The role of the central renin-angiotensin system in Parkinson's disease

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Abstract
Since the discovery of a renin-angiotensin system (RAS) in the brain, several studies have linked this central RAS to neurological disorders such as ischaemia, Alzheimer's disease and depression. In the last decade, evidence has accumulated that the central RAS might also play a role in Parkinson's disease. Although the exact cause of this progressive neurodegenerative disorder of the basal ganglia remains unidentified, inflammation and oxidative stress have been suggested to be key factors in the pathogenesis and the progression of the disease. Since angiotensin II is a pro-inflammatory compound that can induce the production of reactive oxygen species due to activation of the NADPH-dependent oxidase complex, this peptide might contribute to dopaminergic cell death. In this review, three different strategies to interfere with the pathogenesis or the progression of Parkinson's disease are discussed. They include inhibition of the angiotensin-converting enzyme, blockade of the angiotensin II type 1 receptor and stimulation of the angiotensin II type 2 receptor.

Introduction
Today, it is widely accepted that the renin-angiotensin system (RAS) is an important modulator of the salt and water homeostasis, and that angiotensin II is its major effector peptide. Although at least two receptor subtypes, referred to as the angiotensin II type 1 (AT₁) and angiotensin II type 2 (AT₂) receptor, have been described for angiotensin II, most of the classical peripheral actions of this peptide, including vasoconstriction, facilitation of sympathetic transmission and renal water and salt retention, are mediated by the AT₁ receptor subtype. In addition to the well-described peripheral RAS, there is now accumulating evidence for the presence of a central RAS. In this respect, several studies have linked the central RAS to neurological disorders such as ischaemia, Alzheimer's disease and depression. This review focuses on the role of the central RAS in Parkinson's disease, a research area that has received little attention until recently. Although Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease, current therapies remain purely symptomatic. For this reason, research is focusing on treatment strategies that interfere with the underlying neuropathology, i.e. the cell death of dopaminergic neurons in the substantia nigra pars compacta (SNc). Much effort has been made to elucidate the exact cause of the dopaminergic cell death in the idiopathic cases of Parkinson's disease. To date, the trigger of the disease remains unidentified, but research suggests that processes such as oxidative stress, inflammation, mitochondrial dysfunction and excitotoxicity are key factors in the pathogenesis and progression of this neurodegenerative disorder. Several studies have demonstrated that microglial activation and/or NADPH-derived superoxide play a critical role in neurotoxin-induced dopaminergic degeneration in different animal models of Parkinson's disease. Indeed, intracerebral injection of 6-hydroxydopamine (6-OHDA), a selective neurotoxin for dopaminergic neurons, resulted in an increased NADPH subunit expression and NADPH oxidase activity in the striatum and ventral midbrain of the rat, and activation of microglia. In previous studies, other well-known neurotoxins used to create a parkinsonian state in animals such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone had already been associated with microglial activation and/or NADPH-derived free radicals. Furthermore, an increased expression of neuronal NAD(P)H:quinone oxidoreductase was observed in the SNc of patients with Parkinson's disease. Since angiotensin II is a pro-inflammatory compound that activates the NADPH-dependent oxidase complex, a major source of superoxide, it might be possible to slow down the progression of Parkinson's disease by manipulation of the RAS.
Angiotensin-converting enzyme (ACE) inhibitors

ACE is an important component of the enzymatic cascade which generates angiotensin II. After cleavage of the precursor glycoprotein angiotensinogen by renin, ACE converts the produced angiotensin I into angiotensin II. Several observations point towards a role of ACE in the pathogenesis of Parkinson’s disease. First, ACE is widely distributed in the brain including the nigrostriatal pathway and the basal ganglia, and an increased ACE activity in the cerebrospinal fluid of patients with Parkinson’s disease has been reported. Secondly, chronic treatment with the ACE inhibitor perindopril caused an increase in the striatal dopamine content of rats. In line with this observation, treatment of six patients with moderately severe Parkinson’s disease with perindopril resulted in an improvement of motor responses to the dopamine precursor 3,4-dihydroxy-L-phenylalanine. Thirdly, the enzymatic action of ACE is not limited to cleavage of angiotensin I. ACE can metabolise bradykinin and therefore can modulate inflammation, a key factor in Parkinson’s disease. Substance P can also be hydrolysed by ACE, and this feature might be important since a primary loss of substance P has been linked with the pathogenesis of several neurological diseases including Parkinson’s disease. Fourthly, an association between genetic polymorphism of the ACE gene and Parkinson’s disease has been described in Taiwan. However, in the Australian and Italian population, no correlation between genetic polymorphism of the ACE gene and Parkinson’s disease could be detected. Finally, stimulation of AT1 receptors by angiotensin II has been shown to activate NADPH-dependent oxidases which are a major source of reactive oxygen species (ROS).

Based on these arguments, several studies have been undertaken to evaluate the neuroprotective and neurorestorative effects of ACE inhibitors in animal models of Parkinson’s disease. Jenkins et al. were the first to administer an ACE inhibitor to MPTP-treated mice. Oral treatment with perindopril, started two weeks after the last injection of MPTP and continued for seven days, was able to restore dopamine content to control levels. This restorative effect was not present when treatment was started immediately after cessation of the MPTP treatment. In addition, chronic treatment with ACE inhibitors has also been reported to protect dopaminergic neurons in the SNc against neurotoxicity. Pre-treatment with perindopril reduced the MPTP-induced loss of dopaminergic cells in the SNc and the striatal dopamine depletion. Similar results were obtained with the ACE inhibitor captopril, suggesting that the neuroprotective effect is not a unique property of perindopril but probably a characteristic of the whole class of ACE inhibitors. The neuroprotective action of captopril was reproduced in a 6-OHDA rat model of Parkinson’s disease.

A possible explanation of the mechanism behind the beneficial effects of ACE inhibitors in the above animal models might be that they prevent the chronic and/or toxic angiotensin II signalling via AT1 receptor activation. In this respect, AT1 receptors activate the NADPH oxidase complex which is the most important intracellular source of ROS (mainly the superoxide anion) beyond mitochondria. The produced superoxide anion is then converted into H2O2 by superoxide dismutase or combines with nitric oxide to generate peroxynitrite, thereby decreasing the bioactivity of nitric oxide and promoting lipid and protein oxidation. When ROS are used as second messengers, a tightly regulated balance between their production and inactivation is required. Dysregulation of ROS as a consequence of increased production or decreased inactivation, may cause serious damage to the surrounding biological structures, defined as oxidative stress, i.e. DNA mutation, lipid peroxidation, protein damage and ultimately cell death via apoptosis and necrosis. Secondly, AT1 receptor activation also leads to stimulation of the NFκB signal transduction pathway, resulting in an increased expression and production of chemokines, cytokines and adhesion molecules, which contribute to the migration of inflammatory cells into tissue injury. In addition to this indirect effect, angiotensin II can act directly on inflammatory cells. Stimulation of AT1 receptors on neutrophils, monocytes and lymphocytes by angiotensin II induces inflammatory responses and results in the release of high levels of ROS mainly by activation of their NADPH complex.

The interaction between angiotensin II and NADPH oxidase was originally described in vascular tissue. Later studies with cell cultures from hippocampus and brain stem demonstrated that the interaction between angiotensin II and NADPH oxidase is also present in the brain. Thus, angiotensin II is capable of inducing the excessive production of ROS, causing dopaminergic cell death as in Parkinson’s disease. While administration of angiotensin II alone had no effect on the number of dopaminergic cells in vivo, simultaneous administration of angiotensin II and 6-OHDA indeed increased the neurotoxic effect of the latter. The synergistic action of the ROS derived from neuronal NADPH,
activated via neuronal \( \text{AT}_1 \) receptors, and ROS derived from intraneuronal auto-oxidation of 6-OHDA can contribute to the increased neurotoxicity of 6-OHDA. However, activation of microglial NADPH oxidase and superoxide production resulting in enhanced extracellular levels of ROS and increased release of pro-inflammatory factors are required to achieve the increase of 6-OHDA-induced dopaminergic cell death by angiotensin II.\(^{31}\) These observations may explain why compounds that decrease the generation of angiotensin II, such as ACE inhibitors, are neuroprotective in animal models of Parkinson’s disease.

Independent of their ability to inhibit ACE activity, ACE inhibitors are by themselves capable of scavenging ROS.\(^{55}\) Contradictory results have been reported on the role of the sulphhydryl group in these ROS scavenging properties. According to some authors, only ACE inhibitors with a sulphhydryl group are capable of scavenging ROS,\(^{32,35}\) whereas others suggest that the free radical scavenging properties are independent of the sulphhydryl group.\(^{54-57}\) Although ACE inhibitors such as captopril are potent free radical scavengers \( \text{in vitro} \), a scavenger action \( \text{in vivo} \) is unlikely. In the DATATOP (Deprenyl and Tocopherol Antioxidant Therapy of Parkinson’s disease) study, two antioxidants, i.e. alpha-tocopherol (vitamin E) and deprenyl (selegiline), were screened for their neuroprotective effect in patients with Parkinson’s disease. Unfortunately, treatment with alpha-tocopherol had no effect on the primary end point (i.e. point that blinded investigators felt that symptomatic therapy in the form of 3,4-dihydroxy-L-phenylalanine needed to be introduced) of the study. In contrast, hopeful results were obtained with selegiline, but interpretation of these results was complicated by the symptomatic effect of the compound.\(^{58}\) Although compounds such as alpha-tocopherol do scavenge free radicals, the rate constant for this reaction is much lower than the rate constant for the reaction between nitric oxide and the superoxide anion. Therefore, in order to reach compartments where superoxide is formed to scavenge the free radicals, anti-oxidants must be administered in very high concentrations.\(^{59}\) However, tissue concentrations of ACE inhibitors in the brain during therapeutic use will probably be too low to exert any direct scavenging effect.

Finally, the observed beneficial effects of ACE inhibitors in animal models of Parkinson’s disease might also be related to the direct interaction between these compounds and the dopaminergic system. Indeed, as already mentioned above, chronic treatment with ACE inhibitors increased striatal dopamine content and release in intact rats and MPTP-treated mice.\(^{20,24}\) In addition, acute administration of angiotensin II has been reported to increase striatal dopamine release via action on \( \text{AT}_1 \) receptors.\(^{50,64}\)

However, it cannot be excluded that ACE substrates other than angiotensin II are also involved in the protective effects of ACE inhibitors.\(^{57}\) This is possible since ACE is found to be colocalised with its substrates enkephalin, substance P and beta-endorphin in striatal projections, peptides that may also be involved in the modulation of the dopamine turnover.\(^{22,54}\)

**AT\(_1\), receptor antagonists**

From the data above, it can be hypothesised that \( \text{AT}_1 \) receptors play a role in Parkinson’s disease. \( \text{AT}_1 \) receptors are present in the SNc and the striatum of different mammals, including rats and humans.\(^{62,65}\) When compared to rats and other mammals, the density of \( \text{AT}_1 \) receptors is very high in human striatum and substantia nigra.\(^{51}\) In the striatum of patients with Parkinson’s disease, the number of \( \text{AT}_1 \) receptors is markedly reduced, suggesting that this angiotensin receptor subtype is present on the dopaminergic neurons.\(^{66}\) Furthermore, modulation of the striatal dopamine release via these \( \text{AT}_1 \) receptors, as described by several authors, also points towards an interaction between the RAS and the central dopaminergic system.\(^{50,60,61,66}\)

As mentioned above, stimulation of \( \text{AT}_1 \) receptors has been associated with activation of the NADPH oxidase complex. If the neuroprotective action of ACE inhibitors in animal models of Parkinson’s disease is not solely related to their anti-oxidant properties and the reduced breakdown of neuropeptides other than angiotensin II, blockade of the \( \text{AT}_1 \) receptor should result in neuroprotection. This hypothesis was confirmed in three recent studies with \( \text{AT}_1 \) receptor antagonists. In the 6-OHDA rat model of Parkinson’s disease, treatment with ZD 7155, an \( \text{AT}_1 \) receptor antagonist, reduced lipid peroxidation and protein oxidation in the striatum and the ventral midbrain, and protected dopaminergic neurons in the SNc.\(^{51}\) Comparable results on 6-OHDA-induced dopaminergic cell death and markers of oxidative stress were obtained with apocynin, an inhibitor of NADPH oxidase, suggesting that the neuroprotective action of the \( \text{AT}_1 \) receptor antagonist is based on the reduced activation of the NADPH oxidase complex.\(^{51}\) An additional study demonstrated that treatment with \( \text{AT}_1 \) receptor antagonists not only reduced the formation of NADPH oxidase-derived
ROS, but also the activation of microglia after administration of 6-OHDA. The neuroprotective action of AT$_1$ receptor antagonists was not restricted to the 6-OHDA rat model. Indeed, losartan was able to protect dopaminergic neurons against MPTP-induced neurotoxicity.\(^{67}\)

Interestingly, inhibition of AT$_1$ receptors by their antagonists may not only result in a reduced activation of NADPH oxidase, but also in an increased synthesis of angiotensin II, which in turn could lead to a preferential activation of the unopposed AT$_2$ receptor. Several data suggest a neuroprotective role for this receptor subtype which under normal conditions is masked by the opposing role of the AT$_1$ receptor.\(^{68,69}\) According to this hypothesis, AT$_1$ receptor antagonists may be more beneficial than ACE inhibitors in reducing neurotoxicity. By decreasing the generation of angiotensin II in the brain, ACE inhibitors will not only reduce stimulation of the AT$_1$ receptor, but also prevent the neuroprotective actions of AT$_2$ receptor-mediated signalling. In agreement, AT$_2$ receptor blockers are reported to have a greater propensity than ACE inhibitors to cross the blood-brain barrier and block central AT$_1$ receptors,\(^{70}\) while leaving the AT$_2$ receptor stimulation unaffected. A comparative study between the neuroprotective effect of an ACE inhibitor and an AT$_1$ receptor antagonist in an animal model of Parkinson’s disease may provide evidence whether this is indeed taking place.

Recently, a large observational study has been performed in the United Kingdom in order to explore the association between the use of various antihypertensive drugs and the risk of developing a first-time diagnosis of Parkinson’s disease. No such association was found for ACE inhibitors or AT$_1$ receptor antagonists.\(^{71}\)

### The possible role of the AT$_2$ receptor in Parkinson’s disease

AT$_2$ receptors are widely distributed in foetal tissue,\(^{72}\) but their expression is dramatically decreased after birth. In adult animals, AT$_2$ receptor expression is restricted to a few organs such as uterus,\(^{73}\) ovarian granulose cells, adrenal glands, heart, vascular endothelium, kidney, myometrium and brain areas involved in cognition and behaviour.\(^{74,75}\) Conflicting results have been reported concerning the distribution of AT$_2$ receptors in the striatum and the substantia nigra of mammals.\(^{76,78}\) Moreover, studies are complicated by the low expression levels of AT$_2$ receptors and require highly sensitive techniques such as real-time PCR.\(^{67}\)

Whereas most of the classical actions of angiotensin II on blood pressure and water and salt homeostasis in the adult brain appear to be mediated by AT$_1$ receptors,\(^{74,77}\) AT$_2$ receptors have been implicated in processes occurring during brain development and tissue regeneration.\(^{82,83}\) Activation of the AT$_1$ receptor has been associated with cell proliferation, cell differentiation, tissue regeneration, and even with apoptosis in different cell lines from neuronal origin.\(^{74,84-88}\) In mesencephalic precursor cells, angiotensin II has been reported to increase differentiation of the precursor cells into dopaminergic neurons via activation of the AT$_2$ receptor. Based on these data, administration of a mixture of angiotensin II with different factors that increase differentiation, maturation or survival of dopaminergic neurons, may lead to large-scale production of dopaminergic neurons for clinical transplantation in the treatment of Parkinson’s disease.\(^{89}\)

AT$_1$ and AT$_2$ receptors have been reported to exert opposing effects. One of the major roles of the AT$_2$ receptor appears to be protection against overstimulation of AT$_1$ receptors.\(^{86}\) An increase in the AT$_2$ receptor expression has been observed under several pathological conditions including vascular injury, myocardial infarction, congestive heart failure, renal failure, brain ischaemia, scatic or optic nerve transsection and neurodegenerative diseases such as Huntington’s and Alzheimer’s disease.\(^{90,88}\) Upregulation of this receptor subtype under pathological conditions speaks in favour of a general role of the AT$_2$ receptor in the repair of injured tissue. Consequently, stimulation of upregulated AT$_2$ receptors may counteract the deleterious effect of AT$_1$ receptor activation resulting in neuroprotection.\(^{12,86}\) Consistent with this hypothesis, activation of the AT$_2$ receptor has been reported to inhibit NADPH oxidase activation.\(^{92,93}\) In cultured midbrain dopaminergic neurons, angiotensin II protected against rotenone-induced cell death. In this in vitro model, the neuroprotective action of angiotensin II was mediated via the AT$_2$ receptor since the effect was prevented in the presence of the AT$_2$ receptor antagonist PD123319 and significantly increased after blockage of the AT$_1$ receptor.\(^{86}\) In addition to the inhibitory effect on NADPH oxidase, other mechanisms such as an anti-inflammatory action and a stimulatory effect on cell survival, might contribute to the neuroprotective action of AT$_2$ receptor stimulation after administration of angiotensin II.\(^{91,92}\) In another in vitro model of Parkinson’s disease, angiotensin II-mediated protection against alpha-synuclein toxicity seemed to be mediated via a non-AT$_1$ or AT$_2$ receptor mechanism.\(^{94}\) Although these studies speak in favour of a protective role of angiotensin II in vitro, 6-OHDA-induced dopaminergic cell death was not
decreased but increased by angiotensin II in a study by Rodriguez-Pallares et al. Differences in the mechanism of action of the neurotoxins and in the origin of the cell lines used may be responsible for the discrepancy in the obtained results. Furthermore, it is important to note that these contradictory results might also be related to the ratio of AT₁ and AT₂ receptors. When the AT₁ receptor is the dominating receptor subtype, the deleterious effects of the AT₁ receptor will overrule the beneficial effects of the AT₂ receptor. In cells where the AT₂ receptor is more abundantly expressed, pro-survival effects might dominate over the negative effects of the AT₁ receptor.

In patients with Parkinson’s disease, the level of AT₂ receptors was decreased in the caudate nucleus and remained unaltered in the putamen and the SN. Data on the distribution of AT₂ receptors in animal models of Parkinson’s disease are limited to one study performed in mice. AT₂ receptor expression in the SN of these mice was not altered by MPTP-induced neurotoxicity. As AT₁ receptor expression is decreased or unaltered in Parkinson’s disease, administration of an AT₁ receptor antagonist might not be sufficient to result in endogenous stimulation of the AT₂ receptor with a significant intensity.

Until now, AT₂ receptor-mediated effects had to be examined either by treatment with angiotensin II under concomitant blockade of the AT₁ receptor or in genetically altered animals. A more elegant approach could be to use the AT₂ receptor-selective peptide agonist CGP42112. However, the use of this compound is restricted because it is reported to display only partial agonist activity, and peptides in general have a poor blood-brain barrier permeability and are susceptible to enzymatic degradation in in vitro models. To circumvent this, the administration of the recently developed non-peptide AT₂ receptor agonist Compound 21 (C21) could be more successful.

Conceptually, it would be expected that a combination of C21 and AT₁ receptor blockade would result in maximal efficacy. Despite this, in an animal model of myocardial infarction, the combination of C21 and candesartan was not superior to the AT₁ receptor agonist alone. In this study, monotherapy probably elicited a maximal functional improvement, which did not allow any further improvement.

Finally, administration of C21 in in vitro and in vivo models of Parkinson’s disease could be useful in order to obtain better insights into the mechanisms of action of this receptor subtype. In this context, it would be useful to explore the ability of the AT₁ receptor agonist (C21) either alone or in combination with an AT₁ receptor antagonist to improve the pathological parameters in models for Parkinson’s disease.

**Conclusion**

The importance of the RAS in Parkinson’s disease is a relatively new concept. Accumulating evidence presented above suggests that the brain RAS is involved in the degeneration of dopaminergic neurons and the progression of Parkinson’s disease. Consequently, both angiotensin II receptor subtypes as well as ACE might be interesting targets for developing new treatment strategies of this neurodegenerative disease. However, additional research is required in order to further unravel the implications of the RAS in Parkinson’s disease.

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**References**

15. Wu DC, Teissmann P, Tieu K et al. NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson’s disease. Proc Natl Acad Sci U S A 2003;100:6145-50.
41. Beckman JS, Beckman TW, Chen J et al. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 1990;87:1620-4.


