Review Article

The Angiotensin II Type 2 Receptor in Brain Functions: An Update

Marie-Odile Guimond and Nicole Gallo-Payet

Division of Endocrinology, Department of Medicine, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, Sherbrooke, QC, Canada J1H 5N4

Correspondence should be addressed to Nicole Gallo-Payet, nicole.gallo-payet@usherbrooke.ca

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Angiotensin II (Ang II) is the main active product of the renin-angiotensin system (RAS), mediating its action via two major receptors, namely, the Ang II type 1 (AT1) receptor and the type 2 (AT2) receptor. Recent results also implicate several other members of the renin-angiotensin system in various aspects of brain functions. The first aim of this paper is to summarize the current state of knowledge regarding the properties and signaling of the AT2 receptor, its expression in the brain, and its well-established effects. Secondly, we will highlight the potential role of the AT2 receptor in cognitive function, neurological disorders and in the regulation of appetite and the possible link with development of metabolic disorders. The potential utility of novel nonpeptide selective AT2 receptor ligands in clarifying potential roles of this receptor in physiology will also be discussed. If confirmed, these new pharmacological tools should help to improve impaired cognitive performance, not only through its action on brain microcirculation and inflammation, but also through more specific effects on neurons. However, the overall physiological relevance of the AT2 receptor in the brain must also consider the Ang IV/AT4 receptor.

1. Introduction

A major advance in the field of the renin-angiotensin system (RAS) was the discovery of a complete RAS in the brain, independent from the peripheral system, by Jacques Genest’s laboratory in Montreal in 1971 [1] (for reviews see [2–4]). Further studies by Mendelsohn et al. [5, 6] and Unger et al. [7] corroborated these observations, using biochemical, pharmacological, and autoradiographic approaches. It was found that brain levels of angiotensin II (Ang II) are higher than its circulating levels, suggesting independence of the two systems (review in [3, 4]). The various components of RAS (angiotensin-converting enzyme (ACE), Ang II and Ang II receptors) are all found in the adult brain in areas involved in the regulation of fluid and electrolyte balance, in the regulation of arterial pressure and vasopressin release, and regulation of the autonomic system [8, 9]. They are also present in structures involved in cognition, behavior, and locomotion. In particular, over the past 10 years, several advances have been made regarding the role of Ang II in various brain functions, including cerebroprotection, stress, depression, and memory consolidation [3, 4, 10–12]. Moreover, many evidences highlight the potential function of Ang II in the etiology of certain neurodegenerative diseases, including Alzheimer’s and Parkinson’s disease, seizures, and the development of metabolic syndrome and diabetes (review in [4, 13, 14]).

In the classical view, synthesis of Ang II begins with the conversion of angiotensinogen into Ang I by the enzyme renin. Ang I is then converted by ACE into Ang II (a.a. 1–8), which is then metabolized to Ang III (a.a. 2–8) and Ang IV (a.a. 3–8). Ang II and Ang IV can be further converted into Ang (1–7) and Ang (3–7) (Figure 1). Ang II binds to two main receptors, namely, the angiotensin type 1 (AT1) and type 2 (AT2) receptors, both belonging to the G-protein coupled receptor (GPCR) family [4, 12, 13]. Aside from this common link, these two receptors otherwise carry very little similarities. Indeed, their actions are generally opposite and while the AT1 receptor is expressed abundantly in several tissues, expression of AT2 receptor is limited to specific tissues and brain areas, where its concentration is generally low compared to the AT1 receptor. Most of the known effects of Ang II are due to activation of the AT1 receptor, including vasoconstriction, cellular growth, and proliferation. On the
Figure 1: Summary of the brain renin angiotensin system (RAS). The figure summarizes the conversion of angiotensinogen to angiotensins I and II through fragments. The biologically active forms include angiotensins II, III, IV, and (1–7). The main enzymatic pathways are mediated by renin and angiotensin converting enzyme ACE or ACE2, AP-N and AP-A. The major brain effects of angiotensins are mediated by AT1, AT2, AT4, prorenin, and Mas receptors. The functions associated with each receptor are indicated. ACE: angiotensin converting enzyme; AP-A: aminopeptidase A; AP-N: aminopeptidase N, adapted from Phillips and Oliveira, 2008 [3] and from Wright and Harding, 2012 [4].

other hand, it is generally assumed that the AT2 receptor counteracts the action of the AT1 receptor, promoting vasodilatation, apoptosis, and antigrowth effects. In addition to the classical AT1 and AT2 receptors, more recent studies have identified other receptors for RAS components, such as the (pro)renin receptor, the Ang (1–7) Mas receptor, and the Ang IV receptor (AT4 receptor, called IRAP for insulin-regulated aminopeptidase), all of which are expressed in the brain (for reviews, see [4, 12, 13]) (Figure 1). In particular, several studies have shown that Ang IV-AT4 receptor/IRAP have important functions in the brain, related to cognition and memory. In addition, in many situations, Ang IV acts as an inhibitor of AT1 receptor actions [15–17].

The present paper is focused on the known and suggested roles of the AT2 receptor in brain functions related to neuronal activities and cognitive disorders as well as the potential link between metabolic syndrome and cognitive functions. The role of the AT2 receptor in the central regulation of blood pressure, thirst and related pathologies, such as hypertension, stroke, or ischemic damage, will not be discussed here; these latter topics are covered in recent reviews (see [11, 18–20]). For further detail, readers are also invited to consult several excellent reviews describing recent up-to-date advances pertaining to the various active Ang II-derived ligands and their receptors [4, 12, 13] However, considering that there are some similarities between the AT2 receptor and the Ang IV/AT4 receptor, comparisons between the two will be included, when appropriate.

2. The Type 2 Receptor—A Nonclassical GPCR

Three important periods highlight the history of the AT2 receptor: (i) its discovery in the 1980s, (ii) its cloning in 1991–1993, and (iii) the generation of transgenic mice in 1995. However, because Ang II has a similar affinity for both of its receptors, it has been difficult to discriminate between the latter using nonselective ligands such as native Ang II or peptide analogs such as Saralasin or SarIle. At the end of 1980s, tools enabling to distinguish these Ang II receptors became available. The first tools included the nonpeptide antagonists losartan (previously known as DUP753) and PD123,177, and peptide ligands such as CGP42112A and p-aminophenylalanine (review in [2]). Seminal autoradiographic studies [5, 6], followed by biochemical studies by the groups of Marc de Gasparo, Peter Timmermans, and Robert Speth revealed the presence of two binding sites for Ang II, which differed in their expression pattern and biochemical properties [21–25]. However, the conclusive evidence for the existence of two receptors came from their cloning in the early 1990s [26, 27]. Despite the fact that both receptors belong to the large 7-transmembrane domain family of GPCR, AT1 and AT2 share only ~34% amino acid sequence identity. Moreover, while the AT2 receptor displays most of the structural features of a GPCR, it is usually considered as an atypical member of this family. Indeed, although some of its signaling pathways involve a G1-dependent mechanism, most of the known effects of the AT2
receptor are independent from G-protein coupling mechanisms (for reviews, see [14, 20, 28–31]). Moreover it fails to induce all of the classical signaling pathways such as cAMP, production of inositol triphosphate, or intracellular calcium release (for reviews, see [28, 29]).

2.1. Selective Ligands of the AT2 Receptor. In fact, AT2 receptor functions are still unclear both in many physiological and pathophysiological situations, mainly because research on this receptor has long been hampered by at least three challenges, namely, (i) the low and unusual expression of this receptor, (ii) its nonclassical signaling, and (iii) the absence of appropriate selective ligands. While several nonpeptide ligands with a large spectrum of selectivity for the AT1 receptor are readily available, only a few have been developed for the AT2 receptor. Until recently, physiological and pharmacological assessments of the AT2 receptors were obtained using either the agonist PD123,319 (a modification of the initial PD123,177) or the more common AT2 receptor agonist CGP42112A, although two other peptides have also shown agonistic properties on AT2 receptors, namely, the agonist p-aminophenylalanine [22] and novokin [32, 33]. However, the fact that CGP42112A is only a partial agonist, combined with its short half-life, has hampered its further use in in vivo studies (review in [20, 34, 35]). Thus, many hypotheses regarding AT2 receptor functions in physiological situations have emerged from indirect observations, either using transgenic animals, or blockade of the AT1 receptor. In 2004, Wan and collaborators synthesized the first selective nonpeptide AT2 receptor agonist, called compound 21 (C21) [36], now renamed M024 [37]. Since then, an increasing number of studies have used C21/M024 for both in vitro and in vivo studies (see further below) to better investigate the selective role of the AT2 receptor (for review, see [20, 34]).

3. Overview of the Signaling Pathways of the AT2 Receptor in the Brain

Signaling pathways associated with the AT2 receptor primarily involve a balance between phosphatase and kinase activity. The final outcomes vary according to whether the cell is undifferentiated or differentiated and whether angiotensin AT1 receptors are also expressed or not. Although there is still much controversy surrounding the effects of the AT2 receptor in peripheral systems, its role in physiological situations in the brain have gained much greater consensus [14, 31, 38].

3.1. Signaling Pathways of the AT2 Receptor Leading to Neurite Outgrowth. In neuronal models, studies on functionality and signaling have usually been conducted simultaneously. Most of the data, including our own findings using NG108-15 cells, indicate that the effects of the AT2 receptor on neurite outgrowth involve four main complementary signaling cascades (Figure 2). The first cascade entails a decrease in p21 Ras [39] and protein kinase Ca (PKCa) activities [40], both of which are involved in the switch from proliferation to differentiation. Simultaneously, a second cascade, initiated by Rap1/B-Raf, induces a delayed and sustained phosphorylation of p42/p44 mapk [39, 41]. In both NG108-15 [39] and PC12W [42] cells, this sustained activation is essential for inducing neurite outgrowth. The initial activation of Rap1 by the AT2 receptor is not direct, but rather mediated by phosphorylation of the tropomysin-related kinase receptor A (TrkA) [43], through the intervention of a Src family kinase member [44]. The third cascade comprises nitric oxide (NO) and cGMP. In NG108-15 cells, we [45] and others [46] have shown that external application of NO is sufficient to induce neurite outgrowth and elongation. In these cells, neuronal NO synthase (nNOS) activation and cGMP production induced by the AT2 receptor is dependent on the Ga protein. However, cGMP is not involved in Ang II-induced activation of p42/p44 mapk [45]. More recently, Li et al. [47] have shown that after AT2 receptor stimulation, the AT2 receptor interacting protein, ATIP [48], interacts with the tyrosine phosphatase SHP-1. The complex then translocates into the nucleus where it transactivates the ubiquitin-conjugating enzyme variants called methyl methanesulfonate sensitive 2 (MMS2), resulting in neural differentiation and protection (reviewed in [31, 49]). Finally, interaction of AT2 with certain receptor tyrosine kinases may also induce neurite outgrowth. For example, in a model of fructose-induced insulin-resistant rats, authors also demonstrated that neurite outgrowth of dorsal root ganglia (DRG) neurons induced by the AT2 receptor was facilitated by activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, suggesting the existence of a crosstalk between the AT2 receptor and the insulin receptor [50]. In addition, several phosphatases, such as PP2A [51–54] and Src homology region 2 domain-containing phosphatase-1 (SHP-1), are clearly associated with the mechanism of action of the AT2 receptor. Finally, certain pathways associated with AT2 receptor activation may be through interaction with protein partners such as the promyelocytic zinc finger (PLZF) protein [55, 56] or the peroxisome proliferator-activated receptor gamma (PPARy) [57] (for review, see [29, 31, 58–60]).

3.2. Ang-II-Independent Effects of the AT2 Receptor. It should be noted that while the main effects of AT1 and AT2 receptors are dependent of Ang II binding, some evidences also suggest that they may have certain ligand-independent effects (review in [29, 31]). For example, AT1 receptors can be activated by mechanical stress, independently from Ang II binding and/or stimulation of p42/p44 mapk [61, 62]. Similarly, AT2 receptor overexpression in CHO cells, R3T3 fibroblasts, and vascular smooth muscle cells enhances apoptosis signaling simply by its overexpression [63]. Another study also observed that the AT2 receptor, when expressed as a constitutive homooligomer, leads to G-protein dysfunction and symptoms of neurodegeneration without Ang II stimulation [64]. Although the mechanism underpinning this effect still requires further investigation, it appears that the C-terminal portion of the AT2 receptor is essential, since expression of a mutant AT2 receptor truncated in its C-terminal region is unable to form oligomers. Aside from its effect on cell survival, Ang II-independent effects of the AT2 receptor also include modulation of gene expression, at least in human coronary artery endothelial cells, where AT2 receptor overexpression modulates more genes than
CGP42112A stimulation [65]. Although these AT2-regulated genes are associated with many cellular functions, including cell migration, protein processing, intracellular signaling, and DNA repair, it is still unknown whether Ang II-independent effects of the AT2 receptor are associated with protective effects in neuronal function. Moreover, an Ang II-independent effect of the AT2 receptor was observed in a model overexpressing the receptor, and thus its relevance in physiological situations is still unknown. Clearly, many questions still remain to be elucidated in order to fully understand how the AT2 receptor exerts its effects on brain functions. However, new recent insights in AT2 receptor signaling have been achieved which could partly explain some of the observed discrepancies (see Section 8).

3.3. Signaling Pathways Associated with Ang IV and the AT4 Receptor. Interestingly, despite the high similarity between central biological effects associated with AT2 and AT4 receptor stimulation (memory processing, long-term potentiation facilitation, protective function in cognitive loss) (see Section 5), the signaling pathways induced by these two receptors are clearly different. In contrast with the AT2 receptor, the AT4 receptor is a single transmembrane receptor, initially known for its aminopeptidase activity, leading to peptide processing of oxytocin, vasopressin, Ang III, metenkephalin, somatostatin, and other neuropeptides (review in [4, 12]). Ang IV has also been shown to increase endothelial NO synthase activity via mobilization of intracellular calcium [66, 67] and to stimulate the PI3 K/PKB (protein kinase B) pathway [68] in endothelial cells. A recent study also showed that Ang IV treatment in diet-induced hyperglycemic mice increased the interaction between IRAP and PI3 K, leading to activation of Akt and the glucose transporter type-4 (GLUT4) translocation. This pathway was also associated with an improvement in glucose tolerance [69], suggesting that Ang IV may be implicated in insulin signaling and development of diabetes. Finally, in some studies, Ang IV was also found to elicit rapid activation of other selected kinases including ERK1/2, p38 kinase, focal adhesion kinase, and paxillin (review in [70]). Nevertheless, it appears that these signaling effects of Ang IV are model dependent, and none of these pathways have been observed in neuronal cells. Therefore, although renewed interest in Ang IV has recently emerged with the recognition that the Ang IV/IRAP/AT4 receptor plays an important role in cognition and pain [4, 12, 71], it is still unknown to date whether signaling pathways associated with Ang IV stimulation are implicated in these effects.

4. Expression and Roles of the AT2 Receptor in the Brain

All of the various RAS components in the brain, and in particular the AT2 receptor, are highly expressed during fetal life, suggesting that they could play key roles during development. On the other hand, in the adult, AT2 receptor expression in the brain is limited to certain specific areas (review in [72–75]). For instance, the AT2 receptor has been found at high levels in the medulla oblongata (control of autonomous functions), septum and amygdala (associated
with anxiety-like behavior), thalamus (sensory perception), and superior colliculus (control of eye movements in response to visual information) as well as in the subthalamic nucleus and cerebellum (areas associated with learning of motor functions). It is also expressed with the AT1 receptor in areas involved with cardiovascular functions, learning and behavior (cingulate cortex, molecular layer of the cerebellar cortex, superior colliculus, paraventricular nuclei hippocampus) (for extensive mapping, see [72, 73]). More recently, expression of the AT2 receptor was also detected in the substantia nigra pars compacta, the area involved in dopaminergic signals and associated with Parkinson’s disease [76], and in the hippocampus [64, 77]. At the cellular level, the AT2 receptor is expressed in neurons, but not in astrocytes [28, 72, 78]. Evidence also suggests that the AT2 receptor is expressed in the vasculature wall, where it acts on cerebral blood flow (review in [31, 49]). In addition, the presence of a non-AT1/non-AT2 receptor in the CNS has been suggested, which displays high affinity for Ang I, II, and III [79].

Interestingly, the Ang IV receptor IRAP is also observed in structures classically associated with cognitive processes and sensory and motor functions (including hippocampus, thalamic nuclei, caudate putamen, cerebellum, neocortex, lateral geniculate body, inferior olivary nucleus, superior colliculus, ventral tegmental area, and brain stem) (review in [12, 80, 81]). In contrast, the Ang (1–7) Mas receptor is expressed in brain areas involved in central cardiovascular regulation (in particular, the nucleus of the solitary tract (NTS), rostral ventrolateral medulla (RVLM), caudal ventrolateral medulla (CVLM), inferior olive, parvo- and magnocellular portions of the paraventricular nucleus (PVN), supraoptic nucleus, and lateral preoptic area) (review in [82]). In some instances, these areas also contain AT1 and AT2 receptors.

One of the first roles of the AT2 receptor to be identified was the modulation of neuronal excitability (review in [28, 83]). In cells of neuronal origin, activation of the AT2 receptor decreases activity of T-type calcium channels [84, 85] while stimulating a delayed-rectifier K+ current (IKr) and a transient K+ current (Ikur) [86]. Using primary cultures of cortical neurons, studies by Grammatopoulos et al. [87] have shown that II neuroprotection against chemical hypoxia was mediated by activation of a delayed rectifier K+ channel, an effect exemplified by simultaneous blockade of the AT1 receptor. Moreover, in preparations of locus coeruleus brain slices [88], angiotensin II, through the AT2 receptor, was found to depress glutamate depolarization and excitatory postsynaptic potentials. In the superior colliculus, both AT1 and AT2 receptors are involved in sensory visuomotor integration. Lastly, Ang II, through AT2 receptor stimulation with CGP42112A, has also been shown to induce a strong suppressive effect on visual neuronal activity. Together, these results indicate that AT2 receptor modulation of potassium and calcium channels activity may impact neuronal functions.

The second and best recognized effect of the AT2 receptor is stimulation of neurite outgrowth in various cell types from neuronal origin (NG108-15 and PC12W cells) as well as in primary cultures of neurons from retinal explants [89], in neurospheres from mouse fetal brain [90], cerebellar neuronal cells [91], and the cortex [47] (review in [3, 10, 14, 34]). This AT2-induced neurite elongation is characterized by an increase in mature neural cell markers, such as βIII-tubulin and MAP2 [47, 92]. It is also associated with a rise in neuronal migration [54, 91] and neuronal survival following ischemia-induced neuronal injury [93]. These effects may be important not only for developmental differentiation, but also after injury-induced regeneration. Indeed, a beneficial effect of the AT2 receptor in nerve regeneration has been observed following both optic [89] and sciatic [94] nerve crush or in perivascular nerves implicated in vasodilation [95]. This implication of the AT2 receptor in neuronal regeneration has even led to the suggestion that Ang II, via the AT2 receptor, could act as a neurotrophic factor. In summary, the AT2 receptor, by its function in the modulation of neuronal excitability, neurite elongation, migration, and nerve regeneration, may be an important factor in the regulation of central nervous system activity and cognitive function either following nerve injury or during the development of neurodegenerative disease (Figure 3).

In addition to neuronal differentiation, which is of paramount importance in nerve regeneration, the AT2 receptor also stimulates differentiation of hematopoietic cells, a key process during regeneration and reconstruction. Indeed, ischemic damage is characterized by infiltration of a number of hematopoietic cells such as leukocytes, platelets, macrophages, and leukocytes [96]. In particular, the AT2 receptor has the capacity to induce differentiation of human monocytes into dendritic cells [97]. Supporting a protective effect of the AT2 receptor is the observation that ischemic damages were found to be greater in mice with hematopoietic cells deleted in AT2 receptor expression [98]. These findings suggest that expression and activation of the AT2 receptor in hematopoietic cells may be part of its beneficial effect following brain injury, although the mechanism involved remains to be investigated (review in [99]).

Some of the initial hypotheses regarding the potential role of the AT2 receptor in vivo were confirmed in 1995 when two independent groups developed AT2 knockout mice by targeted gene deletion [100, 101]. Surprisingly, despite the fact that the AT2 receptor is highly expressed during fetal development in many tissues, including skin, kidney, brain, and heart, it appears that mice lacking the AT2 receptor do not exhibit any major anatomical defects. However, these mice exhibit markedly reduced exploratory behavior as well as altered thirst reaction, lower body temperature, slightly elevated mean arterial pressure, and a stronger vasoconstrictive response to Ang II [100–102]. Moreover, they exacerbate stronger symptoms when exposed to pathological situations. For example, these mice display an accelerated pathological response when exposed to cardiovascular disease induction. They also exhibit larger cerebral infarct size following medial cerebral artery occlusion (MCAO) [93], stronger cognitive deficits following ischemia [103], and faster progression of atherosclerosis [104]. The various actions of the AT2 receptor currently documented in the brain are summarized in Figure 3.
5. Role of the AT₂ Receptor in Cognitive Function and Neurological Disorders

The first evidence for the implication of the AT₂ receptor in cognition resulted from studies in knockout mice. Indeed, AT₂-deficient mice suffer from perturbations in exploratory behavior and locomotor activity [100, 101], as well as displaying an anxiety-like behavior [105]. Moreover, in the adult, inhibition of the AT₁ receptor with the AT₂ receptor antagonist PD123,319 has been reported to abolish the Ang II-induced acquisition of conditioned avoidance responses [106]. These results strongly support that, in addition to a role during development, the AT₂ receptor may be involved in cognitive processes in the adult. Furthermore, although the AT₁ receptor is expressed at low levels in many areas of the nervous system, it may be reexpressed in certain pathological conditions such as optic [89] or sciatic [94] nerve transection, stroke [107], and certain neurodegenerative diseases such as Alzheimer’s disease [108]. For example, Ge and Barnes [108] found that AT₂ receptor expression is diminished in Parkinson’s disease (caudate nucleus and cerebellum) but enhanced in Huntington’s disease (caudate nucleus). In Alzheimer’s disease, the temporal cortex of the adult brain exhibits an increased expression while the hippocampus displays a decreased expression of the AT₂ receptor. In most of these situations, the AT₂ receptor is described as having beneficial effects in improving neuroprotection by acting not only on neurons, but also on blood circulation. Indeed, recent studies have clearly shown a protective role of the AT₂ receptor following brain ischemia and demonstrated that expression and activation of the AT₂ receptor may decrease brain damage and restore cognitive loss following middle cerebral artery occlusion (for review, see [11, 14, 49]). Altogether, these data suggest that the AT₂ receptor may play an important role in maintaining functions of the human brain.

5.1. AGTR2 Mutations in Intellectual Disability. Intellectual disability, previously described as mental retardation [109], affects approximately 1–3% of the population, of which a large number are associated with mutations on chromosome X. Among these, certain mutations on AGTR2 coding for the angiotensin AT₂ receptor have been identified. Mutations in the AGTR2 gene correlate with the development of human X-linked intellectual disability [110]. Indeed, 9 patients with X-linked intellectual disability were shown to have 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. Clinical features of these mutations ranged from moderate to severe intellectual disability, seizure, and manifestations of autism, thus supporting the hypothesis that the AT₂ receptor is required for brain development and for the maintenance of neuronal connections involved in learning and memory. This hypothesis was further corroborated by two other studies reporting mutations of AGTR2 in patients suffering from intellectual
disability, seizures, restlessness, hyperactivity, and disrupted speech development [111, 112]. However, other studies failed to link any AGTR2 mutations with intellectual disability, observing no difference in mutation incidence between the latter and control groups [113–115]. Differences among control cohort selection, number of patients per group, and ethnical variations between different studies could explain the discrepancies in these findings. Hence, it remains unclear whether mutations in AGTR2 are associated or not with intellectual disability.

5.2. AT2 Receptor in Alzheimer's Disease (AD). Amyloid-β (Aβ) deposition in senile plaques and the presence of neurofibrillary tangles are the main pathological hallmarks of AD. However, other structural and functional alterations, including inflammation, increased oxidative stress and vascular damage/ischemia, are also associated with AD and other neurodegenerative diseases. These alterations may contribute to neuronal and synaptic dysfunction and loss, as well as the ensuing cognitive deficits and dementia of this disorder [116–121] (for recent review, see [11, 14, 49]). Clinical studies have documented that treatment with antihypertensive drugs is associated with an improvement in cognitive function (review in [112–114]). More recent studies have shown that treatment with angiotensin II receptor blockers (ARBs) is associated with a decrease in AD and dementia progression with a greater efficacy compared to ACE inhibitors [125, 126]. In particular, Tsukada et al. [127] have shown that cognitive deficit induced by Aβ (1–42) in mice was improved by pretreatment with a low dose of telmisartan partly because of peroxisome proliferator-activated receptor-gamma (PPARγ) activation. Corroborating the hypothesis that ARBs could be beneficial in reducing the onset of AD, Kume et al. [128] recently observed that AD hypertensive patients treated with telmisartan presented no decrease in cognitive functions test scores, but an increased cerebral blood flow, suggesting that treatment with this ARB could reduce AD progression. Altogether, studies conducted using various models of cognitive disorders have reported improved memory and cognitive processes and/or attenuation of Aβ1–42 oligomerization following treatment with ARBs, particularly valsartan [129], losartan [130], telmisartan [128, 131], and olmesartan [132] (now called metabosartans for ARBs with a PPARγ agonistic effect) (review in [11, 14, 49, 133]). More recently, a study using direct stimulation of the AT2 receptor with the selective agonist C21/M024 demonstrated similar effects in an AD mouse model [134]. In this latter study, Jing et al. observed that intracerebroventricular injections of Aβ (1–40) in mice induced a poorer performance in the Morris water maze and that this effect was reversed by coadministration of C21/M024, indicating that direct stimulation of AT2 receptors improves spatial memory functions. Stroke is also one of the most important causes of cognitive impairment and dementia (review in [11, 49]). There is increasing evidence suggesting that activation of the AT2 receptor could improve cerebral blood flow and microcirculation as well as decrease inflammation [93, 103, 135, 136], both being associated with improvement in cognitive function following cerebral ischemia [90, 93, 103, 137] (for reviews, see [3, 10, 14, 49]). As reported by Iwai et al. [103] and summarized by Horiuchi and Mogi [49] and Mogi and Horiuchi [11], superoxide anion production was found to be more markedly enhanced in AT2 receptor-deficient mice compared to wild-type animals in a model of MCAO. These observations suggest that AT2 receptor stimulation has a protective effect on ischemic brain lesions, at least partly through modulation of cerebral blood flow and superoxide production. Moreover, beneficial effects of ARBs on these parameters were less evident in AT2 receptor-deficient mice [103]. Similar approaches were also used in other studies suggesting a beneficial effect of the AT2 receptor on cognitive functions [90, 135, 136].

However, not only blood circulation but also neuronal functions can be improved by activation of AT2 receptors. Indeed, activation of AT2 receptors in neurons is also associated with a decrease in apoptosis signaling. Grammatopoulos et al. demonstrated in cultures of primary cortical neurons that angiotensin decreased sodium azide-induced apoptosis through AT2 receptor activation [138] by reducing caspase-3 activation [139]. Finally, we and others have shown that the AT2 receptor can activate the tyrosine kinase Fyn [44] and the phosphatase PP2A [51, 52, 54], both of which are key regulators of the phosphorylation of the microtubule associated protein tau. Hyperphosphorylation of tau is of paramount importance in the development of Alzheimer’s disease by forming neurofibrillary tangles and leading to microtubule depolymerization. Thus, AT2 receptor activation may participate to the control of equilibrium between tau phosphorylation and dephosphorylation.

Another key observation is the effect of estrogen receptors on AT2 receptor functions. In 2008, Chakrabarty et al. [140] demonstrated that estrogen, through its E2 receptor, induced neurite elongation of dorsal root ganglion and that this effect was dependent on AT2 receptor activation. Moreover, it has been observed that ischemic damage in AT2-deficient mice was greater in females than in males, while no significant sex-different change was observed in AT1-expressing mice [135]. These results suggest that the existence of some level of crosstalk between AT2 receptor and estrogen and that AT2 receptor could be necessary for E2 receptors to elicit its full effect on neuronal physiology. Thus, while stimulation of the AT2 receptor and/or inhibition of the AT1 receptor could lead to potential therapeutic avenues in neurodegenerative disease, the interaction between AT2 receptor and estrogen should also be considered. Indeed, there are some differences in response to RAS stimulation according to gender (review in [141]). Nonetheless, the clear demonstration of an AT2 receptor effect in Alzheimer’s disease remains to be firmly demonstrated, mainly because in most studies to date the presence of AT1 receptors and AT2 receptors in the hippocampus has not been studied. Indeed, to our knowledge, only two studies have documented the presence of AT2 receptors in the hippocampus, one in a model of Alzheimer’s disease [64], the other in a model of epilepsy [77]. On the other hand, presence of the AT2 receptor was not detected in the hippocampus in various studies from the Llorens-Cortes group [72, 73]. However, these studies were performed with healthy and middle-aged animals.
It should be mentioned that, in addition to the AT₂ receptor, the Ang IV/AT₄ receptor may also have a protective effect on cognitive function. Indeed, Braszkó’s group was the first to report that intracerebroventricular injections of Ang II and Ang IV were equivalent in facilitating exploratory behavior in rats tested in an open field and improved recall of passive avoidance conditioning [142, 143], results that were confirmed by others in subsequent studies (review in [71]). This strongly suggests an important function of Ang IV and its receptor in learning and memory processes and could represent a new therapeutic target in the treatment of memory loss associated with dementia (review in [71]). One mechanism proposed to explain this beneficial effect of Ang IV on cognitive function is the colocalization of IRAP with the glucose transporter GLUT4. In hippocampal pyramidal neurons, IRAP and GLUT4 are localized in secretory vesicles responsive to insulin (review in [70, 71, 80]), suggesting that Ang IV, by binding to IRAP, may increase GLUT4 membrane expression thus facilitating glucose uptake, as observed in adipocytes [144] (review in [145]).

5.3. AT₂ Receptor and Parkinson’s Disease. In addition to AD and stroke, some evidences also suggest that central RAS could be implicated in the development of Parkinson’s disease. Parkinson’s is the second most common neurodegenerative disorder and is characterized by the progressive cell death of midbrain dopaminergic neurons in the substantia nigra and the presence of protein inclusions leading to formation of Lewy bodies (review in [146]). Although the mechanisms leading to Parkinson’s disease are still unclear, it appears that mitochondrial dysfunction, oxidative stress, and inflammation are key factors to its progression [147]. Recently, ARBs have been shown to reduce lipid peroxidation and protein oxidation while protecting dopaminergic neurons in the substantia nigra in a rat model of Parkinson’s disease [76, 148]. However, whether such action is due solely to a blockade of the AT₁ receptor or also from activation of the AT₂ receptor is not yet clearly established (review in [147]). Activation of the AT₂ receptor is able to stimulate differentiation of mesencephalic precursor cells into dopaminergic neurons, suggesting that stimulation of the AT₂ receptor could be useful in increasing the production of dopaminergic neurons in Parkinson’s disease [149]. Moreover, Grammatopoulos et al. observed a protection against rotenone-induced oxidative stress and associated cell death in dopaminergic neurons following Ang II stimulation. This protective effect was prevented by the presence of the AT₂ receptor antagonist PD123,319, but was increased in the presence of the AT₁ receptor antagonist losartan [150]. More recently, it has been observed that during the aging process in rats, AT₂ receptor expression is decreased in dopaminergic neurons, as opposed to an increase in AT₁ receptor expression. This was associated with an enhancement of prooxidative and proinflammatory markers in the substantia nigra, leading to an increase in dopaminergic neuronal death [151]. Although these findings do not indicate a role of AT₂ receptor in the development of Parkinson’s disease, they strongly suggest that such modifications in Ang II receptors during the natural aging process may increase the risk of Parkinson’s disease.

6. Role of the AT₂ Receptor in the Regulation of Appetite

Obesity, which is characterized by excess body fat accumulation [152], is associated with an increased risk of diabetes, hypertension, and dyslipidemia. It is also one of the major components of the metabolic syndrome. Studies have demonstrated AT₂ receptor expression in tissues associated with glucose metabolism, including pancreatic [153–155] and adipose tissues [13, 30]. For example, expression of the AT₂ receptor in the pancreas has been shown to be important for fetal pancreatic development [156] while in the adult, it is associated with protection against pancreatic fibrosis [157] and a decrease in pancreatic tumor growth [158, 159]. The following is a summary of what is currently known regarding the potential function of the AT₂ receptor in the regulation of appetite, glucose metabolism, and its potential role in metabolic syndrome.

There are some evidences suggesting that Ang II could be implicated in food intake: for example, Ang II suppresses food intake after central infusion [32, 160, 161] while blockade of the AT₁ receptor by telmisartan is associated with a decrease in body weight [162]. Furthermore, both AT₁ and AT₂ receptors are expressed in the hypothalamus, which is implicated in the central regulation of food intake. In 2008, Ohinata et al. [32] observed that the decrease in food intake induced by centrally administrated Ang II was inhibited by PD123,319, and absent in AT₁-KO mice, suggesting that this effect was mediated by the AT₂ receptor. A similar effect was observed using novokin, a potent analog of ovoquin with AT₂ receptor agonistic properties [163]. Although the mechanism underlying this AT₂ receptor-associated decrease in food intake remains unclear, it may be linked to its capacity to modulate T-type calcium channels, since a recent study demonstrated that inhibition of these channels inhibits weight gain in mice fed with a high-fat diet [164]. However, this hypothesis remains to be explored. Studies conducted to date with the selective AT₂ agonist C21/M024 do not describe such differences between C21/M024-treated animals compared to the control group [165, 166], suggesting that the duration of C21/M024 treatment may have been too short to induce any modification in body weight. Thus, in this latter instance, even if the AT₂ receptor was observed to decrease food intake, it was probably not sufficient to induce a decrease in body weight, at least following short periods of stimulation (<8 weeks). Moreover, it has also been observed that inhibition of ACE by captopril also reduced body weight of mice fed with a high-fat diet [167]. This decrease in body weight, however, was not associated with a decrease in food intake, suggesting that other mechanisms were regulated by Ang II. Since Ang II is no longer available in captopril-treated mice, these results suggest that other members of the RAS, independent of Ang II, could be implicated in the regulation of food intake and body weight. These observations should therefore be considered when interpreting results obtained with ARBs.
7. Link between Metabolic Syndrome and Alzheimer’s Disease: Is There a Place for the AT₂ Receptor?

A number of excellent reviews have recently been published regarding the potential link between insulin resistance, metabolic syndrome, and neurodegenerative disorders (both AD and vascular dementia) [168–177], including the involvement of RAS in this process [90, 133, 178]. Development of AD is closely associated with a reduction in cerebral glucose utilization, even in the early stages of the disease. In fact, cerebral metabolism in the AD brain decreases prior to the onset of cognitive decline, suggesting that energy failure could represent one of the earliest hallmarks of AD. Induction of insulin resistance in AD animal models aggravate both amyloid and tau accumulation [179, 180], leading several investigators to refer to Alzheimer’s disease as type 3 diabetes (review in [168]). One aspect of this relationship is the loss of insulin signaling in the insulin-resistant brain. In addition to the many peripheral complications associated with dysfunction in insulin sensitivity, it appears that brain insulin signaling plays crucial central functions in the regulation of energy balance (food intake, body weight) as well as in learning and memory (review in [171]). Moreover, inflammation, increase in oxidative stress, and mitochondrial dysfunctions are key features of type 2 diabetes (T2D) that are also shared in Alzheimer’s disease.

In T2D patients, results of a major clinical study (Study on Cognition and Prognosis in the Elderly, SCOPE) [181] and a clinical double-blind study [182] have revealed that ARBs have a further therapeutic effect on impaired cognitive function beyond their antihypertensive effects compared with other antihypertensive drugs. Similarly, Tsukuda et al. [131, 183] have demonstrated that candesartan improves impaired cognitive function induced by T2D, with multiple beneficial effects. Two of these effects may be through PPARγ or through AT₂ receptor activation. Therefore, improvement of metabolic syndrome may also be beneficial in decreasing associated cognitive decline. This would contribute to better insulin signaling in the brain and, therefore, a slowing of cognitive decline associated with brain insulin resistance.

8. New Insights in AT2 Receptor Knowledge and Perspectives: What Remains to Be Done?

As pointed out recently [18, 20], there is still an ongoing debate as to the putative role of the AT₂ receptor in physiology, and whether this role is deleterious or beneficial. Summarized below are some of the new advances in AT₂ receptor signaling that could have important insights in AT₂ receptor-associated brain functions.

8.1. Homo- and Heterodimerization. Although GPCRs have traditionally been thought to act as monomers (review in [184]), it is now well accepted that many GPCRs can form dimers which could affect both their trafficking and function. In this context, homodimerization of AT₁ and AT₂ receptors, as well as AT₁/AT₂ heterodimer formations, has been reported. For example, the AT₂ receptor is known to undergo homodimerization, a property which enhances apoptosis [185]. In addition, AbdAlla et al. [186] reported that the AT₂ receptor undergoes heterodimerization with the AT₁ receptor in transfected PC12 cells, in fetal fibroblasts, and in myometrial biopsies. In an animal model of Alzheimer’s disease, the same group demonstrated that Aβ induces the formation of cross-linked AT₁ receptor oligomers [64, 187]. Notably, oligomers of AT₁ receptors have also been observed in prefrontal cortex specimens of Alzheimer’s disease patients, while being completely absent in specimens of nondemented control individuals, thus lending further support for a role of the AT₂ receptor in cognitive function. Heterodimerization between the AT₂ receptor and bradykinin has also been described in PC12W cells [188]. It is already known that bradykinin mediates AT₂ receptor-induced NO production [189–191]. This interaction between the two receptors has been shown to enhance phosphorylation of various kinases, including p42/p44 MAPK and p38 MAPK. Therefore, it would appear that homo- and heterodimerization of the AT₂ receptor may have a role in its regulation. However, these initial observations still require confirmation before being accepted as important regulatory aspects of Ang II receptor signaling and functions (review in [29, 31]). The use of recently developed methodologies such as FRET/BRET technology has confirmed efficient heterodimerization of the AT₁ receptor with the bradykinin receptor B₂ [192], indicating the potential clinical significance of GPCR oligomerization [29, 31]. Moreover, recent studies have identified intracellular crosstalk pathways between the AT₁ receptor and the AT₂ receptor at the gene expression level. Indeed, AT₁ receptor activation enhances AT₂ receptor mRNA degradation, while AT₂ receptor activation increases its own mRNA transcription [193].

8.2. PPARγ: Could It Be the Missing Link? There is existing confusion regarding the mechanism of action of specific ARBs, since some also have partial PPARγ agonistic activity (such as telmisartan, irbesartan, and candesartan). There is some evidence suggesting that this PPARγ activation following blockade of the AT₁ receptor could be part of its anti-inflammatory and antioxidative effects, leading to neuroprotection against ischemia and Aβ accumulation [127, 194, 195]. Indeed, neuroprotective effects of PPARγ agonists, such as pioglitazone, have been observed during neural cell differentiation and death, and in inflammatory and neurodegenerative conditions, including amyotrophic lateral sclerosis, Alzheimer’s disease and Parkinson’s disease models, as well as stroke [196, 197]. PPARγ is a transcriptional factor regulating the expression of multiple genes, thereby promoting the differentiation and development of various tissues, specifically adipose tissue, brain, placenta, and skin (review in [197]). In addition, certain studies have indicated that AT₂ receptor stimulation increases PPARγ expression and transcriptional activity, at least in PC2W cells [57] and neurons [131]. The final targets of these pathways are gene expression and phosphorylation of various microtubule-associated proteins, which modulate microtubule stability/dynamics responsible for neurite elongation. This observation is noteworthy, especially with regard to
the implication of PPARγ in NGF-induced neurite outgrowth [198] which clearly suggests a possible crosstalk between the AT2 receptor and NGF pathways. This hypothesis is furthermore reinforced by the observation that inhibition of the NGF receptor TrkA significantly decreases AT2 receptor-induced neurite outgrowth [43]. Moreover, Iwai et al., using atherosclerotic ApoE-KO mice with an AT2 receptor deficiency (AT2R/ApoE double knockout mice), observed that the lack of AT2 receptor expression decreased the expression of PPARγ in adipocytes [199]. These observations strongly suggest a link between the AT2 receptor and PPARγ functions. Considering that similar neuroprotective effects were also associated with the AT2 receptor, it may be hypothesized that activation of PPARγ could be shared both by ARBs and AT2 receptor stimulation.

9. Could the AT2 Receptor Be an Attractive Therapeutic Target?

One of the biggest challenges in studying the AT2 receptor is applying observations stemming from the use of cell lines to in vivo models. Indeed, studies using cell lines expressing the AT2 receptor either endogenously or via transfection have provided paramount information regarding its intracellular mechanisms of action. However, associating these mechanisms with biological functions has proven to be much more difficult. As indicated previously, synthesis and characterization of the selective AT2 receptor selective agonist C21/M024 in 2004 or of the recently developed antagonist [200] has provided long-awaited tools to bypass the difficulty of using traditional AT2 receptor ligands such as CGP42112A or PD123,319. Since then, many studies have allowed significant advances in the understanding of AT2 receptor functions. Nonetheless, one would have thought that this new AT2 receptor ligand would have resolved certain controversies surrounding this enigmatic receptor. However, eight years after the first characterization of this compound, the clear demonstration of an AT2 receptor effect in the brain remains to be unequivocally established. One major input since C21/M024 was first described is that selective stimulation of the AT2 receptor does not decrease blood pressure [134, 165, 166, 201–206]. These results are quite surprising, considering previous reports emanating from the indirect in vivo manipulation studies which associated AT2 receptor activation with vasodilation and a decrease in mean arterial pressure. However, blockade of the AT1 receptor with ARBs not only allows stimulation of the AT2 receptor, the only Ang II receptor available in this condition, but also increases the bioavailability of Ang II for ACE2 and aminopeptidase to produce Ang IV and Ang (1–7), both of which exert vasodilatory effects (Figure 1). Nevertheless, beyond its blood-pressure lowering effects, in vivo studies using C21/M024 have described a protective role of the AT2 receptor in vascular remodeling [166, 207], in poststroke cardiac [208] and renal function [165, 205] as well as in cognitive functions [134]. Furthermore, observation of AT2 receptors in tissues associated with glucose metabolism, such as the pancreas and adipose tissue, suggests that the AT2 receptor could also be beneficial in metabolic syndrome and associated cognitive loss. However, the selectivity of C21/M024 for the AT2 receptor has also been recently challenged and differs according to the dosage used and/or route of administration and, importantly, according to experimental conditions. In particular, a recent observation by Verdonk et al. [209], whereby C21/M024 induced vasorelaxation of preconstricted iliac arteries from SHR Wistar rats and C57BL/6 mice as well as in AT1-deficient mice, has rekindled the debate on the relevance of the AT2 receptor and the selectivity of AT2 receptor ligands in physiological functions. Moreover, this effect of C21/M024 in arteries was only partially blocked by PD123,319. These latest findings raise the possibility that C21/M024 could exhibit certain AT2 receptor-independent effects. Therefore, the question still remains: is the AT2 receptor a potential therapeutic target and could it replace or increase beneficial effects associated with ARBs?

10. Conclusion

As described in the aforementioned sections, AT2 receptor activation may act at several stages in the cascade of alterations leading to cognitive impairment and neuronal dysfunction. An increasing number of studies suggest that the protective effects of ARBs on brain damage and cognition may result not only from the inhibition of AT1 receptor effects, but also from the beneficial effect due to unopposed activation of the AT2 receptor. In addition, the relationship between impaired energy metabolism/obesity/insulin resistance and the increased risk of dementia emphasizes the view that the mechanisms of action of the AT2 receptor may have a beneficial protective effect. However, the physiological relevance of the AT2 receptor in the brain will need to be compared with at least two other components of RAS, namely, the ACE2/Ang-(1–7)/Mas complex and Ang IV/AT4 receptor/IRAP in the brain (Figure 1).

Lifestyle-related disorders, such as hypertension, T2D, and obesity, are also implicated as risk factors for dementia. In this regard, two recent publications have established that direct AT2 receptor stimulation with C21/M024 improves insulin sensitivity in a rat model of diet-induced insulin resistance [210] and in type 2 diabetic mice [211]. Thus, any treatment aimed at improving insulin resistance or cognitive functions is likely to slow down symptoms and improve quality of life associated with these age-related disorders. Furthermore, the recent development of selective AT2 receptor agonists should facilitate efforts to elucidate distinct roles of the AT2 receptor in brain physiology, supporting or disproving the hypothesis that the AT2 receptor helps improve a number of brain impairments related to neuronal plasticity and morphology, microcirculation and inflammation (Figure 3), all of which are altered in certain neurological disorders.

There is clearly still much work to be accomplished to fully understand the role of the AT2 receptor in normal versus pathological conditions and to determine whether AT2 receptor agonists could represent an attractive therapeutic target. In this aspect, compounds such as C21/M024 as well as other recently synthesized highly selective nonpeptide AT2 receptor ligands (all leading to neurite outgrowth in NG108-15 cells) and their effectiveness to induce AT2
receptor-dependent effects need to be further explored [212–216].

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References

[1] D. Ganten, A. Marquez-Juilo, P. Granger et al., “Renin in dog brain,” The American Journal of Physiology, vol. 221, no. 6, pp. 1733–1737, 1971.
[2] M. de Gasparo, K. J. Catt, T. Inagami, J. W. Wright, and T. Unger, “International union of pharmacology. XXIII. The angiotensin II receptors,” Pharmacological Reviews, vol. 52, no. 3, pp. 415–472, 2000.
[3] M. I. Phillips and E. M. de Oliveira, “Brain renin angiotensin in disease,” Journal of Molecular Medicine, vol. 86, no. 6, pp. 715–722, 2008.
[4] J. W. Wright and J. W. Harding, “The brain renin-angiotensin system: a diversity of functions and implications for CNS diseases,” Pfliigers Archiv. In press.
[5] F. A. Mendelsohn, R. Quirion, J. M. Saavedra, G. Aguilera, and K. J. Catt, “Autoradiographic localization of angiotensin II receptors in rat brain,” Proceedings of the National Academy of Sciences of the United States of America, vol. 81, no. 5, pp. 1575–1579, 1984.
[6] F. A. O. Mendelsohn, A. M. Allen, J. Clevers, D. A. Denton, E. Tarjan, and M. J. McKinley, “Localization of angiotensin II receptor binding in rabbit brain by in vitro autoradiography,” Journal of Comparative Neurology, vol. 270, no. 3, pp. 372–384, 1988.
[7] T. Unger, E. Badoer, D. Ganten, R. E. Lang, and R. Rettig, “Brain angiotensin: pathways and pharmacology,” Circulation, vol. 77, no. 6, pp. 40–54, 1988.
[8] W. B. Severs and A. E. Daniels-Severs, “Effects of angiotensin on the central nervous system,” Pharmacological Reviews, vol. 25, no. 3, pp. 415–449, 1973.
[9] M. I. Phillips, “Functions of angiotensin in the central nervous system,” Annual Review of Physiology, vol. 49, pp. 413–435, 1987.
[10] M. Horiiuchi, M. Mogi, and M. Iwai, “The angiotensin II type 2 receptor in the brain,” Journal of the Renin-Angiotensin-Aldosterone System, vol. 11, no. 1, pp. 1–6, 2010.
[11] M. Mogi and M. Horiiuchi, “Effect of angiotensin II type 2 receptor on stroke, cognitive impairment and neurodegenerative diseases,” Geriatrics & Gerontology International. In press.
[12] J. W. Wright and J. W. Harding, “Brain renin-angiotensin—a new look at an old system,” Progress in Neurobiology, vol. 95, no. 1, pp. 49–67, 2011.
[13] A. D. de Kloet, E. G. Krause, and S. C. Woods, “The renin angiotensin system and the metabolic syndrome,” Physiology and Behavior, vol. 100, no. 5, pp. 525–534, 2010.
[14] N. Gallo-Payet, M. O. Guimond, L. Bilodeau, C. Wallinder, M. Alterman, and A. Hallberg, “Angiotensin II, a neuropeptide at the frontier between endocrinology and neuroscience: is there a link between the angiotensin II type 2 receptor (AT(R)R) and Alzheimer’s disease?” Frontiers in Endocrinology, vol. 2, article 17, pp. 1–10, 2011.
[15] R. Yang, I. Smolders, D. De Bundel et al., “Brain and peripheral angiotensin II type 1 receptors mediate renal vasoconstrictor and blood pressure responses to angiotensin IV in the rat,” Journal of Hypertension, vol. 26, no. 5, pp. 998–1007, 2008.
[16] J. W. Wright, A. V. Miller-Wing, M. J. Shaffer et al., “Angiotensin II(3-8) (ANG IV) hippocampal binding: potential role in the facilitation of memory,” Brain Research Bulletin, vol. 32, no. 5, pp. 497–502, 1993.
[17] J. W. Wright, A. J. Bechtoldt, S. L. Chambers, and J. W. Harding, “Angiotensin III and IV activation of the brain AT(1) receptor subtype in cardiovascular function,” Peptides, vol. 17, no. 8, pp. 1365–1371, 1996.
[18] U. M. Steckelings, L. Paulis, P. Namsolleck, and T. Unger, “AT(2) receptor agonists: hypertension and beyond,” Current Opinion in Nephrology and Hypertension, vol. 21, pp. 142–146, 2012.
[19] U. M. Steckelings, F. Rompe, E. Kaschina, and T. Unger, “The evolving story of the RAAS in hypertension, diabetes and CV disease—moving from macrovascular to microvascular targets,” Fundamental and Clinical Pharmacology, vol. 23, no. 6, pp. 693–703, 2009.
[20] K. Verdonk, A. H. Danser, and J. H. van Esch, “Angiotensin II type 2 receptor agonists: where should they be applied?” Expert Opinion on Investigational Drugs, vol. 21, no. 4, pp. 501–513, 2012.
[21] S. Whitebread, M. Mele, B. Kamber, and M. De Gasparo, “Preliminary biochemical characterization of two angiotensin II receptor subtypes,” Biochemical and Biophysical Research Communications, vol. 163, no. 1, pp. 284–291, 1989.
[22] R. C. Speth and K. H. Kim, “Discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II, p-aminophenylalanine6 angiotensin II,” Biochemical and Biophysical Research Communications, vol. 169, no. 3, pp. 997–1006, 1990.
[23] A. T. Chiu, W. F. Herblin, D. E. McCall et al., “Identification of angiotensin II receptor subtypes,” Biochemical and Biophysical Research Communications, vol. 165, no. 1, pp. 196–203, 1989.
[24] A. T. Chiu, D. E. McCall, T. T. Nguyen et al., “Discrimination of angiotensin II receptor subtypes by dithiothreitol,” European Journal of Pharmacology, vol. 170, no. 1-2, pp. 117–118, 1989.
[25] R. C. Speth, B. P. Rowe, K. L. Grove, M. R. Carter, and D. Saylor, “Sulphydryl reducing agents distinguish two subtypes of angiotensin II receptors in the rat brain,” Brain Research, vol. 548, no. 1-2, pp. 1–8, 1991.
[26] M. Nakajima, M. Mukoyama, R. E. Pratt, M. Horiiuchi, and V. J. Dzau, “Cloning of cDNA and analysis of the gene for mouse angiotensin II type 2 receptor,” Biochemical and Biophysical Research Communications, vol. 197, no. 2, pp. 393–399, 1993.
[27] Y. Kambayashi, S. Bardhan, K. Takahashi et al., “Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition,” Journal of Biological Chemistry, vol. 268, no. 33, pp. 24543–24546, 1993.
[28] L. Gendron, M. D. Payet, and N. Gallo-Payet, “The angiotensin type 2 receptor of angiotensin II and neuronal differentiation: from observations to mechanisms,” Journal of Molecular Endocrinology, vol. 31, no. 3, pp. 359–372, 2003.

[29] E. R. Porrello, L. M. D. Delbridge, and W. G. Thomas, “The angiotensin II type 2 (AT2) receptor: an enigmatic seven transmembrane receptor,” Frontiers in Bioscience, vol. 14, no. 3, pp. 958–972, 2009.

[30] N. Gallo-Payet, M. Shum, J. P. Baillargeron et al., “AT2 receptor agonists: exploiting the beneficial arm of Ang II signaling,” Current Hypertension Reviews, vol. 8, pp. 47–59, 2012.

[31] M. Horiuchi, J. Iwanami, and M. Mogi, “Regulation of angiotensin II receptors beyond the classical pathway,” Clinical Science, vol. 123, no. 4, pp. 193–203, 2012.

[32] K. Ohinata, Y. Fujiwara, S. Fukumoto, M. Iwai, M. Horii, and M. Yoshikawa, “Angiotensin II and III suppress food intake via angiotensin AT1 receptor and prostaglandin EP4 receptor in mice,” FEBS Letters, vol. 582, no. 5, pp. 773–777, 2008.

[33] Y. Yamada, D. Yamauchi, H. Usui et al., “Hypotensive activity of novokinin, a potent analogue of ovokinin(2–7), is mediated by angiotensin AT1 receptor and prostaglandin IP receptor,” Peptides, vol. 29, no. 3, pp. 412–418, 2008.

[34] U. M. Steckelings, F. Rompe, E. Kaschina et al., “The past, present and future of angiotensin II type 2 receptor stimulation,” Journal of the Renin-Angiotensin-Aldosterone System, vol. 11, no. 1, pp. 67–73, 2010.

[35] T. Unger and B. Dahlof, “Compound 21, the first orally active, selective agonist of the angiotensin type 2 receptor (AT2): implications for AT1 receptor research and therapeutic potential,” Journal of the Renin-Angiotensin-Aldosterone System, vol. 11, no. 1, pp. 75–77, 2010.

[36] Y. Wan, C. Wallinder, B. Plouffe et al., “Design, synthesis, and biological evaluation, of the first selective nonpeptide AT2 receptor agonist,” Journal of Medicinal Chemistry, vol. 47, no. 24, pp. 5995–6008, 2004.

[37] J. Georgsson, C. Skold, M. Botros et al., “Synthesis of a new class of druglike angiotensin II C-terminal mimics with affinity for the AT2 receptor,” Journal of Medicinal Chemistry, vol. 50, no. 7, pp. 1711–1715, 2007.

[38] U. M. Steckelings, E. Kaschina, and T. Unger, “The AT2 receptor—a matter of love and hate,” Peptides, vol. 26, no. 8, pp. 1401–1409, 2005.

[39] L. Gendron, L. Lafamme, N. Rivard, C. Asselin, M. D. Payet, and N. Gallo-Payet, “Signals from the AT2 (angiotensin type 2) receptor of angiotensin II inhibit p21ras and activate MAPK (mitogen-activated protein kinase) to induce morphological neuronal differentiation in NG108–15 cells,” Molecular Endocrinology, vol. 13, no. 9, pp. 1615–1626, 1999.

[40] H. Beaudry, L. Gendron, M. O. Guimond, M. D. Payet, and N. Gallo-Payet, “Involvement of protein kinase Ca (PKCa) in the early action of Angiotensin II type 2 (AT2) effects on neurite outgrowth in NG108–15 cells: AT2 receptor inhibits PKCa and p21 ras activity,” Endocrinology, vol. 147, no. 9, pp. 4263–4272, 2006.

[41] L. Gendron, J. F. Olligny, M. D. Payet, and N. Gallo-Payet, “Cyclic AMP-independent involvement of Rap1/B-Raf in the angiotensin II AT2 receptor signaling pathway in NG108–15 cells,” Journal of Biological Chemistry, vol. 278, no. 6, pp. 3606–3614, 2003.

[42] U. Stroth, A. Blume, K. Mielke, and T. Unger, “Angiotensin AT2 receptor stimulation ERK1 and ERK2 in quiescent but inhibits ERK in NGF-stimulated PC12W cells,” Molecular Brain Research, vol. 78, no. 1–2, pp. 175–180, 2000.

[43] B. Plouffe, M. O. Guimond, H. Beaudry, and N. Gallo-Payet, “Role of tyrosine kinase receptors in angiotensin II AT2 receptor signaling: involvement in neurite outgrowth and in p42/p44MAPK activation in NG108–15 cells,” Endocrinology, vol. 147, no. 10, pp. 4646–4654, 2006.

[44] M. O. Guimond, C. Roberge, and N. Gallo-Payet, “Fyn is involved in angiotensin II type 2 receptor-induced neurite outgrowth, but not in p42/p44MAPK in NG108–15 cells,” Molecular and Cellular Neuroscience, vol. 45, no. 3, pp. 201–212, 2010.

[45] L. Gendron, F. Cotè, M. D. Payet, and N. Gallo-Payet, “Nitric oxide and cyclic GMP are involved in angiotensin II AT2 receptor effects on neurite outgrowth in NG108–15 cells,” Neuroendocrinology, vol. 75, no. 1, pp. 70–81, 2002.

[46] D. Müller, K. J. Greenland, R. C. Speth, and R. Middendorff, “Neuronal differentiation of NG108–15 cells has impact on nitric oxide- and membrane (natriuretic peptide receptor-A) cyclic GMP-generating proteins,” Molecular and Cellular Endocrinology, vol. 320, no. 1–2, pp. 118–127, 2010.

[47] J. M. Li, M. Mogi, K. Tsukuda et al., “Angiotensin II-induced neuronal differentiation via angiotensin II type 2 (AT2) receptor-MM52 cascade involving interaction between AT2 receptor-interacting protein and Src homology 2 domain-containing protein-tyrosine phosphatase 1,” Molecular Endocrinology, vol. 21, no. 2, pp. 499–511, 2007.

[48] S. Nouet, N. Amzallag, J. M. Li et al., “Trans-inactivation of receptor tyrosine kinases by novel angiotensin II AT2 receptor-interacting protein, ATIP,” Journal of Biological Chemistry, vol. 279, no. 28, pp. 28989–28997, 2004.

[49] M. Horiiuchi and M. Mogi, “Role of angiotensin II receptor subtype activation in cognitive function and ischemic brain damage,” British Journal of Pharmacology, vol. 163, no. 6, pp. 1122–1130, 2011.

[50] N. Hashikawa-Hobara, N. Hashikawa, Y. Inoue et al., “Can-desartan cilexetil improves angiotensin II type 2 receptor-mediated neurite outgrowth via the PI3K-Akt pathway in fructose-induced insulin-resistant rats,” Diabetes, vol. 61, no. 4, pp. 925–932, 2012.

[51] X. C. Huang, E. M. Richards, and C. Summers, “Angiotensin II type 2 receptor-mediated stimulation of protein phosphatase 2A in rat hypothalamic/brainstem neuronal cocultures,” Journal of Neurochemistry, vol. 65, no. 5, pp. 2131–2137, 1995.

[52] X. C. Huang, E. M. Richards, and C. Summers, “Mitogen-activated protein kinases in rat brain neuronal cultures are activated by angiotensin II type 1 receptors and inhibited by angiotensin II type 2 receptors,” Journal of Biological Chemistry, vol. 271, no. 26, pp. 15635–15641, 1996.

[53] R. Caballero, R. Gómez, I. Moreno et al., “Interaction of angiotensin II with the angiotensin type 2 receptor inhibits the cardiac transient outward potassium current,” Cardiovascular Research, vol. 62, no. 1, pp. 86–95, 2004.

[54] P. Kilian, S. Campbell, L. Bilodeau et al., “Angiotensin II type 2 receptor stimulation increases the rate of NG108–15 cell migration via actin depolymerization,” Endocrinology, vol. 149, no. 6, pp. 2923–2933, 2008.

[55] K. Seidel, S. Kirsch, K. Lucht et al., “The promyelocytic leukemia zinc finger (PLZF) protein exerts neuroprotective effects in neuronal cells and is dysregulated in experimental stroke,” Brain Pathology, vol. 21, no. 1, pp. 31–43, 2011.

[56] T. Senbonmatsu, T. Saito, E. J. Landon et al., “A novel angiotensin II type 2 receptor signaling pathway: possible role in cardiac hypertrophy,” EMBO Journal, vol. 22, no. 24, pp. 6471–6482, 2003.
Na'/K' ATPase in primary cortical cultures," *Neuroscience Research*, vol. 50, no. 3, pp. 299–306, 2004.

[88] H. Xiong, "Angiotensin II depresses glutamate depolarizations and excitatory postsynaptic potentials in locus coeruleus through angiotensin II subtype 2 receptors," *Neuroscience*, vol. 62, no. 1, pp. 163–175, 1994.

[89] R. Lucius, S. Gallinat, P. Rosenstiel, T. Herdegen, J. Sievers, and T. Unger, "The angiotensin II type 2 (AT2) receptor promotes axonal regeneration in the optic nerve of adult rats," *Journal of Experimental Medicine*, vol. 188, no. 4, pp. 661–670, 1998.

[90] M. Mogi, J. M. Li, I. Iwanami et al., "Angiotensin II type-2 receptor stimulation prevents neural damage by transcriptional activation of methyl methanesulfonate sensitive 2," *Hypertension*, vol. 48, no. 1, pp. 141–148, 2006.

[91] F. Côté, T. H. Do, L. Laflamme, J. M. Gallo, and N. Gallo-Payet, "Activation of the AT2 receptor of angiotensin II induces neurite outgrowth and cell migration in microexplant cultures of the cerebellum," *Journal of Biological Chemistry*, vol. 274, no. 44, pp. 31686–31692, 1999.

[92] L. Laflamme, M. De Gasparo, J. M. Gallo, M. D. Payet, and N. Gallo-Payet, "Angiotensin II induction of neurite outgrowth by AT2 receptors in NG108-15 cells. Effect counteracted by the AT1 receptors," *Journal of Biological Chemistry*, vol. 271, no. 37, pp. 22729–22735, 1996.

[93] J. Li, J. Culman, H. Hörttmagl et al., "Angiotensin AT2 receptor protects against cerebral ischemia-induced neuronal injury," *FASEB Journal*, vol. 19, no. 6, pp. 617–619, 2005.

[94] S. Gallinat, M. Yu, A. Dorst, T. Unger, and T. Herdegen, "Sciatic nerve transection evokes lasting up-regulation of angiotensin AT2 and AT1 receptor mRNA in adult rat dorsal root ganglia and sciatic nerves," *Molecular Brain Research*, vol. 57, no. 1, pp. 111–122, 1998.

[95] N. Hobar, M. Goda, N. Yoshida et al., "Angiotensin II type 2 receptors facilitate reinnervation of phenol-lesioned vascular calcitonin gene-related peptide-containing nerves in rat mesenteric arteries," *Neuroscience*, vol. 150, no. 3, pp. 730–741, 2007.

[96] A. Okamura, H. Rakugi, M. Ohishi et al., "Upregulation of renin-angiotensin system during differentiation of monocytes to macrophages," *Journal of Hypertension*, vol. 17, no. 4, pp. 537–545, 1999.

[97] K. A. Nahmod, M. E. Vermeulen, S. Raiden et al., "Control of dendritic cell differentiation by angiotensin II," *The FASEB Journal*, vol. 17, no. 3, pp. 491–493, 2003.

[98] J. Iwanami, M. Mogi, K. Tsukuda et al., "Effect of angiotensin II type 2 receptor deletion in hematopoietic cells on brain ischemia-reperfusion injury," *Hypertension*, vol. 58, no. 3, pp. 404–409, 2011.

[99] C. V. Borlongan, L. E. Glover, N. Tajiri, Y. Kaneko, and T. B. Freeman, "The great migration of bone marrow-derived stem cells toward the ischemic brain: therapeutic implications for stroke and other neurological disorders," *Progress in Neurobiology*, vol. 95, no. 2, pp. 213–228, 2011.

[100] L. Rein, G. S. Barsh, R. E. Pratt, V. J. Dzau, and B. K. Kobilka, "Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice," *Nature*, vol. 377, no. 6531, pp. 744–747, 1995.

[101] T. Ichiki, P. A. Labosky, C. Shiota et al., "Effects on blood pressure exploratory behaviour of mice lacking angiotensin II type 2 receptor," *Nature*, vol. 377, no. 6551, pp. 748–750, 1995.

[102] H. M. Siragy, T. Inagami, T. Ichiki, and R. M. Carey, "Sustained hypersensitivity to angiotensin II and its mechanism in mice lacking the subtype-2 (AT2) angiotensin receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 11, pp. 6506–6510, 1999.

[103] M. Iwai, H. W. Liu, R. Chen et al., "Possible inhibition of focal cerebral ischemia by angiotensin II type 2 receptor stimulation," *Circulation*, vol. 110, no. 7, pp. 843–848, 2004.

[104] M. Iwai, R. Chen, Z. Li et al., "Deletion of angiotensin II type 2 receptor exaggerated atherosclerosis in apolipoprotein E-null mice," *Circulation*, vol. 112, no. 11, pp. 1636–1643, 2005.

[105] S. Okuyama, T. Sakagawa, S. Chaki, Y. Imagawa, T. Ichiki, and T. Inagami, "Anxiety-like behavior in mice lacking the angiotensin II type-2 receptor," *Brain Research*, vol. 821, no. 1, pp. 150–159, 1999.

[106] J. J. Braszko, "AT2 but not AT1 receptor antagonism abolishes angiotensin II increase of the acquisition of conditioned avoidance responses in rats," *Behavioural Brain Research*, vol. 131, no. 1-2, pp. 79–86, 2002.

[107] I. Makino, K. Shibata, Y. Ohgami, M. Fujiwara, and T. Furukawa, "Transient upregulation of the AT2 receptor mRNA level after global ischemia in the rat brain," *Neuropeptides*, vol. 30, no. 6, pp. 596–601, 1996.

[108] J. Ge and N. M. Barnes, "Alterations in angiotensin AT1 and AT2 receptor subtype levels in brain regions from patients with neurodegenerative disorders," *European Journal of Pharmacology*, vol. 297, no. 3, pp. 299–306, 1996.

[109] R. L. Schalock, R. A. Luckasson, K. A. Shogren et al., "The renaming of mental retardation: understanding the change to the term intellectual disability," *Intelectual and Developmental Disabilities*, vol. 45, no. 2, pp. 116–124, 2007.

[110] V. S. Vervoort, M. A. Beachem, P. S. Edwards et al., "AGTR2 mutations in X-linked mental retardation," *Science*, vol. 296, no. 5577, pp. 2401–2403, 2002.

[111] E. Takeshita, E. Nakagawa, K. Nakatani, M. Sasaki, and Y. I. Goto, "Novel AGTR2 missense mutation in a Japanese boy with severe mental retardation, pervasive developmental disorder, and epilepsy," *Brain & Development*, vol. 34, pp. 776–779, 2012.

[112] T. Ylisaukko-oja, K. Rehnström, R. Vanhala, C. Tengström, J. Lähdetie, and I. Järvelä, "Identification of two AGTR2 mutations in male patients with non-syndromic mental retardation," *Human Genetics*, vol. 114, no. 2, pp. 211–213, 2004.

[113] T. Bienvenu, K. Poirier, H. Van Esch et al., "Rare polymorphic variants of the AGTR2 gene in boys with non-specific mental retardation," *Journal of Medical Genetics*, vol. 40, no. 5, pp. 357–359, 2003.

[114] J. Erdmann, S. Dählman, M. Guse et al., "The assertion that a G21V mutation in AGTR2 causes mental retardation is not supported by other studies," *Human Genetics*, vol. 114, no. 4, pp. 396–397, 2004.

[115] D. Huang, W. Sun, and C. M. Strom, "Sequence variations in AGTR2 are unlikely to be associated with X-linked mental retardation," *American Journal of Medical Genetics*, vol. 139, no. 3, pp. 243–244, 2005.

[116] V. Boissonneault, M. Filali, M. Lessard, J. Relton, G. Wong, and S. Rivest, "Powerful beneficial effects of macrophage colony-stimulating factor on β-amyloid deposition and cognitive impairment in Alzheimer’s disease," *Brain*, vol. 132, no. 4, pp. 1078–1092, 2009.

[117] C. Iadecola, "Neurovascular regulation in the normal brain and in Alzheimer’s disease," *Nature Reviews Neuroscience*, vol. 5, no. 5, pp. 347–360, 2004.

[118] F. M. LaFerla, K. N. Green, and S. Oddo, "Intracellular amyloid-β in Alzheimer’s disease," *Nature Reviews Neuroscience*, vol. 8, no. 7, pp. 499–509, 2007.
[182] M. A. Tedesco, G. Ratti, S. Mennella et al., “Comparison of losartan and hydrochlorothiazide on cognitive function and quality of life in hypertensive patients,” *American Journal of Hypertension*, vol. 12, no. 11, pp. 1130–1134, 1999.

[183] K. Tsukada, M. Mogi, J. M. Li et al., “Amelioration of cognitive impairment in the type-2 diabetic mouse by the angiotensin II type-1 receptor blocker candesartan,” *Hypertension*, vol. 50, no. 6, pp. 1099–1105, 2007.

[184] S. C. Prinster, C. Hague, and R. A. Hall, “Heterodimerization of G protein-coupled receptors: specificity and functional significance,” *Pharmaceutical Reviews*, vol. 57, no. 3, pp. 289–298, 2005.

[185] S. I. Miura, S. S. Karrik, and K. Saku, “Constitutively active homo-oligomeric angiotensin II type 2 receptor induces cell signaling independent of receptor conformation and ligand stimulation,” *Journal of Biological Chemistry*, vol. 280, no. 18, pp. 18237–18244, 2005.

[186] S. AbdAlla, H. Lother, A. M. Abdel-tawab, and U. Quitterer, “The interaction of nitric oxide and angiotensin II type 2 receptor stimulation ameliorates cardiovascular dysfunction,” *Hypertension*, vol. 48, no. 2, pp. 316–322, 2006.

[187] H. M. Siragy and R. M. Carey, “The subtype-2 (AT2) angiotensin receptor regulates renal cyclic guanosine 3′,5′-monophosphate and AT1 receptor-mediated prostaglandin E2 production in conscious rats,” *Journal of Clinical Investigation*, vol. 97, no. 8, pp. 1978–1982, 1996.

[188] P. Gohlke, C. Fees, and T. Unger, “AT2 receptor stimulation increases aortic cyclic GMP in SHRSP by a kinin-dependent mechanism,” *Hypertension*, vol. 31, no. 1, pp. 349–355, 1998.

[189] C. D. Searles and D. G. Harrison, “The interaction of nitric oxide, bradykinin, and the angiotensin II type 2 receptor: lessons learned from transgenic mice,” *Journal of Clinical Investigation*, vol. 104, no. 8, pp. 1013–1014, 1999.

[190] U. Quitterer, A. Pohl, A. Langer, S. Koller, and S. Abdalla, “A small molecule bradykinin B2 receptor antagonist,” *Biochemical and Biophysical Research Communications*, vol. 409, no. 3, pp. 544–549, 2011.

[191] K. Shibata, I. Makino, H. Shibaguchi, M. Niwa, T. Katsuragi, and T. Furukawa, “Up-regulation of angiotensin type II receptor mRNA by angiotensin II in rat cortical cells,” *Biochemical and Biophysical Research Communications*, vol. 239, no. 2, pp. 633–637, 1997.

[192] J. Iwanami, M. Mogi, K. Tsukuda et al., “Low dose of telmisartan prevents ischemic brain damage with peroxisome proliferator-activated receptor-γ activation in diabetic mice,” *Journal of Hypertension*, vol. 28, no. 8, pp. 1730–1737, 2010.

[193] K. Washida, M. Ihara, K. Nishio et al., “Nonhypotensive dose of telmisartan attenuates cognitive impairment partially due to peroxisome proliferator-activated receptor-γ activation in mice with chronic cerebral hypoperfusion,” *Stroke*, vol. 41, no. 8, pp. 1798–1806, 2010.

[194] R. Clasen, M. Schupp, A. Foryst-Ludwig et al., “PPARγ-activating angiotensin type-1 receptor blockers induce adiponectin,” *Hypertension*, vol. 46, no. 1, pp. 137–143, 2005.

[195] W. Gillespie, N. Tyagi, and S. C. Tyagi, “Role of PPARγ, a nuclear hormone receptor in neuroprotection,” *Indian Journal of Biochemistry and Biophysics*, vol. 48, no. 2, pp. 73–81, 2011.

[196] K. M. Fuenzalida, M. C. Aguilar, D. G. Piderit et al., “Peroxisome proliferator-activated receptor γ is a novel target of the nerve growth factor signaling pathway in PC12 cells,” *Journal of Biological Chemistry*, vol. 280, no. 10, pp. 9604–9609, 2005.

[197] M. Iwai, Y. Tomono, S. Inaba et al., “AT2 receptor deficiency attenuates adipocyte differentiation and decreases adipocyte number in atherosclerotic mice,” *American Journal of Hypertension*, vol. 22, no. 7, pp. 784–791, 2009.

[198] A. M. Murugaiyah, X. Wu, C. Wallinder et al., “From the first selective non-peptide AT2 receptor agonist to structurally related antagonists,” *Journal of Medicinal Chemistry*, vol. 55, no. 5, pp. 2265–2278, 2012.

[199] Q. Ali and T. Hussain, “AT2 receptor non-peptide agonist C21 promotes natriuresis in obese Zucker rats,” *Hypertension Research*, vol. 35, pp. 654–660, 2012.

[200] S. Bosnyak, I. K. Welungoda, A. Hallberg, M. Alterman, R. E. Wid Dop, and E. S. Jones, “Stimulation of angiotensin AT2 receptors by the non-peptide agonist Compound 21, evokes vasodepressor effects in conscious spontaneously hypertensive rats,” *British Journal of Pharmacology*, vol. 159, no. 3, pp. 709–716, 2010.

[201] J. Gao, H. Zhang, K. D. Le, J. Chao, and L. Gao, “Activation of central angiotensin type 2 receptors suppresses norpinephrine excretion and blood pressure in conscious rats,” *American Journal of Hypertension*, vol. 24, no. 6, pp. 724–730, 2011.

[202] A. B. Jehle, Y. Xu, J. M. Dimaria et al., “A nonpeptide angiotensin II type 2 receptor agonist does not attenuate post-myocardial infarction left ventricular remodeling in mice,” *Journal of Cardiovascular Pharmacology*, vol. 59, no. 4, pp. 363–368, 2012.

[203] L. C. Matavelli, J. Huang, and H. M. Siragy, “Angiotensin AT2 receptor stimulation inhibits early renal inflammation in renovascular hypertension,” *Hypertension*, vol. 57, no. 2, pp. 308–313, 2011.

[204] L. Paulis, S. T. Becker, K. Lucht et al., “Direct angiotensin II type 2 receptor stimulation in Nomega-nitro-L-arginine-methyl ester-induced hypertension: the effect on pulse wave velocity and aortic remodeling,” *Hypertension*, vol. 59, no. 2, pp. 485–492, 2012.

[205] L. Paulis and T. Unger, “Novel therapeutic targets for hypertension,” *Nature Reviews Cardiology*, vol. 7, no. 8, pp. 431–441, 2010.

[206] E. Kaschina, A. Grzesiak, J. Li et al., “Angiotensin II type 2 receptor stimulation induces vasorelaxation via an endothelium- and angiotensin II type 2 receptor-independent mechanism: a novel option of therapeutic interference with the rein-in-angiotensin system in myocardial infarction?” *Circulation*, vol. 118, no. 24, pp. 2523–2532, 2008.

[207] K. Verdonk, M. Durik, N. Abd-Alla et al., “Compound 21 induces vasorelaxation via an endothelium- and angiotensin II type 2 receptor-independent mechanism,” *Hypertension*, vol. 60, pp. 722–729, 2012.

[208] M. Shum, S. Pinard, M. O. Guimond et al., “Angiotensin II type 2 receptor promotes adipocyte differentiation and restores adipocyte size in high fat/high fructose diet-induced insulin resistance in rats,” *American Journal of Physiology*. In press.

[209] K. Ohshima, M. Mogi, F. Jing et al., “Direct angiotensin II type 2 receptor stimulation ameliorates insulin resistance
in type 2 diabetes mice with PPARgamma activation,” *PLoS One*, vol. 7, no. 11, Article ID e48387, 2012.

[212] A. K. Mahalingam, Y. Wan, A. M. S. Murugaiah et al., “Selective angiotensin II AT$_2$ receptor agonists with reduced CYP 450 inhibition,” *Bioorganic and Medicinal Chemistry*, vol. 18, no. 12, pp. 4570–4590, 2010.

[213] A. M. S. Murugaiah, C. Wallinder, A. K. Mahalingam et al., “Selective angiotensin II AT$_2$ receptor agonists devoid of the imidazole ring system,” *Bioorganic and Medicinal Chemistry*, vol. 15, no. 22, pp. 7166–7183, 2007.

[214] U. Rosenström, C. Sköld, B. Plouffe et al., “New selective AT$_2$ receptor ligands encompassing a γ-turn mimetic replacing the amino acid residues 4-5 of angiotensin II act as agonists,” *Journal of Medicinal Chemistry*, vol. 48, no. 12, pp. 4009–4024, 2005.

[215] C. Wallinder, M. Botros, U. Rosenström et al., “Selective angiotensin II AT$_2$ receptor agonists: benzamide structure-activity relationships,” *Bioorganic and Medicinal Chemistry*, vol. 16, no. 14, pp. 6841–6849, 2008.

[216] X. Wu, Y. Wan, A. K. Mahalingam et al., “Selective angiotensin II AT$_2$ receptor agonists: arylbenzylimidazole structure-activity relationships,” *Journal of Medicinal Chemistry*, vol. 49, no. 24, pp. 7160–7168, 2006.