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The renin-angiotensin system in the brain: an update

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Introduction

Angiotensin II (Ang II), the effector peptide of the renin-angiotensin system (RAS), plays a key role in the regulation of cardiovascular and body fluid and electrolyte homeostasis. The major actions of Ang II are mediated by two subtypes of G-protein-coupled angiotensin receptors, the AT₁- and the AT₂-receptor. Both receptors are seven transmembrane glycoproteins with only 32–34% sequence similarity. Most of the classical actions of Ang II on fluid and blood pressure (BP) homeostasis are mediated by AT₁-receptors. However, this receptor subtype may also initiate proliferation, hypertrophy and growth in various tissues. The AT₂-receptor is expressed with high density during foetal development and is less abundant in adult tissues, including the brain.¹

All components of the RAS have been found in the brain and it has been firmly established that Ang II is synthesised in the brain independently of peripheral sources. Other angiotensin-derived peptides such as angiotensin 2-8 (Ang III), angiotensin 1-7 and angiotensin 3-8 (Ang IV) have also been demonstrated to have biological activities in the brain. The rat and mouse AT₁-receptors exist as two subtypes, termed AT_{1A} and AT_{1B}, which differ in their distribution in the brain. Besides the AT₁- and AT₂-receptors, the AT₄-receptor, which specifically binds Ang IV, is also located in the brain. Its signalling mechanisms are unknown, but it seems to influence local blood flow and sensory and motor functions.¹

It has been well established that Ang II, acting on AT₁-receptors in the brain, influences numerous physiological responses, including BP, drinking behaviour, sodium intake, natriuresis and vasopressin release. Although less abundant in the adult brain, the AT₂-receptor also contributes to the regulation of BP and water intake.² The circumventricular organs (CVOs), the median preoptic nucleus and the lamina terminalis, a strip of periventricular tissue comprising the anterior wall of the third ventricle (AV3V), represent sites of perception and integration of signals generating thirst, sodium intake and cardiovascular control. These areas are interconnected with the hypothalamus, especially with the paraventricular (PVN) and supraoptic (SON) nuclei, limbic system and brainstem areas.³ The CVOs, comprising the subfornical organ, organum vasculosum laminae terminalis and area postrema, which lack the

blood-brain barrier, contain angiotensin receptors belonging to the AT₁ subtype, which are accessible to both blood-borne and cerebrospinal-fluid derived Ang II.⁴ The anatomical projections to the preoptic, anterior hypothalamic and limbic structures initiate drinking and those to the PVN and SON mediate the release of vasopressin into the circulation. The AV3V region, the preoptic and anterior hypothalamus and limbic structures are crucial in the control of sodium appetite.⁵ Recent findings indicate that the increases in BP elicited by centrally-administered Ang II in the mouse are mediated by AT_{1A} receptors and the drinking response to the peptide by AT_{1B} receptors.⁶ The role of noradrenaline and adrenoceptors in the SON and PVN in the mediation of vasopressin release, following stimulation of AT₁-receptors in the CVOs and periventricular brain areas, has been well-established.^{7,8} On the other hand, the mechanisms responsible for the vasopressin release after direct stimulation of AT₁-receptors in the PVN and SON remain poorly understood. *In situ*-hybridisation studies have failed to demonstrate the localisation of AT₁-receptors on magnocellular vasopressin-producing neurones, although Ang II, microinjected into the PVN, elicits an immediate increase in AVP levels in the circulation, which can be completely inhibited by a prior injection of the selective AT₁-receptor antagonist, losartan.^{7,9} Numerous pieces of evidence indicate that overactivity of the brain RAS may be implicated in the development and maintenance of high BP in spontaneously hypertensive rats (SHR). Ang II content is increased in the brain of SHR, along with an overabundant expression of AT₁-receptors.¹⁰ Inhibition of the central RAS by antisense oligodeoxynucleotides in SHR reduced arterial BP.¹¹ Furthermore, normotensive rats developed hypertension if the levels of Ang II in the brain were elevated using *in vivo* gene transfer of human angiotensin-converting enzyme (ACE).¹² The exact mechanisms by which increased RAS activity in the brain contributes to the development of hypertension are not known. Recent findings indicate that an Ang II-induced suppression of the baroreceptive afferent feedback at the level of the nucleus tractus solitarius may be one of the mechanisms by which elevated central Ang II activity could lead to hypertension.¹³

During the past ten years, much information has been gathered about the effects mediated by

angiotensin receptors in the neuronal tissue which are not directly related to the central control of fluid and BP homeostasis. The fact that Ang II, via the AT₂-receptor, may act as modulator of biological programmes involved in embryonic development, tissue regeneration and protection, differentiation and in the initiation of processes leading to programmed cell death, represents the most exciting discovery during this period of time. Furthermore, there is substantial evidence that the AT₂-receptor can offset or counteract the effects mediated by the AT₁-receptor on cell proliferation, BP and water intake.^{2,14,15} This review focuses on the functions of Ang II in neuronal cells and the nervous system, and especially on the role of AT₁- and AT₂-receptors in the processes of regeneration, differentiation and induction of apoptosis in neuronal tissue and the brain.

Signal transduction

Both the AT₁- and AT₂-receptors belong to the family of seven transmembrane, G-protein-coupled receptors. Although coupling to other G-proteins has been described, the AT₁-receptor seems to couple mainly to G_q, initiating the activation of phospholipase C β , which, in turn, results in the hydrolysis of phosphoinositides and ultimately in the release of calcium in the cells and stimulation of protein kinase C. Other G-proteins which have been reported to mediate Ang II-induced AT₁-receptor signal transduction, include the G_{12/13} family (leading to L-channel activation) and G_{i/o} proteins (which inhibit adenylyl cyclase). In addition to these pathways, the binding of Ang II to its AT₁-receptor also leads to the phosphorylation of a number of downstream mediators such as phospholipase C γ or pp60^{src}. G-protein-coupled receptors do not possess an intrinsic ability for tyrosine phosphorylation, and the mechanisms by which the receptor initiates these reactions are still poorly understood. Phosphorylation-dependent pathways which are activated by the AT₁-receptor include various mitogen-activated protein (MAP) kinases, such as ERK1/2 (extracellular regulated kinase1/2), JNK (c-Jun-N-terminal kinase), p38 kinase, the JAK/Stat pathway and the Akt/protein kinase B pathway. Some of these pathways result in the activation of transcription factors such as c-Fos, attributing to Ang II the ability to regulate the expression of genes. Another interesting feature of the AT₁-receptor is its ability to transactivate growth factor receptors, such as the epidermal growth factor receptor or the insulin-like growth factor-1 receptor.¹

Most of the above signal transduction mechanisms have been studied in cells derived from vascular tissues. The first report on AT₁-receptor signal transduction in the brain described an increase in phosphoinositide turnover in the median eminence.¹⁶ Since then, a number of studies, both on neurones in cell culture and *in vivo*, have contributed to elucidating further signalling mechanisms in the brain. Thus, it has been shown in primary cultures of hypothalamic neurones that stimulation of AT₁-receptors induces an

activation of the ras/raf kinase pathway, leading to activation of the MAP kinase, MEK, with subsequent phosphorylation/activation and translocation of the MAP kinase ERK1/2 into the nucleus.¹⁷⁻¹⁹ Ultimately, MAP kinase activation in these cells leads to an induction of c-fos. The stimulation of noradrenaline synthesis and of the expression of the noradrenaline transporter (NAT) protein, which mediates the re-uptake of the transmitter, are also mediated by the Ang II/AT₁-receptor-induced activation of ERK1/2 in these cultures.¹⁷ MAP kinase activation in hypothalamic neurones seems also to be under the control of AT₂-receptors, since stimulation of this receptor subtype leads to a decrease in ERK1/2 activity, which is mediated via activation of the serine/threonine phosphatase, PP-2A.²⁰ Some of these mechanisms have also been shown to operate in the hypothalamus *in vivo*.^{21,22}

Additionally, AT₁-receptor signal transduction pathways in neurones lead to the regulation of membrane currents. Thus, Ang II-induced activation of protein kinase C results in an increase in neuronal Ca²⁺ current, and protein kinase C, together with calcium/calmodulin protein kinase II, inhibits transient K⁺ current, as well as delayed rectifier K⁺ current.^{23,24} For rat SON-neurones, it has been described that Ang II, via AT₁-receptors, activates a non-selective Na⁺/Ca⁺ channel, leading to depolarisation of these cells.²⁵ Activation of Fos-regulating kinase and JNK has also been found in hypothalamic neurones.²⁶ In contrast to normotensive animals, in neuronal cultures derived from hypothalami of SHR, as well as in hypothalamic neurones *in vivo*, the Ang II-induced elevation of mRNA levels of NAT and tyrosine hydroxylase seems to be mediated in part via ERK1/2 and in part via activation of the Akt/protein kinase B pathway.²⁷

Activation of periventricular AT₁-receptors in the brain *in vivo* induces the expression of transcription factors, such as c-Fos or c-Jun, in nuclei which are participating in Ang II-mediated central cardiovascular regulation and osmoregulation.^{28,29} These regions include forebrain or hypothalamic structures, such as the subfornical organ, the organum vasculosum of the lamina terminalis, the PVN and SON, as well as brainstem structures, such as the area postrema/nucleus of the solitary tract. In genetically-hypertensive rats, in which the brain RAS is overactive, the expression of c-Fos, c-Jun and Krox-24, in response to stimulation with Ang II, is higher than in normotensive animals.^{30,31} The targets which are regulated by the Ang II-induced transcription factor expression *in vivo* remain to be elucidated.

In contrast to the AT₁-receptor, a number of studies on AT₂-receptor signalling have been performed in cells of neuronal origin (PC12W and NG 108-15 cell lines). Some features of the AT₂-receptor make it unique among G-protein-coupled receptors.³² G_i proteins seem to be the G-protein subtype which mainly couples to the AT₂-receptor. A variety of signalling mediators have been characterised, with partly contradictory results. The

AT₂-receptor has been shown to mediate an increase, as well as a decrease, in intracellular cGMP, activation or deactivation of Erk1/2 MAP kinases, and up- or down-regulation of phosphatases (PP2A, MKP-1, SHP-1). The nature of the signal transduction pathways for this receptor is obviously dependent to a high degree on the total signal input into the cell at the time of receptor stimulation. Thus, stimulation with Ang II leads to an AT₂-receptor-mediated increase in ERK1/2 activity in PC12W cells; in contrast, a nerve growth factor-stimulated ERK1/2 activation in the same cells was inhibited by AT₂-receptor activation.³³ Other signal transduction molecules which are stimulated by AT₂-receptor activation are particulate guanylate cyclase, T-type Ca²⁺ and K⁺ currents, nitric oxide release and activation of phospholipase A2 (the latter resulting in the release of arachidonic acid and its metabolites and ultimately in the activation of the Ras kinase/MAP kinase pathway).¹ The apoptosis-promoting properties of the AT₂-receptor have been shown to involve ceramide generation, as well as activation of caspase 3.³⁴⁻³⁶ An interesting new aspect of AT₂-receptor signalling, the ligand-independent signalling of the AT₂-receptor, has recently been shown in several cell types. Obviously, overexpression of the receptor without binding of Ang II was sufficient to induce apoptosis.³⁷

As in peripheral tissues and cells of peripheral origin, cross-talk between AT₁- and AT₂-receptors in the brain may be involved in the regulation of various processes, for example vasopressin release.² An interaction at the level of signal transduction pathways, which might play a role in this context, has again been described in primary hypothalamic neurones in culture. Ang II activates ERK1/2 via binding to its AT₁-receptor; blockade of the AT₂-receptor augmented this response, and stimulation of the cultures with an AT₂-receptor agonist decreased ERK1/2 activity via activation of the phosphatase PPA2.²⁰

Apart from the above studies, *in vivo* experiments on AT₂-receptor signalling in the brain have not, as yet, been published, probably because in most brain regions, AT₂-receptor expression is low. The recent findings that brain injury leads to an increase in AT₂-receptor expression in the respective tissues, will presumably enable more detailed *in vivo* experiments in the future.

Cell differentiation and antiproliferation

Over the last ten years, it has become apparent that the brain RAS is not only involved in the regulation of BP, water and sodium intake and secretion of vasopressin. The age-related pattern of angiotensin receptor localisation and expression in the brain and neuronal tissue indicates that Ang II also plays a role in central nervous system development and differentiation. The AT₂-receptor is predominantly expressed in the foetal brain. After birth, the ratio of AT₁- to AT₂-receptor expression reverses, with the AT₁-receptor being the predominant receptor in the majority of brain areas.^{38,39}

Ang II, acting on the AT₁-receptor, has been shown to promote cell growth and proliferation in a variety of cells, such as vascular smooth muscle cells, fibroblasts and cardiac myocytes.⁴⁰ Generally, adult CNS neurones cannot proliferate and are not able to reinnervate their target regions after injury. Effects mediated via AT₁-receptors in neurones are associated with rapid processes involving neurotransmission and neuromodulation, or delayed actions requiring synthesis of new proteins. As described in detail above, activation of the AT₁-receptor in neurones initiates a cascade of signalling events involving the Ras-Raf-MAP kinase pathway or the Janus-kinases, which phosphorylate the signal transducers and activators of transcription (STATs). Phosphorylated STATs translocate to the nucleus, where they regulate transcription of inducible proteins such as c-Fos, c-Jun and c-Myc, which themselves act as transcription factors for other genes.^{17,18,41} In neurones cultured from neonatal rat hypothalamus or brain stem, stimulation of the AT₁-receptor activates MAP kinases Erk1 and Erk2, which are involved in the regulation of growth and/or differentiation.²⁰

While the Ang II-mediated effects via the AT₁-receptor comprise cell growth and hypertrophy, activation of the AT₂-receptor inhibits cell proliferation and promotes neuronal differentiation and regeneration, but, in some cases, can also induce apoptosis.¹⁴

In cells of neuronal origin, such as NG108-15 neuroblastoma x glioma cells and pheochromocytoma-derived PC12W cells, Ang II, via AT₂-receptors, inhibits cell proliferation and induces morphological differentiation, characterised by neurite outgrowth and expression of neurofilaments.^{42,43} PC12W cells, which represent an established model system for studying various aspects of neuronal differentiation and apoptosis, undergo differentiation in response to nerve growth factor (NGF) application. After the withdrawal of NGF, an apoptotic programme is activated.⁴⁴ In low passages, PC12W cells express mainly AT₂- but only a few, if any, AT₁-receptors, and therefore offer an excellent model to investigate the AT₂-receptor-mediated effects on neuronal cells. Stimulation of AT₂-receptors in these cells enhanced NGF-mediated growth arrest, but induced a different pattern of morphological differentiation and regulation of cytoskeleton elements.^{35,45} Protein filaments, such as microtubules, actin filaments and intermediate filaments, play an important role in the stability and function of neuronal cells; they are up-regulated, for instance, upon treatment of PC12W cells with NGF. Both NGF and stimulation of AT₂-receptors in these cells produced up-regulation of polymerised β -tubulin and the microtubule-associated protein, MAP1. In contrast, NGF also stimulated the expression of MAP2, while the AT₂-receptor mediated down-regulation of this protein.⁴⁵ Morphological differentiation induced by activation of AT₂-receptors in NG108-15 cells correlated with an increase in the level of polymerised tubulin and in the levels of the microtubule-associated protein, MAP2c, and was associated with

inhibition of p21ras activity and an increase in MAP kinase activity.⁴⁵ AT₂-receptor-mediated effects on proliferation and differentiation are not limited to immortal tumour cell lines of neural origin. Activation of AT₂-receptors in microexplant cultures of the cerebellum from three day old rats has been shown to accelerate processes involved in morphological differentiation and to stimulate cell migration.⁴⁶ In neurones cultured from the neonatal rat hypothalamus and brainstem, activation of AT₁- and AT₂-receptors exert opposite effects on cell growth and differentiation: stimulation of AT₂-receptors inhibits the AT₁-receptor-induced activation of MAP kinases Erk1 and Erk2.²⁶ All these new and unexpected discoveries have provided a new insight into the role of the RAS in the development and differentiation of neuronal tissue.

Programmed cell death (apoptosis) in neuronal cells

Neurones undergo apoptosis after ischaemic insult or when they are seriously injured, for example after axotomy. The risk of apoptosis and the potency for axonal degeneration are closely related: lesions occurring close to the neuronal cell bodies initiate a potent cell body response directed towards regeneration, but the risk of apoptosis is simultaneously high. Neurones lesioned more distally do not usually undergo apoptosis, but the regeneration processes are weak. These observations suggest that neuronal injury or damage initiates a series of molecular events which are identical for both regeneration and apoptosis. For instance, overexpression of the transcription factor c-Jun, and in some cases of c-Fos, in neurones, represents part of the genetic programme initiating both neuronal survival and apoptosis.⁴⁷ Generally, the classical mitogen-activated protein kinase cascades, such as the ERK1/2 pathway, the stress-activated protein kinase (SAPK) cascade (JNK pathway) and the p38 pathway, have been implicated in apoptosis. ERK1 and ERK2 kinases inhibit apoptosis, whereas SAPK and p38 are pro-apoptotic. It is likely that a dynamic balance between the SAPK cascades and MAPK plays a critical role in cell survival and death.⁴⁸ Many other factors, like the intricate balance between the expression of pro- and anti-apoptotic proteins belonging to the Bcl-2 family, determine whether the cell will undergo apoptosis or will survive. Apoptotic processes in cells are further associated with activation of cysteine proteases (caspases), calpains (calcium-activated neutral proteinases) and ceramides. Calpains have been particularly implicated in the death of neurones in stroke.⁴⁸ Recent findings indicate that Ang II acting on its receptors not only exerts growth promoting effects and promotes neuronal differentiation and regeneration, but is also involved in the regulation of cellular pro- and anti-apoptotic events. It has been demonstrated that the AT₂-receptor exerts growth inhibitory and pro-apoptotic effects by antagonising the AT₁-mediated effects on growth factors in various cell lines, such

as vascular smooth muscle cells, neuronal PC12W cells or fibroblasts. Stimulation of AT₂-receptors in serum-deprived PC12W cells was reported to induce apoptosis.⁴⁹ In these cells, NGF inhibited the internucleosomal DNA fragmentation induced by serum deprivation, whereas Ang II, acting via AT₂-receptors, antagonised the NGF-mediated survival and induced apoptosis. The pro-apoptotic events mediated by the AT₂-receptor include activation of tyrosine phosphatases, such as MAP kinase-phosphatase-1 (MKP-1), and inactivation of MAP kinase (ERK1/2), which results in Bcl-2 protein dephosphorylation (inactivation of an anti-apoptotic factor) and up-regulation of the pro-apoptotic Bax protein.^{50,51} Analysis of functional domains of the AT₂-receptor in neuronal PC12W cells has revealed that the intermediate portion of the intracellular third loop is important for the apoptotic effects mediated by this receptor.³⁶ The axotomy-induced AT₂-receptor up-regulation, associated with the down-regulation of neurofilament M in PC12W cells, can also be interpreted in terms of apoptosis rather than neuroregeneration.⁵² Ceramides, which are generated by a phospholipase-C type reaction from the precursor sphingomyelin, serve as mediators of the cellular responses to a variety of apoptotic stimuli. The molecular mechanisms responsible for ceramide-induced cell death involve activation of stress kinases or caspases.⁵³ We have recently demonstrated that stimulation of AT₂-receptors selectively induces *de novo* synthesis of ceramides in PC12W cells.³⁵ The AT₂-receptor-induced ceramide accumulation preceded the onset of caspase 3 activation and DNA fragmentation.³⁶ All these findings connect the AT₂-receptor to intracellular signalling pathways associated with apoptosis and represent a solid basis for new research directions to investigate the role of the RAS in neuronal cells.

Neuronal regeneration and tissue repair

In general, the process of wound healing and tissue repair is controlled by a variety of mechanisms. Recent findings indicate that Ang II may interfere with these processes, since increased tissue levels of the AT₂-receptor have been observed after skin injury or after myocardial infarction.¹⁴ Since it has become apparent that Ang II, acting via the AT₂-receptor, plays a role in wound healing and repair of peripheral tissues, the attention of investigators has turned to the role of Ang II in processes occurring during regeneration after injury of neuronal tissue. Adult neurones do not usually reinnervate their target regions after injury, probably owing to the absence of requisite neurotrophic agents. However, if they are supplied with growth-promoting substances, they are able to generate new processes over long distances and, eventually, reinnervate their target region.¹⁵ Stimulation of AT₂-receptors in PC12W cells down-regulates neurofilament M as it occurs in nerve fibre regeneration.⁵² Reduced neuronal expression of this protein has been found following nerve transection.⁵⁴

Following sciatic nerve transection, a several-fold up-regulation of mRNA, coding for the AT₁- and AT₂-receptors, was demonstrated in dorsal root ganglion neurones and in sciatic nerve segments, proximal and distal to the cell body. Sciatic nerve crush resulted in a time-dependent up-regulation of AT₂-receptor mRNA levels in sciatic nerve segments which coincided with the successful regeneration of nerve fibres.⁵⁵ These findings indicate that AT₂- and AT₁-receptor-mediated pathways are involved in Schwann cell-mediated myelination and in the control of neuroregenerative responses in the peripheral nervous system.

Ang II-mediated effects have also been investigated in axonal regeneration of post-natal rat retinal explants and cultured dorsal root ganglion cells (DRGCs). In the *in vitro* model of post-natal retinal explants and cultured DRGCs, which are comparable with adult, non-regenerating DRGCs, as well as in the *in vivo* model of optic nerve crush, Ang II induced a concentration-dependent outgrowth of neurites. These effects of Ang II, both *in vitro* and *in vivo*, were mediated by the AT₂-receptor. As in the previous studies carried out on peripheral nerves, the regeneration process was paralleled by a time-dependent increase in AT₂-receptor mRNA expression in the retina and the crushed optic nerve.⁵⁴ These findings point to a role of Ang II and its receptors in the regeneration processes occurring in neuronal tissue following injury and provide direct evidence that stimulation of AT₂-receptors promotes axonal regeneration, not only *in vitro* but also under *in vivo* conditions after neuronal lesion.

Ischaemic stroke

Interruption of cerebral blood flow results in structural damage to neuronal tissue. Brain injury produced by permanent or transient focal cerebral ischaemia comprises a number of pathophysiological events, including loss of the membrane potentials, excitotoxicity, peri-infarct depolarisation, Ca²⁺ overload and inflammation. Both neuronal necrosis and apoptosis can be observed after brain ischaemia.^{56,57} Cerebral ischaemia also leads to alterations in gene expression, which is partly, under the control of inducible transcription factors (ITFs) like c-Fos. Activation of ITFs after hypoxia may represent an important link between the extracellular signals and the initiation of intracellular genomic and metabolic events which are associated with regeneration and survival or lead to delayed neuronal death. During the past decade, a number of studies have indicated that the brain RAS may be involved in the initiation and regulation of processes occurring during and after brain ischaemia. Treatment with ACE inhibitors or AT₁-receptor antagonists has been reported to prevent the occurrence of stroke in SHR or salt-loaded Dahl salt-sensitive rats and to reduce the infarction volume after middle cerebral artery occlusion.^{58,59} It is generally considered that ACE inhibitors protect against brain ischaemia primarily by reducing BP. However, several lines of evidence suggest that additional effects of ACE

inhibitors may also be involved. ACE inhibitors have been reported to exert beneficial effects on the metabolic and circulatory derangement in the ischaemic brain of SHR and to improve the recovery from cerebral ischaemia in normotensive rats.^{60,61} Neuroprotective effects of ACE inhibitors may be related to normalisation of cerebrovascular autoregulation, since they shift the limits of cerebral flow autoregulation to lower BP levels in normotensive and in SHR.⁶² Similarly, chronic treatment of stroke-prone SHR with candesartan, a selective and potent AT₁-receptor antagonist, reduced stroke incidence without affecting BP.⁶³ Nishimura *et al.*⁶⁴ investigated the effects in SHR of peripheral treatment with candesartan on cerebral autoregulation and infarction volume after middle cerebral artery occlusion (MCAO) with reperfusion. Candesartan treatment reversed the alteration in cerebrovascular autoregulation in SHR, shifting the autoregulatory curve to the left, in the direction of improved vasodilation, thus preventing the decrease in blood flow in the marginal zone of ischaemia. The observed reduction in cerebral oedema and in the infarction volume immediately after MCAO probably resulted from normalisation of cerebrovascular autoregulation in the marginal ischaemia zone. AT₁-receptor antagonists may, however, improve recovery from stroke by mechanisms independent of the normalisation of cerebrovascular autoregulation or BP reduction in hypertensive rats. We have recently demonstrated that chronic intracerebroventricular infusion of the selective AT₁-receptor antagonist, irbesartan, improved neurological outcome of focal cerebral ischaemia and markedly reduced the expression of the AP-1 transcription factors, c-Fos and c-Jun. Irbesartan was infused at a dose which inhibited brain, but not vascular, AT₁-receptors.⁶⁵ Although long-term inhibition of brain AT₂-receptors did not affect the recovery from stroke, it prevented the beneficial effects of the AT₁-receptor blockade (unpublished data). When AT₁-receptors are inhibited, Ang II can increasingly interact with AT₂-receptors, as 1) AT₁-receptor antagonists leave the AT₂-receptor unopposed and expose it to elevated Ang II levels, and, 2) ischaemic lesions of the nervous system have been reported to show increased expression of AT₂-receptors in the brain.⁶⁶ Activation of AT₂-receptors in brain tissue that has undergone ischaemic injury may, on one hand, initiate neuroregenerative events or, on the other, induce apoptosis when neurones are severely damaged. Both these effects are important for the recovery from stroke.⁵⁷

Outlook

Intensive research over the past ten years has delivered new insights into the role of angiotensin receptors in neuronal tissue. AT₂-receptors have been connected with processes occurring in neuronal tissue during development, regeneration and repair, but the actual contribution of these receptors to the regulation of events associated with these processes has not been clearly defined. Direct *in vivo* evidence implicating AT₂-receptors

in both neuroregeneration and the induction of apoptosis is still lacking. Further investigations are needed to precisely define the intracellular signalling cascades linked to the activation of angiotensin receptors and their role in the processes mediated by Ang II. The effects mediated by AT₂-receptors generally counteract those mediated by AT₁-receptors; the net effect of Ang II may, therefore, depend on the numbers and expression pattern of both receptor types in neuronal tissue. Other unresolved problems include the mechanisms involved in the cross-talk between AT₁- and AT₂-receptors and the identification of target genes, which are activated by Ang II acting via AT₁- and AT₂-receptors. The findings on the neurotrophic actions of AT₂-receptor stimulation may provide a basis for the design of new, receptor-directed therapeutic strategies in the failure of axonal regeneration or in the treatment of neurodegenerative disorders and stroke. Currently, AT₁-receptor antagonists are widely prescribed for cardiovascular diseases including hypertension. These antagonists have been reported, at least in rats, to cross the blood-brain barrier.⁶⁷⁻⁷⁰ The question remains as to whether long-term treatment with this class of drugs can prevent the incidence of stroke in hypertensive patients and exert beneficial effects on the metabolic and circulatory derangement in the ischaemic brain.

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