Neuropharmacology 76 (2014) 156–168

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Effect of chronic treatment with angiotensin type 1 receptor antagonists on striatal dopamine levels in normal rats and in a rat model of Parkinson's disease treated with L-DOPA

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ARTICLE INFO

Article history:
Received 11 March 2013
Received in revised form
20 June 2013
Accepted 17 July 2013

Keywords:
Angiotensin
Candesartan
L-DOPA
Neuroprotection
Parkinson
Serotonin

ABSTRACT

Beneficial effects of angiotensin type-1 receptor (AT1) inhibition have been observed in a number of brain processes mediated by oxidative stress and neuroinflammation, including Parkinson's disease. However, important counterregulatory interactions between dopamine and angiotensin systems have recently been demonstrated in several peripheral tissues, and it is possible that a decrease in dopamine levels due to AT1 inhibition may interfere with neuroprotective strategies. The present experiments involving rats with normal dopaminergic innervation indicate that chronic treatment with the AT1 antagonist candesartan does not significantly affect striatal levels of dopamine, serotonin or metabolites, as does not significantly affect motor behavior, as evaluated by the rotarod test. Interestingly, chronic administration of candesartan to normal rats induced a marked increase in dopamine D1 and a decrease in dopamine D2 receptor expression. In a rat model of Parkinson's disease treated with L-DOPA, no differences in striatal dopamine and serotonin levels were observed between candesartan-treated rats and untreated, which suggests that chronic treatment with candesartan does not significantly affect the process of L-DOPA decarboxylation and dopamine release in Parkinson's disease patients. Candesartan did not induce any differences in the striatal expression of dopamine D1 and D2 and serotonin 5-HT1B receptors in 6ydroxydopamine-lesioned rats treated with L-DOPA. The results suggest that chronic treatment with AT1 antagonists as a neuroprotective strategy does not significantly affect striatal dopamine release or motor behavior.

This article is part of the Special Issue entitled 'The Synaptic Basis of Neurodegenerative Disorders'.

1. Introduction

It is now known that in addition to the circulating renin-angiotensin system (RAS), local (tissue or paracrine) RAS exist in many tissues, including brain tissue (Re, 2004). The actions of angiotensin II (AII), which is the most important effector peptide of the RAS, are mediated by two main cell receptors: AII type 1 and 2 (AT1 and AT2) receptors. Hyperactivation of local RAS has been associated to decreased longevity and age-related degenerative changes in a number of tissues (Cassis et al., 2010; Min et al., 2009), because local RAS mediates, via AT1 receptors and NADPH oxidase activation, oxidative stress and several key events in inflammatory processes (Marchesi et al., 2008; Mehta and Griendling, 2007). Furthermore, recent studies have shown that the brain RAS is

Abbreviations: 5-HIAA, metabolite 5-hydroxyindolacetic acid; 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine; AII, angiotensin II; AT1, angiotensin type-1 receptor; AT2, angiotensin type-2 receptor; AUC, area under the curve; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC, high performance liquid chromatograph; HVA, homovanillic acid; L-DOPA, L-3,4-dihydroxyphenylalanine methyl ester hydrochloride; ORP, overall rod performance; RAS, renin-angiotensin system; SEM, standard error of the mean; TH, tyrosine hydroxylase; WB, Western blot analysis.

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http://dx.doi.org/10.1016/j.neuropharm.2013.07.016

Table 1
Experimental design.

Series	Group	Dopamine denervation	L-Dopa	Treatment	*n*	Via	Survival period	Analysis	Methodology
I	A_1	No	No	Candesartan	14	Oral	2 weeks	DA, 5-HT, metabolites,	HPLC + WB, Rotarod
				Vehicle	8	Oral	2 weeks	D1R, D2R, TH, behavior	
	A_2	No	No	Candesartan	6	i.p.	150 min	D1R, D2R	WB
				Vehicle	6	i.p.	150 min		
	B	No	No	Candesartan	8	Oral	2 weeks	DA, metabolites, behavior	HPLC + microdialysis, Rotarod,
				Vehicle	8	Oral	2 weeks		immunohistochemistry (TH + CV)
II	C	Yes	6 mg/kg i.p.	Candesartan	12	Oral	1 + 2 weeks	DA, 5-HT, metabolites,	HPLC + WB, Cylinder, rotometer
				Vehicle	13	Oral	1 + 2 weeks	D1R, D2R, 5-HT1BR, TH, behavior	
	D	Yes	6 mg/kg i.p.	Candesartan	8	Oral	1 + 2 weeks	DA, metabolites, behavior	HPLC + microdialysis, Cylinder,
				Vehicle	9	Oral	1 + 2 weeks		rotometer, Immunohistochemistry,
	E	Yes	18 mg/kg i.p.	Candesartan	4	Oral	1 + 2 weeks		(TH + CV)
	F	Yes	6 mg/kg i.p. + deprenyl	Candesartan	5	Oral	1 + 2 weeks		

DA = Dopamine; 5-HT = Serotonin; D1R = Dopamine D1 receptor; D2R = Dopamine D2 receptor; TH = Tyrosine hydroxylase.
5-HT1BR = Serotonin 5-HT1B receptor; HPLC = High Performance Liquid Chromatography; WB = Western Blot; CV = Cresyl Violet.

involved in several brain functions and disorders (for reviews, see Labandeira-Garcia et al., 2012; Phillips and de Oliveira, 2008; Wright and Harding, 2013). Beneficial effects of AT1 inhibition have been observed in a number of brain processes mediated by oxidative stress and neuroinflammation, including animal models of Alzheimer's disease (Kehoe and Wilcock, 2007; Mogi and Horiuchi, 2009), brain ischemia (Lou et al., 2004), traumatic brain injury (Villapol et al., 2012), multiple sclerosis (Platten et al., 2009; Stegbauer et al., 2009) and Parkinson's disease (PD; Grammatopoulos et al., 2007; Labandeira-Garcia et al., 2012; Rodriguez-Pallares et al., 2008). Furthermore, AT1 receptor antagonists have also been shown to provide neuroprotection in rats with high vulnerability to dopaminergic degeneration (Rodriguez-Perez et al., 2012, 2013; Villar-Cheda et al., 2012).

As important interactions between of renin-angiotensin and dopaminergic systems have been demonstrated in peripheral tissues, the effect on dopaminergic function of possible neuroprotective strategies based on manipulation of brain RAS must be clarified. Dopamine and angiotensin systems directly counterregulate each other in renal cells (Gildea, 2009; Khan et al., 2008; Zeng et al., 2006), in which both D1-like agonists and D2-like agonists decrease AT1 receptor expression (Hussain et al., 1998; Zeng et al., 2003), and the signaling from D1 receptors is inhibited after activation of AT1 receptors (Khan et al., 2008). Consistent with these observations, a counterregulatory interaction between dopamine and angiotensin receptors has been observed in rat striatum and substantia nigra, i.e. dopamine depletion or denervation with 6-hydroxydopamine (6-OHDA) induced a significant increase in the expression of AT1 receptors and the NADPH-oxidase complex activity, which decreased as the dopamine function was restored (Villar-Cheda et al., 2010). Furthermore, it has also been found that abnormal brain RAS hyperactivation enhances dopaminergic neuron degeneration, which is inhibited by treatment with AT1 receptor antagonists (Grammatopoulos et al., 2007; Joglar et al., 2009; Mertens et al., 2011; Rey et al., 2007; Rodriguez-Pallares et al., 2008). However, it is possible that inhibition of AT1 receptors as a protective strategy against the above mentioned brain diseases may also induce a counterregulatory decrease in striatal dopamine levels and lead to parkinsonism. Moreover, it is particularly interesting to know if AT1 inhibition may affect levels of L-DOPA-derived dopamine in Parkinson's disease patients, which may lead to enhancement of parkinsonian symptoms. In the present study, we investigated both questions by using rats with normal dopaminergic innervation and rats subjected to dopaminergic denervation (i.e. a 6-hydroxydiopamine model of PD) and treated with L-DOPA.

2. Materials and methods

2.1. Experimental design

Adult male Sprague-Dawley rats (10 weeks old at the beginning of the experiments) were used in the present study. All experiments were carried out in accordance with Directive 2010/63/EU and the Directive 86/609/CEE and were approved by the relevant committee at the University of Santiago de Compostela. All rats were anaesthetized by ketamine/xylazine prior surgery. Neuroprotective treatment with AT1 antagonists has been suggested for several brain diseases unrelated to dopaminergic degeneration (see above). However, this neuroprotective treatment may induce parkinsonism. Therefore, a first series of experiments (Table 1) was carried out to investigate the effects of chronic treatment with the AT1 antagonist candesartan on dopamine and serotonin striatal levels and motor behavior in rats with normal dopaminergic innervation. Rats were treated with candesartan cilexetil in the drinking water (1 mg/kg/day; Astra Zeneca) or vehicle for 2 weeks (i.e. 14 consecutive days). It has been reported that candesartan is the most effective AT1 antagonist in crossing the blood–brain barrier, and that low doses have no effect on blood pressure and inhibit brain AII effects (see for details Gohlke et al., 2002; Unger, 2004). A first group of rats (group A) treated with either candesartan ($n = 14$) or vehicle ($n = 8$) were killed after 2 weeks of treatment and the striata were dissected out and homogenized to study tissue levels of dopamine, serotonin and metabolites as well as the striatal expression of dopamine D1 and D2 receptors (group A1). As the highest effect of candesartan is 2–4 h post injection (Gohlke et al., 2002), some additional group-A rats were killed 150 min after a single intraperitoneal injection of candesartan (1 mg/kg/day; $n = 6$) or vehicle (sodium carbonate and hydrogen chloride in saline, pH 8; $n = 6$) to study the effect of acute treatment with candesartan on the striatal expression of dopamine receptors (group A2). Another group of rats treated with candesartan ($n = 8$) or vehicle ($n = 8$) via the drinking water for 2 weeks were used to study extracellular levels of dopamine and metabolites in the striatum by intracerebral microdialysis in freely moving rats (group B). Motor behavior of rats in group A and B were analyzed by the rotarod tests before sacrifice (group A) or before implantation of microdialysis guide cannulae (group B), as detailed below.

Treatment with AT1 antagonists has also been suggested as a neuroprotective treatment against progression of PD. Therefore, a second series of experiments (Table 1) was carried out to investigate the effects of chronic treatment with the AT1 antagonist candesartan on dopamine and serotonin striatal levels and motor behavior in rats subjected to dopaminergic denervation and treated with L-DOPA, as an animal model of PD patients under L-DOPA treatment. Rats were subjected to unilateral DA denervation with 6-hydroxydopamine (6-OHDA). Rats with maximal lesions were selected with a rotometer (see below), and then treated with candesartan (1 mg/kg/day) or vehicle via the drinking water until being killed. One week after the beginning of the candesartan treatment, the rats were treated with L-DOPA (L-3,4-dihydroxyphenylalanine methyl ester hydrochloride; plus 10 mg/kg benserazide; in a single intraperitoneal injection/day) for two additional weeks treatment (i.e. until the rats were killed) to ensure that animals were under effects of candesartan during the entire period of L-DOPA treatment. A group of 6-OHDA-lesioned rats (group C) treated with candesartan ($n = 12$) or vehicle ($n = 13$) plus L-DOPA (6 mg/kg/day; i.e. equivalent to a low dose in humans; Brodell et al., 2012) were killed and the striata dissected out and homogenized to study tissue levels of dopamine, serotonin and metabolites, as well as the striatal expression of serotonin 5-HT1B and dopamine D1 and D2 receptors. A second group of 6-OHDA-lesioned rats (group D) treated with candesartan ($n = 8$) or vehicle ($n = 9$) and L-DOPA (6 mg/kg/day) were used to study extracellular levels of dopamine and metabolites in the striatum by intracerebral microdialysis in freely moving rats.