REVIEW

The angiotensin type 2 receptor of angiotensin II and neuronal differentiation: from observations to mechanisms

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Abstract

The angiotensin II (Ang II) type 2 receptor (AT₂) is a member of the seven-transmembrane domain, G-protein coupled receptor family. This receptor is ubiquitously distributed in the fetus but, in most tissues, its expression dramatically falls in the first few hours after birth. Based on this observation, the hypothesis that this receptor could be involved in fetal development was raised and, over the past ten years, many studies have tried to identify a role for the AT₂ receptor using many different tissues and cell lines. To date, one of the major roles associated with the Ang II AT₂ receptor concerns its ability to induce neuronal differentiation. Indeed, in cells of neuronal origin, activation of the AT₂ receptor was shown to induce neurite outgrowth and elongation, modulate neuronal excitability, promote cellular migration and, in particular conditions, induce neuronal cell death. Regarding its signaling mechanisms, the AT₂ receptor still represents one of the most controversial G-protein coupled receptors since it does not stimulate the production of any of the classical second messengers. This review summarizes knowledge of the functions and the signaling mechanisms involved in the actions of the AT₂ receptor in neurons and cells of neuronal origin. Based on its altered expression in neurological disorders, a role for the AT₂ receptor in control of neuronal plasticity is proposed.

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Introduction

The angiotensin II (Ang II) octapeptide, the active component of the renin–angiotensin system, binds and activates two major types of serpentine, seven-transmembrane domain G-protein coupled receptors, namely AT₁ and AT₂. Most of Ang II's well-known effects, including modulation of blood pressure, control of fluid/electrolyte balance and cellular proliferation, are associated with activation of the AT₁ receptor. The functions of the AT₂ receptor, however, were less well known until recently. During the last decade, the AT₂ receptor has been shown to be involved in the regulation of cell proliferation, programmed cell death

(apoptosis), and cellular differentiation; most of these results were obtained from cells of neuronal origin. Observations that in the fetus the AT₂ receptor is widely distributed in many brain structures and steroid-producing glands, such as the adrenal glands or the ovaries (Tanaka *et al.* 1995, Breault *et al.* 1996, Schutz *et al.* 1996, Nuyt *et al.* 1999), led to a hypothesis for a role in cellular differentiation. In the adult, AT₂ receptor expression is restricted to the uterus (Nielsen *et al.* 1997), ovarian granulosa cells (Pucell *et al.* 1991, Yoshimura *et al.* 1996, Johnson *et al.* 1997), adrenal glands (Belloni *et al.* 1998, Wang *et al.* 1998) and some areas of the brain involved in cognition and behavior (Song *et al.* 1992, Lenkei *et al.* 1996, 1997).

Brain renin–angiotensin system

Early studies from Mendelsohn *et al.* (1988) and Unger *et al.* (1988) established, using biochemical and pharmacological approaches, the existence of a renin–angiotensin system in the brain. The various components (angiotensin-converting enzyme, Ang II and Ang II receptors) are found in areas of the brain involved in the regulation of fluid and electrolyte balance and in the regulation of arterial pressure (Severs & Daniels-Severs 1973, Phillips 1987), as well as in structures involved in cognition, behavior and locomotion. Interestingly, all of these components, and in particular the AT₂ receptor, are highly expressed during fetal life, suggesting that they could play important roles during development. As reported by Nuyt *et al.* (1999) based on studies conducted in fetal and neonatal rats, AT₂ receptor mRNA appeared early (as early as embryonic day 13) in the differentiating lateral hypothalamic area, but transiently in various developing/differentiating brain structures. In most areas, ontogeny of AT₂ receptor mRNA expression is highly correlated with the maturation and differentiation of the different areas themselves (as in the cerebellum, inferior olivary complex, and medullary motor nuclei innervating the tongue, perioral, and jaw muscles, where AT₂ receptor expression dramatically diminished in the mature neurons). From studies conducted in cell lines, one can hypothesize that during development activation of the AT₂ receptor is involved in neurite elongation, neuron migration, neuronal death/survival balance, as well as in the establishment and maintenance of synaptic connections. In the adult rat, the AT₂ receptor was found at high levels in the medulla oblongata (control of autonomous functions), in septum and amygdala (associated with anxiety-like behavior), in the thalamus (sensory perception), in the superior colliculus (control of eye movements in response to visual information) as well as in the subthalamic nucleus and in the cerebellum (areas associated with learning of motor functions) (Song *et al.* 1992, Lenkei *et al.* 1996, 1997). According to Bottari *et al.* (1992) and our observations in rat cerebellar granule cells in culture (Fig. 1A), AT₂ receptors are found on neurons, but not on astrocytes or glial cells. The presence of the AT₂ receptor in restricted brain areas of the adult and its wide distribution in the fetus (in many differentiating structures and

nuclei) are suggestive of a role in neuronal function and neuronal development respectively. Accordingly, using cells of neuronal origin and models of neuronal regeneration, the AT₂ receptor was found to be involved in the induction of apoptosis and cell differentiation.

AT₂ receptor and neuronal differentiation

Differentiation of cells from neuronal origin required a progression through four essential steps: (1) cellular growth, (2) cellular migration, (3) elongation of axons and dendrites and (4) synaptogenesis (for review see Hatten & Heintz 1995). Supporting a role for the AT₂ receptor in the development/differentiation of fetal and perinatal brain structures, various studies have shown that its activation with angiotensin II could be involved in at least three steps of the differentiation process: cell growth, migration and elongation of axons and dendrites.

AT₂ receptor and neurite outgrowth

Induction of neurite outgrowth and elongation is one of the best characterized roles of the AT₂ receptor in cells of neuronal origin. We and others have demonstrated that specific activation of the AT₂ receptor induces morphological differentiation of several cell types (Laflamme *et al.* 1996, Meffert *et al.* 1996). We have shown that NG108–15 cells treated for 3 days with Ang II resulted in an increase in both the number and length of neurites (Fig. 1B, C). This effect was accompanied by an increase in the levels of polymerized β -tubulin and the amounts of microtubule-associated protein, also called mitogen-associated protein (MAP2c), associated with microtubules (Laflamme *et al.* 1996), a protein known to stabilize tubulin in a polymerized state, thus actively participating in differentiation (Sanchez *et al.* 2000). Similar results have been reported in the pheochromocytoma-derived cell line, PC12W (Meffert *et al.* 1996). As in NG108–15 cells, treatment of PC12W cells with Ang II promoted neuronal differentiation (characterized by an increase in neurite elongation) (Meffert *et al.* 1996). In this model, Ang II-induced neurite outgrowth is also associated with enhanced levels of polymerized β -tubulin and MAP2

Figure 1 Role of the AT₂ receptor in neuronal differentiation. (A) Epifluorescent localization of the AT₂ receptor was performed in cerebellar granule cells using an antibody against the AT₂ receptor revealed with an anti-rabbit rhodamine-coupled antibody (red) and an anti-glial fibrillary acidic protein (GFAP) antibody revealed with an anti-mouse FITC-coupled antibody (green). As shown in the merged image, the AT₂ receptor is localized on neurons, but not on glial cells. (The anti-AT₂ antibody was a generous gift from Dr Ian Bird, Department of Obstetrics and Gynecology, University of Wisconsin, Madison, USA.) (B) Control NG108-15 cells have neurites which are few in number and short. Daily application of 100 nM Ang II for three consecutive days induces an increase in the number and the length of neurites (C).

associated with microtubules (Stroth *et al.* 1998), but showed reduced expression of MAP1B (Stroth *et al.* 1998) and neurofilament M (Gallinat *et al.* 1997), two proteins specifically associated with

elongation of axons (Gordon-Weeks 1991, Shea & Flanagan 2001).

Angiotensin II-induced neurite outgrowth was also studied in rat neuronal cells in culture.

Activation of the AT₂ receptor in postnatal rat retinal explants (Lucius *et al.* 1998) and in microexplant cultures from rat cerebellum (Côté *et al.* 1999) promoted neurite extension. In addition to increased levels of polymerized β -tubulin, neurite outgrowth in microexplant cultures of the cerebellum was accompanied by enhanced expression of the neuron-specific β III-tubulin and increased levels of tau and MAP2 associated with microtubules (Côté *et al.* 1999). Altogether, these results suggest that activation of the AT₂ receptor is associated with important rearrangements of the cytoskeleton.

AT₂ receptor and neuronal migration

Accumulating evidence suggests that Ang II could be involved in the organization of several brain areas. Indeed, it was postulated that high levels of the AT₂ receptor in the inferior olivary and in the cerebellum are associated with neuronal plasticity and development (Jöhren *et al.* 1998, Arce *et al.* 2001). Within their study on the role of the AT₂ receptor in the induction of neurite outgrowth, Côté *et al.* (1999) observed that Ang II was able to influence cell migration. Indeed, in cerebellar granular cells cultured in microexplants, blockage of the AT₁ receptor or specific activation of the AT₂ receptor with CGP42112 (a specific AT₂ receptor agonist) remarkably induced migration of neuronal cells away from microexplants (where neurons migrate on glial cells, presumably on specialized structures called Bergmann fibers). Control of cellular migration by the AT₂ receptor was also reported in retinal explants during regeneration (Lucius *et al.* 1998) but the mechanisms involved in this process remain to be determined.

AT₂ receptor and neuronal excitability

Calcium and neuronal excitability play a crucial role during neuronal differentiation, regulating neuronal orientation, guidance and differentiation (Komuro & Rakic 1996, Rakic *et al.* 1996, Komuro & Rakic 1998*a,b*). In particular, T-type calcium channels are highly expressed in the early steps of neuronal differentiation, where they are involved in cellular growth and proliferation as well as in protein synthesis, excitability (Spitzer 1991, Spitzer *et al.* 2000), and growth cone guidance (Kater &

Mills 1991). However, their expression decreases as differentiation progresses (Yaari *et al.* 1987, McCobb *et al.* 1989, Kostyuk *et al.* 1993, Schmid & Guenther 1999). In cells of neuronal origin, activation of the AT₂ receptor was shown to promote changes in the expression of different ion channels leading to modifications in neuronal excitability. In NG108–15 cells, Buisson *et al.* (1992, 1995) showed that Ang II induced an inhibition of current amplitude attributed to T-type calcium channels by a mechanism involving activation of an unidentified phosphotyrosine phosphatase and a pertussis toxin (PTX)-insensitive G-protein. On the other hand, in rat brain neuronal cultures Kang *et al.* (1993) showed that activation of the AT₂ receptor stimulated a delayed-rectifier K⁺ current (I_K) and a transient K⁺ current (I_A) through both a PTX-sensitive G-protein (G_i) and PP2A, a serine/threonine phosphatase (Kang *et al.* 1994). In this model, activation of PP2A was mediated by phospholipase A₂ (PLA₂) activation and arachidonic acid release (Zhu *et al.* 1998). Physiologically, AT₂-induced activation of potassium currents is associated with a reduction in the length of action potential as well as with a shortening of the refractory period, both of which lead to an increase in membrane excitability (promoting an enhanced firing rate) (Xiong & Marshall 1994, Zhu *et al.* 2001) (Fig. 2).

AT₂ receptor and neuronal regeneration

Apart from its transient expression in many structures during development, expression of the AT₂ receptor increases after cellular damage suggesting a role in wound healing. Indeed, myocardial infarction (Nio *et al.* 1995), lesions in the nervous system or axotomy of dorsal root ganglia and sciatic (Gallinat *et al.* 1998) or optic nerves induced a substantial increase in AT₂ receptor expression (Lucius *et al.* 1998) the latter of which was shown to be associated with Ang II-induced axonal regeneration (Lucius *et al.* 1998). Accordingly, Ang II and CGP42112 (a specific AT₂ receptor agonist) increased the number of neuronal fibers crossing the lesion site (Lucius *et al.* 1998), thus providing evidence for a neurotropic action of Ang II in the central nervous system. Mechanisms involved in these AT₂-mediated effects are still unidentified, but probably involve signals that promote neuronal

Figure 2 AT_2 receptor and control of neuronal excitability. Activation of the AT_2 receptor induces an inhibition of T-type Ca^{2+} channels involving an unknown phosphotyrosine phosphatase and a PTX-insensitive G-protein. In addition to inhibition of T-type Ca^{2+} channels, Ang II increases phospholipase A_2 (PLA_2) activity thus leading to arachidonic acid release and activation of the Ser/Thr phosphatase 2A (PP2A). Activation of PP2A, in turn, stimulates delayed-rectifier K^+ current (I_K) and transient K^+ current (I_A) responsible for increased cell excitability characterized by an enhanced firing rate of neurons. CGP42112, an agonist of the AT_2 receptor; PTPase, phosphotyrosine phosphatase.

survival, polymerization and/or reorganization of microtubules and actin filaments, in addition to cell migration.

AT_2 receptor and neuronal apoptosis

Recently, many studies have focused on the role of the AT_2 receptor in apoptosis. It is well known that, in many cell types, serum-starvation induces apoptosis which can be rescued by the addition of neurotrophic factors, such as nerve growth factor (NGF) (Pittman *et al.* 1993) or insulin (Parrizas *et al.* 1997). Interestingly, it was found that neurotrophic factor-induced rescue from apoptosis can be blocked by Ang II in cells expressing the AT_2 receptor. Indeed, in PC12W cells, a cell line of neuronal origin, activation of the AT_2 receptor was shown to reverse the protective effects of NGF (Yamada *et al.* 1996) and to inhibit insulin receptor signaling (Elbaz *et al.* 2000), both effects associated with induction of apoptosis. The

pro-apoptotic effects of the AT_2 receptor were also reported in many other cell types such as vascular smooth muscle cells (Cui *et al.* 2001), ovarian granulosa cells (Tanaka *et al.* 1995), rat neuronal cells in culture (Shenoy *et al.* 1999), and cells from the fetal zone of the human adrenal gland (Chamoux *et al.* 1999). Based on the latter observations, it would appear paradoxical that activation of the AT_2 receptor can, in a same cell line (PC12W cells), induce different responses such as apoptosis (Yamada *et al.* 1996, Elbaz *et al.* 2000) and differentiation (Meffert *et al.* 1996). An important difference is observed between the experimental conditions used to study these two functions: while the former is conducted in the absence of serum, the latter is induced in a serum-containing medium. These observations suggest that the effect of the AT_2 receptor is highly sensitive (or dependent) on the cellular environment. It can thus be hypothesized that the

Table 1 Signaling pathways of the AT₂ receptor associated with Gα_i. Depending on the cellular model and/or the specific cascade studied, many events associated with activation of the AT₂ receptor were shown to be sensitive to pertussis toxin (PTX) (Gα_i-mediated) while others are not

	Effect	Reference
PTX-sensitive (Gα_i-coupled)		
PC12W	↑ MKP-1 activity	Horiuchi <i>et al.</i> (1997) Yamada <i>et al.</i> (1996)
VSMC	↑ MKP-1 activity	Hayashida <i>et al.</i> (1996)
Rat Neurons	↑ I _{K+} activity	Kang <i>et al.</i> (1994)
NIH3T3	↓ cell growth	Ozawa <i>et al.</i> (1996)
NG108-15	↑ cGMP	Gendron <i>et al.</i> (2002)
PTX-insensitive		
NG 108-15	↓ T-type Ca ²⁺ -channel	Buisson <i>et al.</i> (1995)
N1E 115	↑ SHP-1 activity	Bedecs <i>et al.</i> (1997)
N1E 115, CHO, AR42J	↑ SHP-1 activity	Elbaz <i>et al.</i> (2000)
CMLV	↑ SHP-1 activity	Shibasaki <i>et al.</i> (2001)

PTX, pertussis toxin; MKP-1, MAP kinase phosphatase; I_{K+}, delayed-rectifier potassium current; SHP-1, SH2 domain containing phosphatase 1.

AT₂ receptor cooperates with several proteins such as growth factor receptors or integrins, to mediate its differentiating effect.

Signaling mechanisms associated with the AT₂ receptor

An up-to-date overview on the AT₂ receptor coupling

Signaling mechanisms associated with activation of the AT₂ receptor are still a matter of debate. One of the most controversial features regarding the AT₂ receptor is its functional coupling to a heterotrimeric G-protein. Even if the AT₂ receptor shares similarities with other seven-transmembrane domain receptors, unlike most of them its affinity for its ligand, the Ang II octapeptide, is unaffected in the presence of GTPγS, a non-hydrolysable analog of GTP (Bottari *et al.* 1991, Pucell *et al.* 1991, Buisson *et al.* 1995). However, it should be mentioned that the AT₂ receptor displays different sensitivity to GTP analogs, depending on the brain structure where it is expressed (Tsutsumi & Saavedra 1992). All these data suggest that the AT₂ receptor could be differentially coupled, since only one isoform has been cloned (Kambayashi *et al.* 1993, Mukoyama *et al.* 1993).

Moreover, it was shown that the AT₂ receptor is not coupled to any of the classical second

messengers (cyclic adenosine monophosphate, Ca²⁺, inositol phosphates), thus suggesting that it does not activate Gα_s or Gα_q proteins (Leung *et al.* 1992, Webb *et al.* 1992). Some studies have demonstrated, however, that signaling mechanisms of the AT₂ receptor were associated with activation of a PTX-sensitive G-protein. Indeed, several groups showed, using *in vitro* reconstitution studies, that the AT₂ receptor can physically associate with different isoforms of Gα_i subunits (Hansen *et al.* 2000), in particular Gα_{i2} (Zhang & Pratt 1996, Sasamura *et al.* 2000) and Gα_{i3} (Zhang & Pratt 1996).

Signaling pathways of the AT₂ receptor associated or not with Gα_i subunits are reported in Table 1. Among the effects associated with the AT₂ receptor via activation of Gα_i proteins are the expression of the phosphatase MAP kinase phosphatase-1 (MKP-1) in PC12W and in vascular smooth muscle cells (Hayashida *et al.* 1996, Yamada *et al.* 1996, Horiuchi *et al.* 1997), activation of potassium channels in neurons (Kang *et al.* 1994), growth inhibition of NIH3T3 cells (Ozawa *et al.* 1996), and cGMP production during neuronal differentiation of NG108–15 cells (Gendron *et al.* 2002). PTX-insensitive effects include inhibition of T-type calcium currents (Buisson *et al.* 1995) as well as activation of the tyrosine phosphatase SHP-1 (SH2 domain containing phosphatase 1) (Bedecs *et al.* 1997, Elbaz *et al.* 2000, Shibasaki *et al.* 2001) involved in the AT₂-mediated inhibition of

different tyrosine kinase receptors and in the AT₂-induced apoptosis (Nouet & Nahmias 2000, Cui *et al.* 2001, 2002).

In COS-7 cells expressing the AT₂ receptor, Feng *et al.* (2002) recently demonstrated that the AT₂ receptor couples to Gα_s subunits. The AT₂ receptor was constitutively associated with SHP-1 and a Gα_s subunit to form an inactive complex. After Ang II application, the complex (AT₂ receptor and Gα_s/SHP-1) dissociates and SHP-1 is activated. Activation of SHP-1 was shown to be independent of Gβγ and Gα_s protein activity, suggesting a scaffolding role for G-proteins in AT₂-mediated signal mechanisms. This study was the first to propose that the AT₂ receptor could physically associate with Gα_s subunits.

AT₂ receptor-induced neuronal apoptosis

In addition to signaling cascades participating in AT₂ receptor-induced neuronal differentiation, cellular mechanisms involved in the induction of apoptosis by Ang II have also been extensively studied. Induction of apoptosis by the AT₂ receptor involved an inhibition of signals from different neurotrophic factor receptors (NGF, epidermal growth factor (EGF), insulin and fibroblast growth factor (FGF)). In serum-starved PC12W cells, Yamada *et al.* (1996) demonstrated that AT₂-induced apoptosis implicated an inhibition of NGF-stimulated p42/p44^{mapk}. In this model, activation of the AT₂ receptor was associated with an increase in the expression of MKP-1 (a phosphatase with dual specificity). This enhanced expression of MKP-1 was responsible for the observed inactivation of p42/p44^{mapk} (Yamada *et al.* 1996) and Bcl-2, an anti-apoptotic protein (Horiuchi *et al.* 1997). In cultured newborn rat hypothalamus and brain stem neurons, activation of the AT₂ receptor enhanced neuronal apoptosis induced by UV radiation. This effect of the AT₂ receptor involved activation of PP2A by a PTX-sensitive mechanism (Shenoy *et al.* 1999).

In non-neuronal cells, induction of apoptosis by the AT₂ receptor can also involve activation of SHP-1. SHP-1 is implicated in the AT₂-dependent inhibition of phosphorylation and signaling of EGF, FGF, insulin and AT₁ receptors (Bedecs *et al.* 1997, Lehtonen *et al.* 1999, Elbaz *et al.* 2000, Cui

et al. 2001, 2002, Shibasaki *et al.* 2001). Further investigations will be needed to identify the complete mechanisms of SHP-1 activation by the AT₂ receptor as well as its role in cellular apoptosis.

The MAP kinase (MAPK) pathway and differentiation

Over the past five years, in our goal to elucidate AT₂ receptor involvement in Ang II-induced neuronal differentiation, we and others have investigated signaling mechanisms activated by this receptor. In NG108–15 cells, we have found that specific activation of the AT₂ receptor induced an increase in p42/p44^{mapk} activities (Gendron *et al.* 1999). Similar results were obtained in PC12W (Stroth *et al.* 2000) as well as in non-neuronal COS-7 (Hansen *et al.* 2000) and NIH3T3 cells (De Paolis *et al.* 2002) overexpressing the AT₂ receptor. In both NG108–15 (Gendron *et al.* 1999) and PC12W cells (Stroth *et al.* 2000), Ang II-induced p42/p44^{mapk} activation appears essential for the induction of neurite outgrowth.

In contrast to the AT₁ receptor, activation of the MAPK cascade via the AT₂ receptor is delayed, but sustained, as seen with NGF in PC12W cells (Yaka *et al.* 1998, York *et al.* 1998). Surprisingly, concomitantly with AT₂-induced p42/p44^{mapk} activation, a decrease in the activity of p21^{ras} is observed (Gendron *et al.* 1999). Accordingly, expression of a dominant negative mutant form of p21^{ras} (RasN17) in NG108–15 cells failed to impair the effect of Ang II on p42/p44^{mapk} (Gendron *et al.* 1999). This observation was the first to document that inhibition of Ras could be associated with neurite elongation.

As illustrated in Fig. 3, we have recently shown that activation of the AT₂ receptor by Ang II and CGP42112 was followed by stimulation of p21^{rap1} and B-Raf activities. Moreover, expression of RapN17 (a dominant negative mutant of p21^{rap1}) or Rap1 GAP (a natural, specific inhibitor of Rap1) completely blocked both AT₂-induced p42/p44^{mapk} activation and neurite outgrowth. These observations indicate that the related Ras/Raf-1, namely Rap1/B-Raf, is involved in the AT₂ receptor signaling mechanisms leading to morphological neuronal differentiation (Gendron *et al.* 2003). In several models, such as PC12W cells stimulated

Figure 3 Pathways involved in the AT₂-induced neuronal differentiation. The Ang II AT₂ receptor induction of neurite outgrowth and elongation involves at least two distinct, independent, but complementary pathways. First, after binding of Ang II, the activated AT₂ receptor rapidly inactivates p21^{ras} and activates the Rap1-B-Raf pathway, leading to a delayed p42/p44^{mapk} phosphorylation. Another pathway involving the NO/sGC/cGMP cascade of signaling is also necessary to observe neurite outgrowth. Stimulation of these two parallel pathways could modulate gene expression and the phosphorylation state of different microtubule-associated proteins (MAPs) to control microtubule stability/dynamic responsible for neurite elongation. NOS, nitric oxide synthase; NO, nitric oxide; sGC, soluble guanylyl cyclase; MEK, mitogen-activated protein kinase kinase; unP-MAPs, unphosphorylated MAPs; P-MAPs, phosphorylated MAPs. CGP42112, an agonist of the AT₂ receptor.

with NGF, cAMP and cAMP-activated proteins (such as protein kinase A (PKA)) are required for activation of p21^{rap1} and p42/p44^{mapk} (York *et al.* 1998). However, in NG108–15 cells, we demonstrated that dbcAMP and forskolin did not stimulate Rap1 or p42/p44^{mapk} activities. Furthermore, addition of H-89, an inhibitor of PKA, or Rp-8-Br-cAMPS, an inactive cAMP analog, failed to impair p42/p44^{mapk} activity and neurite outgrowth induced by Ang II. These observations indicate that the AT₂ signaling is independent of cAMP, and further indicate that neurite elongation induced by cAMP did not involve the Rap1/B-Raf pathway of signaling, at least in the NG108–15 cells (Hamprecht *et al.* 1985, Beaman-Hall & Vallano 1993, Cowley *et al.* 1994, Laflamme *et al.* 1996). The mechanisms involved in the AT₂-induced Rap1/B-Raf activation remain unclear but it could be hypothesized that the AT₂ receptor transactivates other receptors, such as growth factor receptors. In fact, we and others have demonstrated that activation of p42/p44^{mapk} requires the presence of serum (rich in growth factors) in the culture medium (Gendron *et al.* 1999, Hansen *et al.* 2000), suggesting that at least one as yet unidentified growth factor participates in the initial actions of the AT₂ receptor, such as activation of Rap1.

The nitric oxide/cGMP pathway and differentiation

Previous studies have reported that Ang II, through the AT₂ receptor, is able to induce nitric oxide (NO) production and cGMP accumulation in different models such as the kidney (Siragy & Carey 1996, 1997), the aorta (Maeso *et al.* 1996, Gohlke *et al.* 1998), vascular smooth muscle cells from transgenic mice overexpressing the AT₂ receptor (Tsutsumi *et al.* 1999), and NG108–15 cells (Schelman *et al.* 1997, Côté *et al.* 1998). Interestingly, NO, when produced at low levels, is known to induce neuronal differentiation of different cell types such as PC12W (Poluha *et al.* 1997, Phung *et al.* 1999). In NG108–15 cells, we showed that application of NO donors in the culture medium was sufficient to induce neurite outgrowth and elongation (Côté *et al.* 1998). In these cells, AT₂-induced differentiation involved activation of the neuronal NO synthase (nNOS) and cGMP production (Gα_i-dependent). Indeed,

neurite elongation was abolished when the NO signaling cascade was inhibited at the level of nNOS, soluble guanylyl cyclase (sGC) or PKG. Our results demonstrated that this cascade of signaling is involved in neurite outgrowth and elongation as well as in growth cone maturation (Gendron *et al.* 2002).

In NG108–15 and in PC12W cells, MAPK activation is an essential event for neurite elongation (Gendron *et al.* 1999, Stroth *et al.* 2000). For this reason, it has been hypothesized that the NO cascade of signaling could be involved in the activation of p42/p44^{mapk}. However, our results indicate that the two pathways - Rap1/B-Raf/MAPK and NO/cGMP/PKG - are necessary but act in an independent, parallel manner to induce morphological changes, possibly mediating microtubule dynamics (Fig. 3).

From apoptosis to neurite elongation

As outlined in this review, the AT₂ receptor is involved in and/or controls important steps of differentiation. Indeed, Ang II and the AT₂ receptor activate migration, controlled cell death, and neurite elongation.

In the developing brain, after migration is completed and cells have reached their appropriate location, more than 50% of undifferentiated neuronal cells will die by an apoptosis-mediated process (Hatten & Heintz 1995, Huang & Reichardt 2001, Valverde 2002). During this period of differentiation of various brain areas and layers, only cells expressing appropriate receptors for neurotropic factors, and cells adequately stimulated by those factors will survive (Green & Evan 2002). For example, in the absence of NGF, sympathetic neurons expressing both AT₂ and TrkA receptors undergo an apoptosis program (Yamada *et al.* 1996). In the presence of NGF, cells survive and undergo a differentiation process characterized by neurite elongation of both axons and dendrites (Valtorta & Leoni 1999, Tang 2001, Bokel & Brown 2002, Milner & Campbell 2002, Nikolic 2002). These functions of survival, migration and elongation are controlled by specific intracellular signaling cascades, activated not only by neurotropic factors, but also by hormones, such as pituitary adenylate cyclase-activating polypeptide (PACAP) (Vaudry *et al.* 2002) and angiotensin

II, through the AT₂ receptor. Moreover, specific interactions between cells and the surrounding matrix components, through integrins and adhesion factors, are also important to integrate various intracellular cascades (Milner & Campbell 2002) necessary to control cell behavior. Indeed, on plastic, activation of the AT₂ receptor promotes migration in NG108–15 cells (Côté *et al.* 1999), while on fibronectin, the AT₂ receptor inhibits migration in smooth muscle cells (Chassagne *et al.* 2002).

Lessons from the AT₂ receptor alterations: a role for the AT₂ receptor in the developing brain

Knockout mice

In 1995, a mouse model lacking the gene encoding the AT₂ receptor was generated (Hein *et al.* 1995, Ichiki *et al.* 1995). Studies from these knockout mice reinforced the hypothesis that the AT₂ receptor plays a role in neuronal development. In fact, even if these mutant mice appeared to develop normally, significantly more cells were found in many brain structures (cortex, hippocampus, amygdala, thalamus) (von Bohlen und Halbach *et al.* 2001). Based on the roles of the AT₂ receptor described in cells of neuronal origin, it could be hypothesized that the increase of cells in these structures could be the result of the combination of an impaired growth arrest, diminished apoptosis, and/or a reduction of cell migration. The AT₂-deficient mice suffer from perturbations in exploratory behavior and locomotor activity (Hein *et al.* 1995, Ichiki *et al.* 1995), as well as an anxiety-like behavior (Okuyama *et al.* 1999), impaired drinking responses (Hein *et al.* 1995), and hypersensitivity to Ang II on blood pressure and sodium excretion (Siragy *et al.* 1999). Altogether, these results demonstrated that the AT₂ receptor plays an important role in the development and in the maturation of various brain structures.

In the adult rat, Braszko (2002) recently reported that inhibition of the AT₂ receptor with PD123319 (a specific AT₂ receptor antagonist) abolished the Ang II-induced acquisition of conditioned avoidance responses. These results strongly support the hypothesis that, in addition to a role during development, the AT₂ receptor

may also be involved in cognitive processes in the adult.

AT₂ receptor expression in neurological disorders

Over the last decade, it has been found that the levels of expression of the AT₂ receptor were altered in specific areas of the brain in the course of different neurological disorders. For example, Ge and Barnes (1996) found that AT₂ receptor expression is diminished in Parkinson's disease (caudate nucleus and cerebellum) but is enhanced in Huntington's disease (caudate nucleus). In Alzheimer's disease, the temporal cortex of the adult brain showed an increased expression while the hippocampus displayed a decreased expression of the AT₂ receptor. These results suggest that the AT₂ receptor may play an important role in maintaining functions of the human brain.

AT₂ in mental retardation

Recently, Vervoort *et al.* (2002) have published results that show that mutations in the *Agtr2* gene correlate with the development of human X-linked mental retardation. Indeed, 9 patients with X-linked mental retardation had mutations in the *Agtr2* gene associated with decreased expression of the AT₂ receptor, including a complete loss of expression in a woman with an IQ of 44. Again, these observations support the hypothesis that the AT₂ receptor is required for brain development and for the maintenance of neuronal connections involved in learning and memory.

Conclusions and perspectives

As described in this review, the role of the AT₂ receptor of Ang II in neuronal differentiation and brain development is now well documented. Indeed, a number of studies report the role of the AT₂ receptor in neurite elongation, neuronal excitability and axonal regeneration. Over the past 5 years, important progress has been made regarding elucidation of the signaling mechanisms associated with neurite outgrowth, neuronal differentiation and brain development. The increased knowledge of the mechanisms of action

of the AT₂ receptor will certainly contribute to a better understanding of the role of Ang II in the brain, particularly in relation to several neurological disorders (Alzheimer, Huntington, Parkinson, mental retardation). However, the earliest events associated with activation of the AT₂ receptor remain unclear. Apart from some effects which are sensitive to pertussis toxin (see Table 1) and the recently described interaction with a Gα_s/SHP-1 complex (Feng *et al.* 2002), immediate partners of the AT₂ receptor remain to be determined. Although activation of the AT₂ receptor clearly stimulates neurite outgrowth, the cellular targets are not yet clearly identified. Several questions concerning microtubule polymerization and AT₂ signaling partners leading to MAP2, MAP1B and tau phosphorylation remain unanswered.

Because of its correlation with some neurological disorders, it could be expected that the effects of the AT₂ receptor on the different steps of neurite elongation may also play an important role in maintaining/controlling the neuronal plasticity necessary to maintain appropriate synapse connections and plasticity (Arendt 2001, Segal 2002). Taken together, these observations concerning roles for the AT₂ receptor in neuronal functions (differentiation, migration, regeneration), as well as involvement in neurological disorders, contribute to reinforce the hypothesis that activation of this receptor involves important rearrangements of at least microtubule networks as witnessed by neurite growth and elongation. The challenge regarding elucidation of the signaling mechanisms associated with the AT₂ receptor will now be to identify the cytoskeletal targets that control dynamic structures at the growth cone level and the stability of the microtubule axon shaft.

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