylation [86]. A neuromodulatory action of angiotensin via stimulation of the AT_1 receptor of the brain norepinephrine system has been reported by Gelband et al. [87]: neuronal synthesis and reuptake of norepinephrine is enhanced via induction of Fos and Jun proteins. In addition to the ras/raf/MAP kinase pathway, the authors recently demonstrated involvement of the protein PKC β subtype and phosphorylation and redistribution of myristolated alanine-rich C kinase substrate (MARCKS) in neurites, leading to persistent stimulation of the neuromodulatory actions of ANG II [88]. Most of these signaling cascades transform the AT_1-receptor-mediated signal into activation

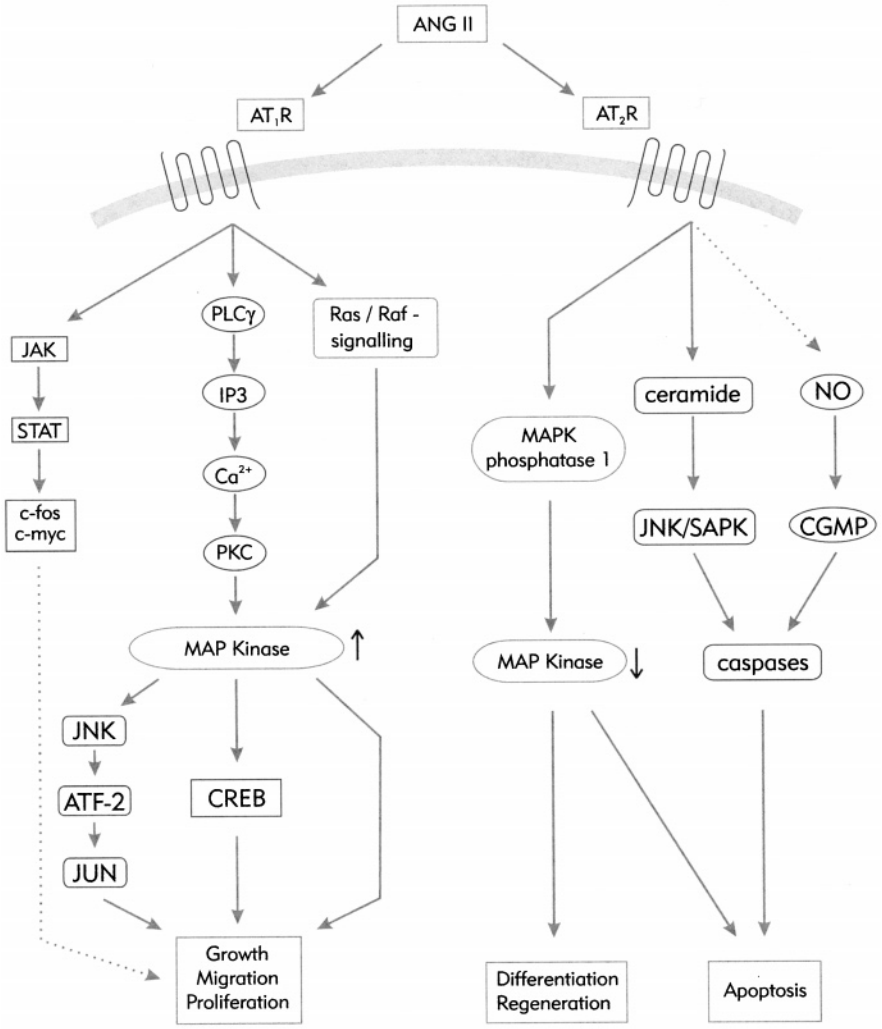

Figure 2. Angiotensin signalling pathways. Stimulation of the AT_1 receptor produces cell growth, proliferation and migration through stimulation of various kinases. AT_2 receptor stimulation induces differentiation and regeneration, although it may also cause apoptosis via ceramide or inhibition of MAPK. PLC, phospholipase C; IP3, inositol triphosphate, PKC, protein kinase C; JAK, Janus kinase; STAT, signal transducers and activators of transcription; MAPK, mitogen-activated protein kinase; JNK, c-Jun terminal kinase; for further details and abbreviations see text.

of inducible transcription factors (ITFs) such as c-Jun, c-Fos or KROX [83]. These DNA-binding proteins regulate gene expression and thus transform the signal into cellular function. ANG II is therefore involved—via the AT_1 receptor—in processes of growth, modulation and proliferation in various cell types (fig. 2).

Compared to the cellular effects of AT_1 receptors, description of the AT_2-receptor-mediated signaling cascades still seems to be far from complete. After the recognition of the AT_2 receptor as a member of the G-protein-linked 7-transmembrane receptor family, several attempts failed to demonstrate G-protein-related pathways. As mentioned above, AT_2 receptor stimulation mediates antagonizing actions in terms of growth modulation. In an AT_2 receptor knock-out model, expression of AT_2 receptors was recently shown to influence fetal vascular growth via modulation of the extracellular signal-regulated kinase (ERK) activity [89]. In nervous tissue, stimulation of AT_2 receptors in cultured neurons stimulates PLA_2 activity [90], and numerous studies including tumour cell lines and neurons indicated an inhibition of MAP kinase activity and subsequent apoptosis after AT_2 receptor stimulation (fig. 2) [91, 92], pointing to involvement of an inhibitory G protein (G_i)-related mechanism. The issue is complicated by increasing evidence of cross-talk between AT_1 and AT_2 receptor signaling, e.g. the recently demonstrated AT_2-receptor-mediated inhibition of ERK and STAT activation, which is seen as a result of AT_1 receptor activation [93]. Thus, the experimental data

obtained from different cell types have further elucidated our knowledge of AT_2 receptor signaling, but some aspects are still an enigma.

AT_2 receptors and neuronal differentiation

Neuronal cell proliferation, differentiation and apoptosis are controlled by various neurotrophic factors. For example, PC12W cells, which represent an established model system for studying various aspects of neuronal differentiation and apoptosis, undergo differentiation to sympathetic neuron-like cells in response to nerve growth factor (NGF) application [94]. After growth factor withdrawal, an apoptotic programme is activated [95]. Since PC12W cells express abundant AT_2 and only few, if any, AT_1 receptors, these cells offer an excellent model to investigate AT_2-receptor-mediated effects on neuronal cells.

AT_2 receptors are not only involved in the initiation of cell differentiation by shifting cells into cell cycling arrest but, furthermore, mediate neurite extension in PC12W cells, as recently demonstrated [52]. ANG II treatment induced neurite outgrowth and enhanced the NGF-mediated morphological changes. These effects were completely suppressed by coincubation with the specific AT_2 receptor antagonist, PD 123177. Such AT_2-receptor-mediated neurite extension has also been observed by Laflamme et al. [96] using NG108-15 cells, a neuroblastoma/glioma cell line. Neurite outgrowth after angiotensin treatment was also detectable in neurons derived from postnatal rat dorsal root ganglia (R. Lucius, R. Rosenstiel, S. Gallinat, J. Sievers, T. Unger unpublished data) and in postnatal retinal explants [51]. Therefore, AT_2 receptors are not only prerequisite for differentiation by exerting antiproliferative effects but appear to actively contribute to neuronal differentiation, visible e.g. by induction of neurite extension.

Neuronal differentiation is accompanied by dramatic changes in gene and protein expression. The stability and function of neurons is dependent on protein filaments like microtubules, actin filaments and intermediate filaments. These changes are reflected, for instance, in PC12W cells upon NGF treatment.

One intermediate filament protein class we were interested in were neurofilament (NF) triplet proteins, which are exclusively expressed in neurons and which can be detected at early stages of embryogenesis [97, 98]. Three subunits of NFs with molecular masses of 68 kDa (NF-L), 160 kDa (NF-M) and 200 kDa (NF-H) are known. Besides being responsible for the stability of axons, this type of intermediate filament protein is involved in axial growth of neurons following the elongation phase [99, 100], leading to increased conduction velocity [100].

To further characterize the AT_2-receptor-induced morphological changes in PC12W cells, we investigated the effects of AT_2 receptors on the expression pattern of NF-M. In contrast to NGF stimulation [101, 102], the morphological changes after AT_2 receptor activation were paralleled by an AT_2-receptor-mediated down-regulation of NF-M [53]. We also demonstrated that these receptors modulate the expression of MAP1B, MAP2 and β tubulin in PC12W cells [103]. MAP1B expression was attenuated, whereas AT_2 receptor stimulation up-regulated β tubulin and MAP2.

These results are in line with recent results from other scientists. For example, Laflamme et al. [96] have shown that the AT_2-receptor-induced neurite outgrowth in NG108-15 cells was accompanied by the regulation of β tubulin, tau and MAP2C, suggesting a role for AT_2 receptors in the regulation of protein filaments in neuronal cells.

The interaction of AT_2 receptors with T-type calcium channels observed in non-differentiated NG108-15 cells [81] together with the observation of the crucial role of calcium in neuronal differentiation [82] also indicate the involvement of AT_2 receptors in this process.

In summary, these data show that in different cell lines of neuronal origin, AT_2 receptor stimulation exerts antiproliferative actions and induces neurite extension. In addition to these visible morphological changes, modulation of the expression of important protein filaments through AT_2 receptor activation shows that these receptors are directly involved in neuronal development by cytoskeletal reorganization.

AT_2 receptors in tissue repair and regeneration

As already mentioned, AT_2 receptors have been implicated in the process of wound healing and tissue repair [48, 49, 71]. Increased tissue AT_2 levels as early as 24 h after myocardial infarction [49] and after brain injury [54, 104] have been reported. In myocardial infarction, this effect was neither influenced by pretreatment with the AT_1 receptor antagonist, losartan, nor the ACE inhibitor, ramipril [105].

In coronary endothelial cells, both angiotensin receptor subtypes regulate several extracellular matrix proteins, i.e. fibronectin, thrombospondin and tenascin [106]. This observation not only points to the potency of both receptor subtypes to alter the adhesion and migration of coronary endothelial cells, it further suggests that angiotensin receptors are capable of promoting neurite extension by influencing the extracellular matrix composition, of which molecules like fibronectin or laminin are known to promote neurite outgrowth. Another aspect of interest in tissue repair processes is the involvement of angiotensin receptors in angiogenic processes

[107]. The authors could show that ANG II not only facilitated the activation of pre-existing collateral vessels in the rabbit cornea but, furthermore, exerted angiogenic properties. Unfortunately, the ANG-II-induced angiogenesis was not attributed to any angiotensin receptor subtype, but these effects of ANG II can be explained by AT_1 receptor stimulation, since all known growth-promoting effects of ANG II are mediated by AT_1 receptors.

However, AT_2 receptors have not only been implicated in multicellular repair systems like the process of wound healing but also in nerve regeneration after injury. Whereas the immature mammalian central nervous system (CNS) can regenerate [108], adult neurons are in most cases unable to reinnervate their target regions after injury, probably due to the actions of inhibitory molecules from CNS myelin [109] or the absence of requisite neurotrophic molecules. However, it has been shown that CNS neurons are able to regenerate new processes over long distances and to reinnervate their target region [110, 111] if they are supplied with growth-promoting substrates, e.g. transplants of fetal CNS tissue [112] or peripheral nerves [113]. The first evidence for an involvement of AT_2 receptors in either apoptotic or neuroregenerative events came from our observation of the AT_2-receptor-mediated down-regulation of NF-M in PC12W cells [53]. Diminished NF-M expression has also been observed in neurons following nerve transection [114–119] and in neurons undergoing apoptosis [120]. Additionally, the modulation of MAP1B expression involves AT_2 receptors in PC12W cells [103], a process which is of interest, since down-regulation of MAP1B has been observed in regenerating neurons following axotomy [121, 122]. In view of this fact, it is intriguing to speculate about a physiological role for AT_2 receptors in these events.

Book et al. [123] detected a correlation between a decrease in MAP2 levels and death of neurons, using peripheral and central nerve lesions. Therefore, the AT_2-receptor-induced MAP2 up-regulation in PC12W cells would support a role for AT_2 receptors in the inhibition of PCD and the promotion of regenerative processes (fig. 3).

Using a peripheral nervous system lesion approach, we could demonstrate a several-fold up-regulation of AT_2 and AT_1 receptor mRNA after sciatic nerve transection in dorsal root ganglion neurons and in sciatic nerve segments of adult rats [124]. Successful regeneration in this model under regeneration-permissive conditions, i.e. sciatic nerve crush, additionally revealed a transient up-regulation of AT_2 receptor mRNA with its expression declining in parallel with axonal elongation. These results suggest that in the peripheral nervous system, AT_2 receptors exert their actions by modulating Schwann-cell-mediated activity, e.g. the production of neurotrophic factors, myelin degeneration or preparation of Schwann cells for subsequent synthesis of the myelin sheath.

These observations in conjunction with the above-mentioned evidence for AT_2-receptor-mediated neuronal differentiation prompted us to investigate the potential role of ANG II, acting through the AT_2 receptor, as a neurotrophic factor for CNS neurons in vitro and in vivo.

We investigated the effect of ANG II receptor stimulation on axonal regeneration of postnatal rat retinal explants and cultured dorsal root ganglia (DRG) cells in vitro and after optic nerve crush in vivo [51]. In the in vitro model, the retinal ganglion cells (RGCs) are comparable to adult, non-regenerating RGCs [125, 126]. ANG II (10^{-7}–10^{-5} M) induced regeneration of neurites in a concentration-dependent manner, as shown by significant neurite outgrowth in vitro (retinal explants and the DRG cells) and in vivo after lesion of the optic nerve. The effects of ANG II—both in vitro and in vivo—were mediated by the AT_2 receptor, since (i) the effects were mimicked by CGP 42112 (an AT_2 receptor agonist), (ii) they were not suppressed by losartan (an AT_1 receptor antagonist) and (iii) they were abolished by coincubation with PD 123177 (an AT_2 receptor antagonist). The involvement of AT_2 receptors in these events was also demonstrated by a reverse transcription-polymerase chain reaction assay in which a time-dependent increase of AT_2 receptor mRNA—as in the previous studies in the peripheral nerve—could be seen both in the retina and the crushed optic nerve. These results clearly demonstrate that ANG II via its

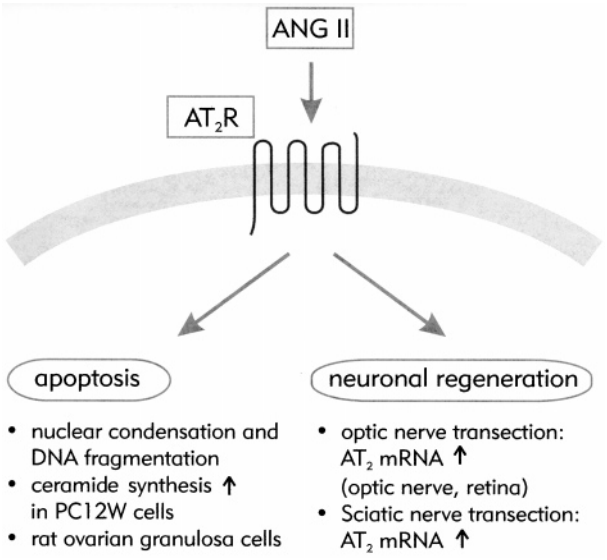

Figure 3. AT_2 receptors: apoptosis versus regeneration. For details see text.

AT_2 receptor induces axonal regeneration not only in postnatal retinal explants or DRG neurons in vitro, but also in the adult optic nerve after lesion.

Evidence for AT_2-receptor-mediated programmed cell death in neurons

Axon interruption elicits a complex neuronal response that leaves neurons poised between death and regeneration. The risk of apoptosis and the potency for axonal regeneration are closely related: mammalian CNS neurons lesioned close to their cell bodies show a strong cell body response and high regenerative capacity but, simultaneously, there is a high risk for cell death. If CNS neurons are lesioned more distally, regenerative potency is weak, but the neurons are somehow protected from apoptosis. The transcription factor c-Jun is, for example, one of the earliest markers for neurons responding to nerve fibre transection and its expression can be related to both degeneration and survival [127]. These observations suggest that neuronal injury initiates—at least in the early events of injury—a series of molecular events that are identical for both regeneration and apoptosis. Therefore, the axotomy-induced AT_2 receptor up-regulation and the AT_2 receptor-mediated NF-M down-regulation in PC12W cells [53] might also be interpreted in terms of apoptosis instead of neuroregeneration. In addition, AT_1 and AT_2 receptors were reported to be involved in modulation of the proteolytic activity of the extracellular microenvironment of neurons, e.g. by down-regulation of the serine protease inhibitor protease nexin-1 (PN-1) in primary cultures of Schwann cells [128]. PN-1 is induced after lesion of the sciatic nerve, has been demonstrated to rescue motoneurons from axotomy-induced cell death [129] and is markedly decreased in Alzheimer's disease brain [130, 131]. In PC12W cells, AT_2 receptors induce chromatin condensation and internucleosomal DNA fragmentation—molecular changes typical for apoptosis. Furthermore, the authors observed antagonization of NGF-induced MAP kinase activity by AT_2 receptors [91]. Since selective inhibition of the MAP kinase pathway induces apoptosis and thus prevents survival of PC12W cells [132], AT_2 receptors were concluded to play a role in programmed cell death (PCD). Outside the nervous system, AT_2 receptors have been associated with PCD in rat ovarian granulosa cells [133] and in GN4 liver epithelial cells, where ANG II was able to activate the cell-death-signaling JNK pathway [134].

To further elucidate AT_2-receptor-mediated neuronal regeneration or apoptosis, we could recently demonstrate that stimulation of the AT_2 receptor selectively induces de novo synthesis of ceramides in PC12W cells [57]. Ceramide acts as a lipid second messenger and is an important mediator of PCD. This apoptotic effect is accompanied by activation of mitogen-activated protein kinase phosphatase-1 (MKP-1) and the subsequent dephosporylation of bcl-2, an antiapoptotic protein [91], and activation of the cell death protease caspase-3 (CPP32), leading to induction of apoptosis [135]. Our finding of AT_2-receptor-mediated ceramide generation in PC12W cells—confirmed in the meantime by Lehtonen et al. [136]—connects this receptor to important apoptotic pathways.

Concluding remarks and perspectives

In this review article, we have focused on the role of the octapeptide ANG II both in regeneration/tissue repair and in apoptosis, mainly mediated via the AT_2 receptor. It appears now that the well-known growth-promoting effects of the AT_1 receptor, which can engender neuroplastic as well as pathological structural changes in several organs, are counteracted within the RAS itself by growth arrest, differentiation and tissue repair, effected through the AT_2 receptor, and a (disturbed) balance between the opposing actions of these two receptors determines the net effects of the RAS in a given disease situation. The AT_2 receptor appears to act as a modulator of complex biological programmes in development, cell differentiation, tissue repair and PCD. Many observations cited in this review could be interpreted in terms of both PCD and survival—especially neuronal survival—but at this point in time it is difficult to assess whether ANG II mediates PCD, regeneration or both.

In the CNS, the signal transduction cascades leading to either apoptosis or nerve fibre regeneration are not completely understood. How a common initial signaling pathway leads to the apparently opposed responses of cell death and axonal regeneration is a matter of intense debate.

That AT_2 receptors are involved in cell differentiation processes and exert antiproliferative actions is widely accepted. However, direct in vivo evidence implicating these receptors in both neuroregeneration and PCD is still lacking. Even if participation in these events has been suggested by several studies, further investigations will have to determine precisely the physiological role of this receptor subtype. The findings on the neurotrophic actions of the AT_2 receptor may provide a basis for the design of new, receptor-directed, therapeutic strategies in the failure of axonal regeneration in the mammalian CNS. This is of particular interest considering the current difficulties in applying neurotrophic factors after nerve fibre damage. Moreover, AT_2-mediated tissue regeneration may not be confined to axonal regrowth but may constitute a general phenomenon to be exploited

by therapeutic intervention. It will be interesting to see to what extent the results obtained from neuronal cells can be applied to the cardiovascular system, under physiological and pathophysiological situations. The clinical relevance of this approach is already apparent with the increasing use of AT_1 receptor antagonists as antihypertensive drugs in, to date, more than two million patients worldwide. Since AT_2 receptors are unmasked and ANG II levels increased by AT_1 receptor antagonists, part of the organ-protective actions of these drugs might be ascribed to an agonistic action of ANG II at the AT_2 receptor site. Beyond the 'classical' actions of ANG II—a story that began 100 years ago—future investigations will shed new light on the complex function of the RAS both in the CNS and periphery.

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