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.
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.

Figure 1 Role of the AT$_2$ receptor in neuronal differentiation. (A) Epifluorescent localization of the AT$_2$ receptor was performed in cerebellar granule cells using an antibody against the AT$_2$ 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 AT$_2$ receptor is localized on neurons, but not on glial cells. (The anti-AT$_2$ 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).
Activation of the AT$_2$ 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 βIII-tubulin and increased levels of tau and MAP2 associated with microtubules (Côté et al. 1999). Altogether, these results suggest that activation of the AT$_2$ receptor is associated with important rearrangements of the cytoskeleton.

**AT$_2$ 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 AT$_2$ 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 AT$_2$ 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 AT$_1$ receptor or specific activation of the AT$_2$ receptor with CGP42112 (a specific AT$_2$ 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 AT$_2$ receptor was also reported in retinal explants during regeneration (Lucius et al. 1998) but the mechanisms involved in this process remain to be determined.

**AT$_2$ 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 1998a,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 AT$_2$ 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 AT$_2$ 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 A$_2$ (PLA$_2$) activation and arachidonic acid release (Zhu et al. 1998). Physiologically, AT$_2$-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).

**AT$_2$ receptor and neuronal regeneration**

Apart from its transient expression in many structures during development, expression of the AT$_2$ 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 AT$_2$ 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 AT$_2$ 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 AT$_2$-mediated effects are still unidentified, but probably involve signals that promote neuronal
survival, polymerization and/or reorganization of microtubules and actin filaments, in addition to cell migration.

**AT₂ receptor and neuronal apoptosis**

Recently, many studies have focused on the role of the AT₂ receptor in apoptosis. It is well known that, in many cell types, serum-starvation induces apoptosis which can be rescued by the addition of neurotropic factors, such as nerve growth factor (NGF) (Pittman et al. 1993) or insulin (Parrizas et al. 1997). Interestingly, it was found that neurotropic factor-induced rescue from apoptosis can be blocked by Ang II in cells expressing the AT₂ receptor. Indeed, in PC12W cells, a cell line of neuronal origin, activation of the AT₂ receptor was shown to reverse the protective effect 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 AT₂ 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 AT₂ 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 AT₂ receptor is highly sensitive (or dependent) on the cellular environment. It can thus be hypothesized that the

**Figure 2** AT₂ receptor and control of neuronal excitability. Activation of the AT₂ receptor induces an inhibition of T-type Ca²⁺ channels involving an unknown phosphotyrosine phosphatase and a PTX-insensitive G-protein. In addition to inhibition of T-type Ca²⁺ channels, Ang II increases phospholipase A₂ (PLA₂) 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 (Iₖ) and transient K⁺ current (Iₐ) responsible for increased cell excitability characterized by an enhanced firing rate of neurons. CGP42112, an agonist of the AT₂ receptor; PTPase, phosphotyrosine phosphatase.
The AT$_2$ receptor cooperates with several proteins such as growth factor receptors or integrins, to mediate its differentiating effect.

### Signaling mechanisms associated with the AT$_2$ receptor

#### An up-to-date overview on the AT$_2$ receptor coupling

Signaling mechanisms associated with activation of the AT$_2$ receptor are still a matter of debate. One of the most controversial features regarding the AT$_2$ receptor is its functional coupling to a heterotrimeric G-protein. Unlike most of them, its affinity for its ligand, the Ang II octapeptide, is unaffected in the presence of GTP$_{\alpha}$S, 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 AT$_2$ 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 AT$_2$ 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 AT$_2$ receptor is not coupled to any of the classical second messengers (cyclic adenosine monophosphate, Ca$^{2+}$, inositol phosphates), thus suggesting that it does not activate G$_{\alpha}$ or G$_{\beta\gamma}$ proteins (Leung et al. 1992, Webb et al. 1992). Some studies have demonstrated, however, that signaling mechanisms of the AT$_2$ receptor were associated with activation of a PTX-sensitive G-protein. Indeed, several groups showed, using in vitro reconstitution studies, that the AT$_2$ receptor can physically associate with different isoforms of G$_{\alpha}i$ subunits (Hansen et al. 2000), in particular G$_{\alpha}i2$ (Zhang & Pratt 1996, Sasamura et al. 2000) and G$_{\alpha}i3$ (Zhang & Pratt 1996).

#### Table 1: Signaling pathways of the AT$_2$ receptor associated with G$_{\alpha}i$

Depending on the cellular model and/or the specific cascade studied, many events associated with activation of the AT$_2$ receptor were shown to be sensitive to pertussis toxin (PTX) (G$_{\alpha}i$-mediated) while others are not.

<table>
<thead>
<tr>
<th>PTX-sensitive (G$_{\alpha}i$-coupled)</th>
<th>Effect</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>PC12W</td>
<td>↑ MKP-1 activity</td>
<td>Horiuchi et al. (1997)</td>
</tr>
<tr>
<td>VSMC</td>
<td>↑ MKP-1 activity</td>
<td>Yamada et al. (1996)</td>
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<tr>
<td>Rat Neurons</td>
<td>↑ $I_{Ks}$ activity</td>
<td>Kang et al. (1994)</td>
</tr>
<tr>
<td>NIH3T3</td>
<td>↓ cell growth</td>
<td>Ozawa et al. (1996)</td>
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<tr>
<td>NG108-15</td>
<td>↑ cGMP</td>
<td>Gendron et al. (2002)</td>
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<tr>
<th>PTX-insensitive</th>
<th>Effect</th>
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<tr>
<td>NG 108-15</td>
<td>↓ T-type Ca$^{2+}$-channel</td>
<td>Buisson et al. (1995)</td>
</tr>
<tr>
<td>N1E 115</td>
<td>↑ SHP-1 activity</td>
<td>Bedecs et al. (1997)</td>
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<tr>
<td>N1E 115, CHO, AR42J</td>
<td>↑ SHP-1 activity</td>
<td>Elbaz et al. (2000)</td>
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<tr>
<td>CMLV</td>
<td>↑ SHP-1 activity</td>
<td>Shibasaki et al. (2001)</td>
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PTX, pertussis toxin; MKP-1, MAP kinase phosphatase; $I_{Ks}$, delayed-rectifier potassium current; SHP-1, SH2 domain containing phosphatase 1.
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 Gaq subunits. The AT2 receptor was constitutively associated with SHP-1 and a Gaq subunit to form an inactive complex. After Ang II application, the complex (AT2 receptor and Gaq/SHP-1) dissociates and SHP-1 is activated. Activation of SHP-1 was shown to be independent of Gβγ and Gaq 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 Gaq 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 neurotropic 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 SHP-1 (a phosphatase with dual specificity). This enhanced expression of SHP-1 was responsible for the observed inactivation of p42/p44\textsuperscript{mapk} (Yamada et al. 1996) and Bel-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\textsuperscript{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\textsuperscript{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\textsuperscript{mapk} activation, a decrease in the activity of p21\textsuperscript{ras} is observed (Gendron et al. 1999). Accordingly, expression of a dominant negative mutant form of p21\textsuperscript{ras} (RasN17) in NG108–15 cells failed to impair the effect of Ang II on p42/p44\textsuperscript{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 AT3 receptor by Ang II and CGP42112 was followed by stimulation of p21\textsuperscript{rap1} and B-Raf activities. Moreover, expression of RapN17 (a dominant negative mutant of p21\textsuperscript{rap1}) or Rap1 GAP (a natural, specific inhibitor of Rap1) completely blocked both AT3-induced p42/p44\textsuperscript{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 via free access.
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 p21ras and activates the Rap1-B-Raf pathway, leading to a delayed p42/p44mapk 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\(\text{mapk}\) and p42/p44\(\text{mapk}\) (York et al. 1998). However, in NG108–15 cells, we demonstrated that dbcAMP and forskolin did not stimulate Rap1 or p42/p44\(\text{mapk}\) activities. Furthermore, addition of H-89, an inhibitor of PKA, or Rp-8-Br-cAMPS, an inactive cAMP analog, failed to impair p42/p44\(\text{mapk}\) activity and neurite outgrowth induced by Ang II. These observations indicate that the AT\(_2\) 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 AT\(_2\)-induced Rap1/B-Raf activation remain unclear but it could be hypothesized that the AT\(_2\) receptor transactivates other receptors, such as growth factor receptors. In fact, we and others have demonstrated that activation of p42/p44\(\text{mapk}\) 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 AT\(_2\) receptor, such as activation of Rap1.

### The nitric oxide/cGMP pathway and differentiation

Previous studies have reported that Ang II, through the AT\(_2\) 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 AT\(_2\) 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, AT\(_2\)-induced differentiation involved activation of the neuronal NO synthase (nNOS) and cGMP production (\(\text{G}_{\alpha}\)-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\(\text{mapk}\). 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 AT\(_2\) receptor is involved in and/or controls important steps of differentiation. Indeed, Ang II and the AT\(_2\) 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 AT\(_2\) 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 Gaₒ/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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References


Braszkó JJ 2002 AT₂ receptor but not AT₁ receptor antagonism abolishes angiotensin II increase of the acquisition of conditioned avoidance responses in rats. Behavioural Brain Research 131 79–86.


Komuro H & Rakic P 1998b Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular Ca2+ fluctuations. *Journal of Neurobiology* 37 110–130.


Côté F, Do TH, Lafamme L, Gallo JM & Gallo-Payet N 1999 Activation of the AT2 receptor of angiotensin II induces neurite outgrowth and cell migration in microexplant cultures of the cerebellum. *Journal of Biological Chemistry* 274 31686–31692.


Gendron L, Côté F, Payet MD & Gallo-Payet N 2002 Nitric oxide and cyclic GMP are involved in angiotensin II AT2 receptor effects on neurite outgrowth in NG108–15 cells. *Neuroendocrinology* 75 70–81.


McCobb DP, Best PM & Beam KG 1989 Development alters the
Mendelsohn FA, Allen AM, Clevers J, Denton DA, Tarjan E &
Nouet S & Nahmias C 2000 Signal transduction from the
Lucius R, Gallinat S, Rosenstiel P, Herdegen T, Sievers J & Unger T
Ozawa Y, Suzuki Y, Murakami K & Miyazaki H 1996 The
Nuyt AM, Lenkei Z, Palkovits M, Corvol P & Llorens-Cortes C 1996
Expression of angiotensin type-1 (AT1) and type-2 (AT2)
receptor mRNAs in the adult rat brain: a functional neuroanatomical
Leung KH, Roscoe WA, Smith RD, Timmermans PB & Chiu AT
1992 Characterization of biochemical responses of angiotensin II
(AT2) binding sites in the rat pheochromocytoma PC12W cells.
European Journal of Pharmacology 277 65–70.
Lucius R, Gallinat S, Rosenstiel P, Herdegen T, Sievers J & Unger T
1998 The angiotensin II type 2 (AT2) receptor promotes axonal
regeneration in the optic nerve of adult rats. Journal of Experimental
Brain Research 138 661–670.
McCobb DP, Hillger PM & Beam KG 1989 Development alters the
expression of calcium currents in chick limb motoneurons.
Neuron 2 1633–1643.
phenylephrine constrictor response in aortic rings from
spontaneously hypertensive rats. Role of nitric oxide and
angiotensin II type 2 receptors. Hypertension 28 967–972.
Meffert S, Stoll M, Steckelings UM, Bottari SP & Unger T 1996
The angiotensin II AT1 receptor inhibitor prevents proliferation
and promotes differentiation in PC12W cells. Molecular and Cellular
Endocrinology 122 59–67.
Mendelsohn FA, Allen AM, Clevers J, Denton DA, Tarjan E &
McKinley MJ 1998 Localization of angiotensin II receptor
binding in rabbit brain by in vivo autoradiography. Journal of
Comparative Neurology 370 372–384.
Milner R & Campbell IL 2002 The integrin family of cell adhesion
molecules has multiple functions within the CNS. Journal of
Neuroscience Research 69 286–291.
Mukoyama M, Nakajima M, Horiuchi M, Sasahara H, Pratt RE &
Dzau VJ 1993 Expression cloning of type 2 angiotensin II
receptor reveals a unique class of seven-transmembrane receptors.
Journal of Biological Chemistry 268 24539–24542.
Angiotensin II receptors and renin in the porcine uterus: myometrial AT2 and endometrial AT1 receptors are down-
regulated during gestation. Clinical and Experimental Pharmacology
and Physiology 24 309–314.
Nikolic M 2002 The role of Rho GTPases and associated kinases in
regulating neurite outgrowth. International Journal of Biochemistry
and Cell Biology 34 731–743.
Nio Y, Matsubara H, Murasawa S, Kanasaki M & Inada M 1995
Regulation of gene transcription of angiotensin II receptor
subtypes in myocardial infarction. Journal of Clinical Investigation
95 46–54.
Nouet S & Nahmias C 2000 Signal transduction from the
angiotensin II AT2 receptor. Trends in Endocrinology and Metabolism
11 1–6.
Nuyt AM, Lenkei Z, Palkovits M, Corvol P & Llorens-Cortes C 1999
Ontogeny of angiotensin II type 2 receptor mRNA
expression in fetal and neonatal rat brain. Journal of Comparative
Okumaya S, Sakagawa T, Chaki S, Imagawa Y, Ichiki T &
Imagami T 1999 Anxiety-like behavior in mice lacking
the angiotensin II type-2 receptor. Brain Research 821 150–159.
Ozawa Y, Suzuki Y, Murakami K & Miyazaki H 1996 The
angiotensin II type 2 receptor primarily inhibits cell growth via
pertussis toxin-sensitive G proteins. Biochemical and Biophysical
Research Communications 228 328–333.
Parrizas M, Saltiel AR & LeRoith D 1997 Insulin-like growth
factor-I inhibits apoptosis using the phosphatidylinositol 3-kinase
and mitogen-activated protein kinase pathways. Journal of Biological
Chemistry 272 154–161.
Phillips MI 1987 Functions of angiotensin in the central nervous
Phung YT, Bekker JM, Hallmark OG & Black SM 1999 Both
neuronal NO synthase and nitric oxide are required for PC12 cell
differentiation: a cGMP independent pathway. Molecular Brain
Research 64 163–178.
characterizing cellular and molecular events in programmed
Poluha W, Schonhoff CM, Harrington KS, Lachyankar MB,
Crosbie RH, Bulsoco DA & Ross AH 1997 A novel, nerve growth
factor-activated pathway involving nitric oxide, p53, and p21
WAF1 regulates neuronal differentiation of PC12 cells. Journal of
Biological Chemistry 272 24002–24007.
Pucell AG, Hodges JC, Sen I, Bumpus FM & Husain A 1991
Biochemical properties of the ovarian granulosa cell type
Rakic P, Knyihar-Csillik E & Csillik B 1996 Polarity of
microtubule assemblies during neuronal cell migration. PMBS 93
9218–9222.
Sanchez C, Diaz-Nedo J & Avila J 2000 Phosphorylation of
microtubule-associated protein 2 (MAP2) and its regulatory role in
the regulation of the neuronal cytoskeleton function. Progress in
Neurobiology 61 135–168.
Sasamura H, Mifune M, Nakaya H, Amemiya T, Hiraki T,
Nishimoto I & Saruta T 2000 Analysis of Gm protein recognition
profiles of angiotensin II receptors using chimeric Gm proteins.
Molecular and Cellular Endocrinology 170 113–121.
Schelman WR, Kurth JL, Berdeaux RL, Norby SW &
Weyhenmeyer JA 1997 Angiotensin II type-2 (AT2) receptor-
mediated inhibition of NMDA receptor signaling in neuronal cells.
Molecular Brain Research 48 197–203.
Schmidt S & Guenther E 1999 Voltage-activated calcium currents in
rat retinal ganglion cells in situ: changes during prenatal and
Schutz S, Le Mouiller JM, Corvol P & Gasc JM 1996 Early
expression of all the components of the renin-angiotensin system
in human development. American Journal of Pathology 149
2067–2079.
Segal M 2002 Dendritic spines: elementary structural units of
Severs WB & Daniels-Severs AE 1973 Effects of angiotensin on the
Shea TB & Flanagan LA 2001 Kinesin, dynein and neurofilament
Shenoy UV, Richards EM, Huang XC & Summers C 1999
Angiotensin II type 2 receptor-mediated apoptosis of
cultured neurons from newborn rat brain. Endocrinology 140
500–509.
Shibahara Y, Matsubara H, Nozawa Y, Mori Y, Masaki H, Kosaki
A, Tsutsumi Y, Uchiyama Y, Fujiyama S, Nose A et al. 2001
Angiotensin II type 2 receptor inhibits epidermal growth factor
receptor transactivation by increasing association of SHP-1
Siraig H & Carey R 1996 The subtype-2 (AT2) angiotensin
receptor regulates renal cyclic guanosine 3',5'-monophosphate and
AT1 receptor-mediated prostaglandin E2 production in conscious rats.
Siraig HM & Carey RM 1997 The subtype-2 (AT2) angiotensin
receptor mediates renal production of nitric oxide in conscious rats.
Journal of Clinical Investigation 100 264–269.


Zhang J & Pratt RE 1996 The AT_2 receptor selectively associates with G_{as} and G_{ai} in the rat uterus. **Journal of Biological Chemistry** 271 15026–15033.


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