Chapter

RENIN-ANGIOTENSIN SYSTEM MODULATING FUNCTIONS IN THE CPU

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ABSTRACT

The caudate-putamen (CPu) of the striatum is one of the main entrances to the basal ganglia. The CPu is fundamentally a dopaminergic area receiving dopamine innervation from the substantia nigra, ventral tegmental area, and mesencephalic structures, but also has noradrenergic inputs from a post-encephalic area, the locus coeruleus, and glutamatergic innervation from cortical structures and cholinergic and GABAergic interneurons. It is well known that functional interactions between different neurotransmission systems play a crucial integrative role in the caudate-putamen, and are widely recognized as contributing to central motor activity and movements, and also to the processing of cognitive and limbic functions, despite autonomic responses across the noradrenergic system. Not only does typical neurotransmission regulate these functions, but peptidergic systems also have an important role.

The brain renin-angiotensin system (RAS) is involved not only in the regulation of blood pressure, but also in the modulation of multiple additional functions in the brain, including processes of sensory information, learning and memory, and the regulation of emotional and behavioral responses. There is increasing ontogenetic, anatomic and functional evidence of the existence of a brain renin-angiotensin system and of its interaction with other putative neurotransmitters and their receptors. All components of the RAS have been observed in the striatum, and Ang II modulates dopamine release from striatal dopaminergic terminals, in vivo and in vitro, via their AT_1 receptors. There is considerable evidence supporting a key role for dopamine (DA) neurotransmission in the Cpu in long-term neuroadaptative changes induced by stress or psychostimulants, such as cocaine or amphetamine. Repeated amphetamine or cocaine administration

results in progressive and enduring enhancement of their psychomotor and positive reinforcing effects (sensitization phenomenon). We recently found evidence of the participation of Ang II, through its AT_1 receptors, in the development of the locomotor sensitization induced by psychostimulant drugs.

Moreover, the brain RAS may play a role in the pathogenesis and progression of Parkinson's disease and aging-related loss of DA neurons. Manipulation of RAS components may be useful for neuroprotection in Parkinson's disease patients because local RAS plays a major role in proinflammatory and pro-oxidative changes in aged substantia nigra. RAS is involved in modulating neurotransmission systems in the CPu and their functions, and for this reason it could be a possible target in the treatment of stress related diseases, drug abuse or neurodegenerative disorders.

GENERAL FUNCTIONS OF THE CAUDATE-PUTAMEN

The caudate-putamen (CPu) of the striatum is one of the main entrances to the basal ganglia. It is fundamentally a dopaminergic area, located at the midbrain, and has functional interactions with different neurotransmission systems that are known to play a crucial integrative role. It is now widely recognized that it contributes to central motor activity and also to the processing of cognitive and limbic functions, despite autonomic responses across the noradrenergic system. Not only does typical neurotransmission regulate these functions, but peptidergic systems also have an important role.

In comparison to typical neurotransmitter synthesis, the neuropeptides require complex metabolic pathways, including proteolytic processing from neuropeptide precursors to smaller pro-neuropeptides and to the final mature neuropeptides. In several instances, the common precursor can give rise to different biologically active neuropeptides. An example of the latter is the renin-angiotensin system (RAS) in the brain [1].

Neurochemistry and Physiology of the Caudate-Putamen

The CPu receives dopaminergic innervations from substantia nigra and ventral tegmental area mesencephalic structures. It also has noradrenergic inputs from the locus coeruleus, glutamatergic innervation from cortical structures, hippocampus and basolateral amygdala, and serotoninergic input from the raphe nucleus as well as the cholinergic and GABAergic interneurons. The CPu has GABAergic neurons which principally project to the ventral pallidum and substantia nigra. The ventral pallidum receives dopaminergic innervations from the midbrain and it is believed to play a significant role in several behavioral aspects, in particular those related to drug sensitization [2].

The main actions of dopamine (DA) may best be described, not in terms of inhibition or excitation, but rather as related to the gating of inputs and modulation of states of neuronal elements. It does not directly produce a motor output or reward signal, but instead modulates inputs and adjusts the states of the organism in order to redirect the stimulus-response output to achieve the most effective behavioral strategy [2].

DA is released primarily in a spike-dependent manner, because inactivation of DA neuron firing blunts DA release within the striatum. The striatal CPu provides a powerful

feedback regulation of DA neuron firing. Thus, alterations in striatal activity potently affect DA cell activity states.

DA has also been shown to affect the response of striatal neurons to other neurotransmitters. Thus, DA was found to modulate the response of striatal neurons during glutamatergic excitation. D1 receptors induce facilitation of glutamate transmission mediated by burstfiring–dependent phasic DA release. In contrast, D2 receptor stimulation appears to preferentially attenuate non–NMDA-mediated responses under physiologic conditions. It also attenuates the responses to GABAergic input. In addition to its ability to modulate postsynaptic neurotransmitter actions, DA also plays a significant role in the presynaptic regulation of neurotransmitter release. Dopamine D2 autoreceptor stimulation has been reported to presynaptically decrease glutamate release from corticostriatal terminals and GABA release from intrinsic neurons.

DA acts on presynaptic terminals containing glutamate, as well as affecting the action of glutamate on postsynaptic neurons. Combined with the reciprocal feedback interactions between glutamate and DA terminals, this system appears to be designed to facilitate rapid changes in input states while attenuating any long-term alterations that may occur.

BRAIN RENIN-ANGIOTENSIN SYSTEM

The RAS was initially described as a circulating humoral system influencing blood pressure and fluid and electrolyte homeostasis through effects on vascular smooth muscle, the adrenal cortex and the kidney [3]. It is now known that a tissue-based system also exists in many regions, including the vasculature, heart, kidney, adrenal gland, ovaries, placenta and brain, with actions largely complementary to those of the systemic peptide [4, 5].

Most available evidence indicates that a complete brain RAS exists that is distinctly separate from the peripheral system and comprises all necessary precursors and enzymes required for formation and metabolism of the biologically active forms of angiotensin [6-8]. The principal active neuropeptide is the octapeptide angiotensin II (Ang II); it does not cross the blood-brain barrier, but, though generated at the periphery, can stimulate the brain RAS at specific brain sites, such as the circumventricular organs (specific sites in the central nervous system that lack the blood-brain barrier). Circumventricular organs are critically involved in the regulation of many homeostatic processes, including the control of cardiovascular functions, hydromineral balance, body temperature, and hormone secretion [9]. The action of peripherally generated Ang II at these sites is thought to influence classical behavioral (drinking), endocrine (vasopressin, oxytocin, and ACTH secretion), and autonomic functions [10, 11]. Ang II belongs to the group of peptides known to stimulate DA release [12-14]. Moreover, Ang II receptors are located in DA-rich brain areas [14, 15]. Central actions of Ang II are not exclusively associated with their traditional roles. Indeed, several studies have shown that central Ang II is also involved in sexual behavior, stress, learning and memory and included in psychostimulant behaviors [7].

In the brain, Ang II is generated from the precursor angiotensinogen, which is cleaved by renin to form the inactive decapeptide angiotensin I. By the activity of the angiotensin-converting enzyme (ACE), angiotensin I is hydrolyzed at its carboxy-terminus. This leads to generation of the active octapeptide Ang II. Ang II seems to represent the first neuroactive

form of the angiotensins [16] and is not only generated in the brain via this classical pathway, involving renin and ACE, but can also be produced directly from angiotensinogen by cathepsin G or tonin [17]. Subsequently, Ang II is metabolized to Ang III, which is itself converted to Ang IV by aminopeptidases. There are further hypotheses that the brain processes alternative enzymatic mechanisms for the formation of neuroactive forms of angiotensin that are distinct from those involved in the classical pathway [18]. In this chapter, we are interested principally in Ang II and its role in modulating CPu functions, so the other angiotensins will not be discussed here.

The biological actions of Ang II are mediated by seven specific transmembrane-spanning G protein-coupled angiotensin receptors. Studies with non-peptide antagonists have led to the identification of two pharmacologically distinct Ang II receptor subtypes: AT_1 and AT_2 [19]. Both receptors have been cloned in rodents (30% homology between them), finding two types of AT_1 receptors, AT_{1A} and AT_{1B}, and AT_2. [20, 21]. Most species, like humans, express a single autosomal AT_1 gene [22], but two related AT_{1A} and AT_{1B} receptor genes are expressed in rodents. These two receptors are 95% identical in their amino acid sequences. They also seem to be similar in terms of ligand-binding activation but differ in their tissue distribution, chromosomal localization and transcriptional regulation [23-25]. AT_1 and AT_2 receptors are both post-translationally modified by N-linked glycosylation, which is required for efficient folding on cell surface expression. Ang II-stimulated AT_1 receptors, but not AT_2 receptors, are robustly phosphorylated at serine residues within the central core of the carboxyl-terminus [26]. This modification recruits proteins known as arrestins to the activated AT_1 receptor, leading to rapid and vigorous endocytosis of AT_1 receptors into clathrin-coated vesicles. Internalized receptors are either trafficked to lysosomes (or degraded), or dephosphorylated and recycled to the cell surface. AT_2 receptors do not undergo Ang II-stimulated internalization in accordance with their very weak phosphorylation [23].

Northern blot and RT-PCR analysis has identified mRNA for AT_1 and AT_2 in many cells and tissues corresponding to known sites of receptor expression and action of Ang II [23]. AT_1 receptors are expressed in blood vessels (to promote vasoconstriction), the adrenal cortex (to release the salt-retaining hormone, aldosterone), liver (glycogen metabolism), kidney (water and salt retention), brain (vasopressin release, thirst, salt appetite, sympathetic output, blood pressure regulation); AT_2 receptors are highest in fetal mesenchymal tissue, adrenal medulla, uterus, and atretic ovarian follicles, corresponding to putative roles in development and apoptosis [27].

Angiotensinogen is synthesized by astrocytes [28], so a mechanism must exist to transport angiotensinogen, or a metabolite, from astrocytes to neurons, because it has also been found in neurons. Renin is present in the brain in very low concentrations [4] and ACE is widely distributed in the brain [29]. The distribution of angiotensin-like immunoreactivity in nerve terminals is well defined [30] and has a good correlation with angiotensin AT_1 and AT_2 receptors, defined by in vitro autoradiography with 125I-angiotensin II, or by in situ hybridization histochemistry [31, 32]. In addition, angiotensin receptors and angiotensin-like immunoreactive nerve terminals occur in sites where microinjections of Ang II produce changes in physiological parameters such as blood pressure, drinking behavior, salt appetite and neuroendocrine function [33]. These observations provide strong support to the hypothesis that angiotensin acts in the brain as a neurotransmitter or neuromodulator.

The distribution pattern of cells expressing the various components of the brain RAS has been reviewed in detail elsewhere [34, 35].

Brain Renin-Angiotensin System Functions

Brain Ang II is involved in fluid and salt ingestion, neuroendocrine system modulation, including vasopressin and corticotropin-releasing factor release, and interaction with the autonomic control of the cardiovascular system to influence blood pressure [36, 37]. In many instances, these effects are complementary to those of the systemic peptide on peripheral target organs. Thus, systemic Ang II affects the brain through AT_1 receptors located in the circumventricular organs (subfornical organ, vascular organ of the lamina terminalis, median eminence, anterior pituitary and the area postrema of the hindbrain), [31, 32]. In addition, endogenous neutrally-derived Ang II appears to act at many central nervous system sites behind the blood brain barrier [1, 38], such as the median preoptic nucleus, hypothalamic paraventricular nucleus, anteroventral preoptic, suprachiasmatic and periventricular nuclei, and discrete regions of the lateral and dorsomedial hypothalamus. Most of the classical actions of Ang II are mediated via the AT_1 receptors present in large amounts in these areas, whereas AT_2 receptor stimulation may cause opposite effects.

Ang II generated within the brain may act on AT_1 receptors as a neurotransmitter or neuromodulator in neural pathways, influencing the cardiovascular system and fluid and electrolyte balance. Angiotensinergic neural pathways within the brain may have important homeostatic functions, particularly related to the control of arterial pressure, fluid and electrolyte homeostasis and thermoregulation.

The brain RAS is involved not only in the regulation of blood pressure, but also in the modulation of multiple additional functions, including processes of sensory information [18, 39], learning and memory [40, 41], and the regulation of emotional [39] and behavioral responses [42]. Georgiev et al. reported that Ang II influenced rat behavior in an open field [43], locomotion and stereotypy [44, 45].

Brain Ang II was found to regulate some responses induced by drugs of abuse such as cocaine, amphetamine, among others. It was also found that Ang II enhanced the stereotypy induced by apomorphine (DA receptor agonist), and this response was blocked by Ang II AT_1 receptor antagonists [46]. The presence of Ang II AT_1 receptors has been described in pre- and postsynaptic CPu dopaminergic neurons [12], which are involved in the motor and behavioral responses induced by psychostimulants, as well as their modulatory action on noradrenergic [47], serotoninergic [48], GABAergic and glutamatergic neurotransmission [49, 50].

In this chapter we focus principally on evidence related to the role of brain RAS in behavioral responses mediated by CPu and the modulation of the dopaminergic system.

Renin-Angiotensin System in the Caudate-Putamen

Increasing ontogenetic, anatomic and functional evidence has indicated the existence of a brain RAS and its interaction with other putative neurotransmitters and their receptors. During the embriologyc period, it was shown that Ang II increased the differentiation of mesencephalic precursors towards the dopaminergic phenotype [51]. Moreover, all RAS components have been observed in the CPu, as well as in the other basal ganglia structures. AT_1 receptors were observed in the cell body in the substantia nigra (pars compacta), and at the presynaptic terminal in the CPu [52, 53]. Studies in adult human brain revealed that, in the

substantia nigra [54], 90% of the receptors were AT_1 and that the remainder were AT_2. Lower concentrations of receptors were found within the putamen and caudate nucleus, where the AT_1/AT_2 ratio was 70/30 [54, 55]. Despite the fact that AT_1 receptor density is low in the rat striatum, Allen et al. and others were able to demonstrate that Ang II acts presynaptically in the rat CPu to potentiate DA release [12, 56].

The localization of ACE in the brain is associated with the endothelium of cerebral blood vessels, epithelial cells of the choroid plexus and the plasma membranes of astrocytes in the circumventricular organs; moderate levels occur in neurons in the paraventricular nucleus and supraoptic hypothalamic nuclei and the dorsal vagal complex, where its distribution coincides with those of Ang II immunoreactivity and AT_1 receptors. ACE is also found in other brain regions not associated with the presence of Ang II immunoreactivity, including the basal ganglia, hippocampus and cerebellum, thereby suggesting other novel actions for the enzyme in the brain [57]. In human basal ganglia, ACE is located in the SNi (pars reticulata) and enriched in striosomes of the striatum [58].

Renin-Angiotensin System-Dopamine in the Caudate-Putamen

There is a large body of evidence to support the concept of a relationship between brain Ang II and catecholamine systems. This interaction may participate in some central actions of Ang II such as cardiovascular control, dipsogenesis, and complex behaviors. It also extends to the nigrostriatal DA system, which bears AT_1 receptors where Ang II can markedly potentiate DA release. This observation suggests that drugs which modulate central Ang II may be useful in regulating central DA activity.

Dopaminergic neurotransmission in the CPu plays a critical role in locomotor and stereotypic behaviors. There is strong evidence supporting a close relationship between Ang II and DA neurotransmission in the brain. The regulatory role of the RAS in various physiological processes, such as release of pituitary gland hormones [59], body temperature control [60], water balance [61], locomotion and stereotypy [44, 45], is exerted by its modulator influence on main dopaminergic pathways. These functional interactions correlate well with anatomical findings that demonstrate high AT_1 receptor density in DA-rich regions, in CPu, hypothalamus, NAcc, etc. Autoradiographic studies showed that AT_1 receptor bindings are distributed on the ascending DA-containing nigrostriatal neurons, both in cell bodies in the substantia nigra and on their terminals in the striatum (see above). There is functional evidence that the RAS is involved in modulating DA release in the CPu, an effect that is mediated by AT_1 receptors. It was shown in rats with unilateral 6-hydroxydopamine lesions in the nigrostriatal pathway that injection of Ang II (2 nmol) into the unlesioned striatum elicited dose-related tight rotations, ipsilateral to the lesion; this rotation was suppressed by co-administration of the Ang II AT_1 receptor antagonist, losartan (2 nmol), which had no significant effect when injected alone; and pre-administration of the DA antagonist, haloperidol (2 mg/Kg, i.p.) completely blocked Ang II-induced turning [62].

A facilitatory role of Ang II, via AT_1 receptors, has been demonstrated in DA release in the striatum, which is involved in Ang II behavioral effects [56, 62]. Ang II increased DA release in a concentration-related manner, in experiments using *in vitro* striatal slice preparations and *in vivo* striatal microdialysis, in conscious freely moving rats, and this study revealed that acute central or peripheral administration of losartan significantly decreased striatal DA levels [12]. Another study revealed that acute administration of losartan resulted in a significant decrease of the high DA levels induced by a single administration of Ang II,

while chronic AT_1 non-peptide antagonist resulted in an increase of DOPAC levels, without changes in DA levels [63].

Losartan, mainly used for the treatment of hypertension, has been reported to cross the blood-brain barrier, and decrease striatal DA levels [63]. Losartan administration, reversed the hyperlocomotion and the stereotypy induced by apomorphine [44, 45]. Not only does the manipulation of AT_1 receptors modulate motor functions, but also ACE inhibitors such as captopril modulate the expression of apomorphine-induced oral stereotypy, a response that is thought to be mediated by postsynaptic DA receptors [46]. Moreover, it has been shown that Ang II increased exploratory behavior in an open field, which was increased by apomorphine and nomifensine (a DA reuptake inhibitor) and decreased by haloperidol and alpha-para-tyrosine, and the same pharmacological treatment decreased the apomorphine-increased stereotypy [64]. Other authors reported that acute treatment with losartan (i.p.) decreased locomotor activity without producing sedation or muscle relaxation, and diminished apomorphine-induced stereotypy [44]. These results suggest close interactions between central dopaminergic transmission and Ang II.

Apart from the modulation of brain DA activity by Ang II via AT_1 receptors, ACE may play additional roles in the brain other than Ang II formation. One of the novel actions of brain ACE is modulation of DA turnover in the striatum. It was observed that chronic perindopril treatment significantly inhibited brain ACE, and increased both striatal DA release and content in rats [58]. The distribution of ACE in the basal ganglia, in contrast to the AT_1 receptors which are at the terminals of the nigrostriatal dopaminergic projections, occurs in striatopallidal and striatonigral neurons; in addition to this, it is thought that the effect of ACE inhibitor on DA release may be mediated, at least in part, by increasing the levels of pre-pro-enkephaline mRNA within the striatum, so that the results of DA levels after ACE inhibition are opposite to those obtained with AT_1 antagonist treatment. This is in line with the fact that it is possible to modulate dopaminergic functions through manipulating ACE activity. This may have clinical implications for Parkinson's disease, a movement disorder associated with the loss of DA-synthesizing neurons. ACE inhibitors are currently undergoing clinical evaluation for treating Parkinson's disease.

Renin-Angiotensin System-Noradrenaline in the Caudate-Putamen

As mentioned above, the CPu not only receives dopaminergic terminals, but also has noradrenergic innervations from locus coeruleus and serotoninergic (5-HT) innervations from the raphe nucleus. There is evidence that not only dopaminergic neurotransmission is modulated by RAS. Ang II, via AT_1 receptors, mediates the enhancement of noradrenaline (NA) transporter expression, tyrosine hydroxilase and DA-beta-hydroxylase transcription [47]. More recent evidence revealed Ang II stimulation of NA neuromodulation, increasing vesicular trafficking via AT_1 receptors in brain neurons [65]. In our laboratory, we found that repeated oral treatment (5 days) with a non-peptide AT_1 receptor antagonist (candesartan cilexetil, 3 mg/kg) decreases the spontaneous firing of NA neurons (unpublished data table 1). Our results supported a role of RAS in catecholaminergic neuromodulation. Ang II not only modulates NA release, but also regulates the synthesis and release of serotonin in the brain [48].

Table 1. Spontaneously firing unitary cell registers in locus coeruleus from rats pretreated with candesartan (cv) 3 mg/kg, p.o; 1 dose (acute candesartan) or 5 doses, one per day

administration (repeated candesartan). The registers were done 2 hours after the last administration of the cv. The preliminary data are expressed as means ± SEM of the number of spontaneously active cells per tract (left column), and means ± SEM of firing rate (firing/sec) of spontaneously active cells (right column)

GROUP	Active cells (cells/tract)	Firing rate (firing/sec)
Vehicle	3.20 ± 0.24 (3)	1.59 ± 0.28 (7)
Acute candesartan	4.60 ± 0.28 (2)	1.20 ± 0.15 (13)
Repeated candesartan	3.25 ± 0.67 (4)	0.90 ± 0.08 (24)

Brain Renin-Angiotensin System and Drug Abuse

There is considerable evidence that DA neurotransmission in the Cpu plays a key role in long-term neuroadaptative changes induced by stress, or psychostimulants such as cocaine or amphetamine. Repeated exposure to amphetamine, as with most addictive drugs, results in a progressive and enduring enhancement of its psychomotor and positive reinforcing effects.

The enhanced response to psychostimulants, a phenomenon termed behavioral sensitization, relies on time-dependent neuroplastic changes in the brain circuitry involved in motivational behavior [66-68]. These changes are associated with long-lasting hyperreactivity of the mesolimbic dopaminergic pathway [69-71]. The evidence indicates that exposure to a drug of abuse did not need to be repeated to induce locomotor sensitization; thus studies in mice and rats showed that a single exposure to psychostimulants (amphetamine or cocaine) induced behavioral sensitization [72, 73]. The sensitization process encompasses two temporally distinct phases: induction and expression [69, 74]. Neuroadaptive changes in mesotelencephalic dopaminergic projections play a key role in the induction and expression of amphetamine sensitization. Sensitization can be induced by microinjection of amphetamine into the ventral tegmental area; its expression is associated with time-dependent adaptations in forebrain DA-innervated areas, such as the nucleus accumbens and CPu.

Dopaminergic neurotransmission in the nucleus accumbens and CPu plays a critical role in the locomotor and stereotypic effects of psychostimulant drugs. However, other neurotransmitter or neuromodulator systems are linked to DA neurons that may also contribute to the regulation underlying drug dependence [75]. All anatomical and functional evidence described above in this chapter may support the hypothesis that RAS could be involved in the neuroadaptative changes induced by drugs of abuse, such as amphetamine, changes that are related to the development of behavioral and neurochemical sensitization.

There is indirect evidence of RAS participation in these neuroadaptative changes. A history of sodium depletion, which activates RAS and Ang II synthesis, was found to have cross-sensitization effects, leading to enhanced psychostimulant responses to amphetamine [76]. In another study, previous depleted and repleted sodium rats showed enhanced locomotor activity in an open field test when challenged with morphine (1 mg/kg, s.c.). These studies showed that behavioral responses induced by sodium deficiency and morphine treatment cross-sensitize [77]. Recently, behavioral cross-sensitization was also described

between sodium depletion and cocaine. In rats, repeated administration of the mineralocorticoid agonist, deoxycorticosterone acetate (DOCA), initially induces incremental increases in daily hypertonic saline consumption (i.e., sensitization of sodium appetite) in spite of the sodium retention [78]. It was found that animals pretreated with DOCA without access to saline showed greater locomotor responses to cocaine than animals receiving vehicle treatment. The result of this experiment indicates that treatments generating a sustained salt appetite (which would imply activation of RAS), and producing cocaine-induced psychomotor responses, show reciprocal behavioral cross-sensitization [78].

There is recent evidence that supports a direct relationship between RAS and behavioral sensitization. It was found in our laboratory that Ang II AT_1 receptors are involved in the neuroadaptative changes induced by a single exposure to amphetamine and that such changes are related to the development of behavioral and neurochemical sensitization. The study examined the expression of amphetamine-enhanced (0,5 mg/kg, i.p.) locomotor activity in animals pretreated with candesartan cilexetil (3 mg/kg, p.o. x 5 days) 3 weeks after an amphetamine injection (5 mg/kg, i.p.) figure1..

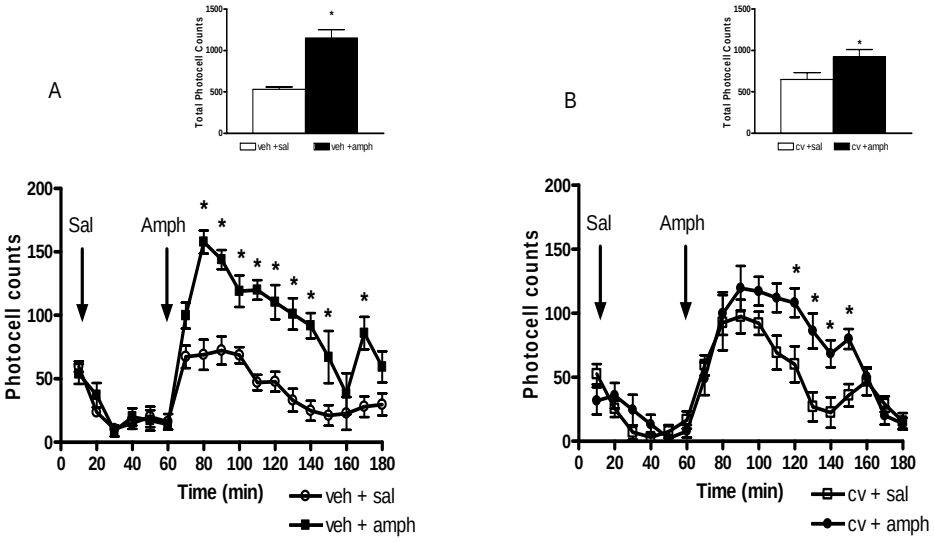

Figure 1. AT_1 blockade attenuated behavioral sensitization to amphetamine challenge (0.5 mg/kg,i.p.) in rats with candesartan (cv) 3 mg/kg, p.o. or vehicle (veh) 1 ml/kg, p.o.; and amphetamine (amph) 5 mg/kg, i.p. or saline (sal) 1 ml/kg, i.p. (n=8); 3 weeks after treatment. Data are expressed as mean ± SEM of photocell counts per 10 min interval. (A) veh + sal vs. veh + amph group, photocell counts per 10 min block. (B) cv +sal vs. cv + amph, photocell counts per 10 min block. *$p \leq 0.01$ (two-way ANOVA, Bonferroni post test). The figures below correspond to the data of graphics (A) and (B) expressed as mean ± SEM of total photocell counts per 120 min after amphetamine challenge. *$p \leq 0.01$ (one-way ANOVA, Student-Newman-Keuls post test).

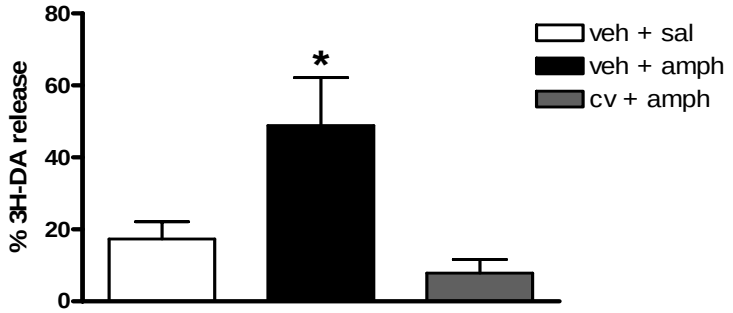

Figure 2. AT_1 receptor blockade attenuated neurochemical sensitization to amphetamine K+ 28 mM-evoked 3H-DA release from superfused CPu slices of rats pretreated with candesartan (cv) 3 mg/kg, p.o. or vehicle (veh) 1 ml/kg, p.o.; and amphetamine (amph) 5 mg/kg, i.p.; or saline (sal) 1 ml/kg, i.p. (n= 6); 3 weeks after pretreatment. Data are expressed as percent increase above their basal 3H-DA release (mean ± SEM). *p ≤ 0.01 vs. all treatments (one-way ANOVA, Student- Newman-Keuls post test).

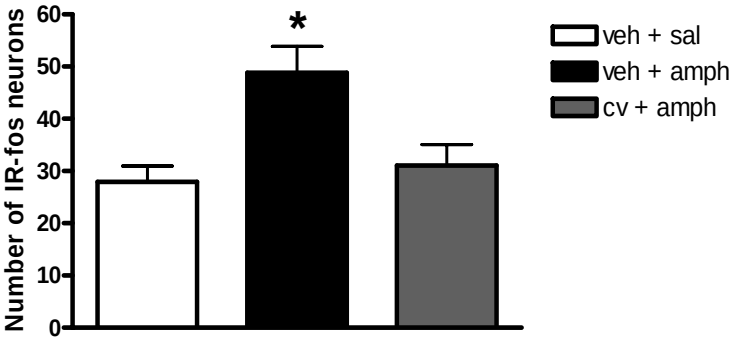

Figure 3. AT_1 receptor blockade attenuated neuronal activity induced by amphetamine. Average number of ir-FOS neurons in the CPu induced by amphetamine challenge 0.5 mg/kg, i.p. from rats pretreated with candesartan (cv) 3 mg/kg, p.o. or vehicle (veh) 1 ml/kg, p.o.; and amphetamine (amph) 5 mg/kg, i.p.; or saline (sal) 1 ml/kg, i.p. three weeks after pretreatment. Values are mean ± SEM; n= 6. *p≤ 0.01 vs. all treatments (one-way ANOVA, Student Newman-Keuls post test).

The AT_1 blockade effects became evident 3 weeks after pretreatment with a single exposure to amphetamine, when the adaptive changes in behavioral response have been described to be more pronounced [73]. Dopaminergic hyperactivity associated with sensitization was tested by measuring 3H-DA release in vitro from CPu slices, induced by K+ stimulus. Behavioral sensitization to amphetamine was confirmed in the two-injection protocol, and pretreatment with an AT_1 blocker, candesartan, attenuated this response [79] figure2. With the same purpose, the involvement of brain ang II AT_1 receptors was studied in the development of neuronal activity changes, and so the immunoreactivity of CPu neurons to c-fos antibody (FOS-ir) was measured after 3 weeks of the same treatment described before figure 3 (unpublished data). These three experimental approaches provide evidence that supports the involvement of brain Ang II AT_1 receptors in the development of amphetamine-induced behavior sensitization. Since it has been suggested that the phenomenon of behavioral sensitization is an adaptative process within addiction to psychostimulants and other drugs of abuse [80], a new role of brain RAS may be indicated

Brain Renin-Angiotensin System and Parkinson´S Disease

The brain RAS may play a key role in the self-propelling mechanism of Parkinson's disease. In support of this, brains obtained postmortem from patients with advanced Parkinson's disease showed markedly reduced levels of AT_1 receptors in the basal ganglia [53], and moreover increased angiotensin-converting enzyme activity was found in cerebrospinal fluid of treated patients [81, 82].

It has been shown that the loss of dopaminergic neurons induced by neurotoxins is amplified by local Ang II, via AT_1 receptors, and moreover Ang II is one of the most important inflammation and oxidative stress inducers, and also produces reactive oxygen species by activation of the NADPH-oxidase complex [82-84]. Microglial activation is involved in this effect and AT_1 receptor antagonists inhibited both dopaminergic neurons degeneration and early microglial and NADPH activation [85].

Rats subjected to intraventricular injection in the 6-hydroxydopamine model of Parkinson's disease showed bilateral reduction in the number of dopaminergic neurons and terminals, with increased expression of AT_1 receptors and decreased expression of AT_2 receptors and these effects were reduced by AT_1 antagonist treatment [83, 84].

The substantia nigra and striatal damage induced by the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) was significantly attenuated by the administration of the ACE inhibitor perindopril [86].

Taking all this into account, Ang II may play a pivotal role, via AT_1 receptors, in increasing the oxidative damage of dopaminergic cells, and treatment with AT_1 antagonists or ACE inhibitors may reduce the progression of Parkinson's disease.

The experimental evidence suggests that manipulation of the brain RAS may constitute an effective unexplored neuroprotective strategy against aging-related risks of dopaminergic degeneration.

CONCLUSION

The aim of this chapter was to summarize the data about the newly-found roles of CPu brain RAS, via AT_1 receptors.

The evidence presented here points to brain RAS as a possible target in the treatment of drugs of abuse or neurodegenerative related disorders. Moreover, the available compounds interfering with RAS, ACE inhibitors and AT_1 blockers, are currently used in hypertension treatment, and they are very well tolerated.

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