Experimental Neurology 224 (2010) 517–526

Contents lists available at ScienceDirect

Experimental Neurology

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

Estrogen and angiotensin interaction in the substantia nigra. Relevance to postmenopausal Parkinson's disease

Ana I. Rodriguez-Perez [a], Rita Valenzuela [a], Begoña Villar-Cheda [a], Maria J. Guerra [a], Jose L. Lanciego [b], Jose L. Labandeira-Garcia [a,*]

[a] Laboratory of Neuroanatomy and Experimental Neurology, Dept. of Morphological Sciences, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela; Spain
[b] Neurosciences Division, CIMA, University of Navarra, Pamplona, Spain, and (1,2) Networking Research Center on Neurodegenerative Diseases (CIBERNED), Spain

ARTICLE INFO

Article history:
Received 13 January 2010
Revised 11 May 2010
Accepted 18 May 2010
Available online 24 May 2010

Keywords:
Angiotensin
Dopamine
Estrogen
Menopause
NADPH
Neuroinflammation
Neuroprotection
Parkinson
Replacement therapy

ABSTRACT

Epidemiological studies have reported that the incidence of Parkinson's disease (PD) is higher in postmenopausal than in premenopausal women of similar age. Several laboratory observations have revealed that estrogen has protective effects against dopaminergic toxins. The mechanism by which estrogen protects dopaminergic neurons has not been clarified, although estrogen-induced attenuation of the neuroinflammatory response plays a major role. We have recently shown that activation of the nigral renin-angiotensin system (RAS), via type 1 (AT1) receptors, leads to NADPH complex and microglial activation and induces dopaminergic neuron death. In the present study we investigated the effect of ovariectomy and estrogen replacement on the nigral RAS and on dopaminergic degeneration induced by intrastriatal injection of 6-OHDA. We observed a marked loss of dopaminergic neurons in ovariectomized rats treated with 6-OHDA, which was significantly reduced by estrogen replacement or treatment with the AT1 receptor antagonist candesartan. We also observed that estrogen replacement induces significant downregulation of the activity of the angiotensin converting enzyme as well as downregulation of AT1 receptors, upregulation of AT2 receptors and downregulation of the NADPH complex activity in the substantia nigra in comparison with ovariectomized rats. The present results suggest that estrogen-induced down-regulation of RAS and NADPH activity may be associated with the reduced risk of PD in premenopausal women, and increased risk in conditions causing early reduction in endogenous estrogen, and that manipulation of brain RAS system may be an efficient approach for the prevention or coadjutant treatment of PD in estrogen-deficient women.

Introduction

Premenopausal women are less likely to suffer from cardiovascular disease than similarly-aged postmenopausal women (Antonicelli et al., 2008; Kannel and Wilson, 1995). Several studies have shown that estrogen-mediated regulation of the renin angiotensin system (RAS) contributes to the cardiovascular benefits of estrogen (Mirza et al., 2008; Mosca et al., 1997), and interaction between estrogen and angiotensin receptors have been observed (Hoshi-Fukushima et al., 2008; Liu et al., 2002; Tsuda et al., 2005; Xue et al., 2007). The peptide angiotensin II (AII), via type 1 receptors (AT1), is one of the most important inducers of inflammation and oxidative stress (OS), and produces reactive oxygen species (ROS) via activation of the NADPH-oxidase complex in various tissues (Li and Shah, 2003; Phillips and Kagiyama, 2002; Touyz et al., 2002). AII plays a major role in cardiovascular disease (Ruiz-Ortega et al., 2001) and other disorders related to OS, inflammation and ageing (Benigni et al., 2009), including neurodegenerative diseases (Kehoe et al., 2009; Mertens et al., 2010.

Over the past decade, evidence has emerged suggesting that the ovarian steroid hormone 17β-estradiol (E2) exerts a neuroprotective effect against Parkinson's disease (PD), and a number of epidemiological studies have reported that the incidence and prevalence of PD is higher postmenopausal than in premenopausal women of similar age (Currie et al., 2004; Ragonese et al., 2006a,b). However, controversial effects of estrogen replacement therapy (ERT) have been also reported (Popat et al., 2005; Shulman, 2002). Various laboratory studies revealed that E2 has protective effects against dopaminergic (DA) cell degeneration (Callier et al., 2002; Dluzen, 1997; Leranth et al., 2000), although the mechanism by which E2 protects DA neurons has not been clarified. Recent studies have suggested that modulation of the glial neuroinflammatory response by E2 is involved in the neuroprotective effects (Morale et al., 2006; Tripanichkul et al., 2006), and it is known that neuroinflammation and microglial activation play a major role in the progression of PD (Rodriguez-Pallares et al., 2007; Wu et al. 2002, 2003a).

The brain possesses a local RAS (Mckinley et al., 2003; Saavedra, 2005), which has been implicated in the regulation of the cardiovascular

* Corresponding author. Dept. of Morphological Sciences, Faculty of Medicine, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain. Fax: +34 981547078.
E-mail address: joseluis.labandeira@usc.es (J.L. Labandeira-Garcia).

0014-4886/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2010.05.015

homeostasis and dopamine release (Brown et al., 1996). However, AII also induces OS, neuroinflammation and NADPH activation, which are key factors in PD. We have recently shown that AII, via AT1 receptors, increases the DA degeneration process triggered by low doses of DA neurotoxins by amplifying the inflammatory response and activation of microglial NADPH oxidase (Rey et al., 2007; Rodriguez-Pallares et al., 2008; Joglar et al., 2009). Although the limits of animal models must be taken into account (Meissner et al., 2004), it is possible that E2 modulates RAS activity in the substantia nigra (SN), and that higher activation of RAS by the absence of E2 plays a major role in the above-mentioned higher risk of postmenopausal women, as previously observed for cardiovascular disease. In the present study we investigated the effect of ovariectomy (ovx) and E2 replacement on the brain RAS in the substantia nigra, and compared RAS activity between rats with estrogen (ovx + E2) and ovariectomized (ovx) rats. In parallel experiments, we confirmed that E2 and AT1 antagonists protect against 6-OHDA-induced DA cell death in the same experimental conditions.

Materials and Methods

Experimental design

Adult female Sprague-Dawley rats (ten weeks old at the beginning of the experiments) were used. Since a potential effect of increased body weight on the RAS activity has been reported (Bokil and Porter, 2000; Krause et al., 2006), rats with very similar body weigh were selected at the beginning of the experiments for ovx and ovx + E2 groups (233.6 ± 3.4 and 232.3 ± 3.7) and a few ovx rats with considerable increase in body weigh at the end of the experiments (i.e. three weeks post ovx) were excluded from the study in order to compare groups of ovx rats and ovx + E2 rats without significant differences in body weigh (286. 1 ± 3.6 and 275.5 ± 5.2, respectively).

Rats were fed with 2014S Teklad Rodent Maintenance Diet (Harlan Laboratories) to minimize the occurrence of natural phytoestrogens. All experiments were carried out in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985). All surgery was performed under ketamine/xylazine anesthesia. Two series of experiments were carried out with different groups of rats (see Table 1). In the first series of experiments ovx rats (group A) were used to determine the effect of E2 (group A3), candesartan (A4; i.e. inhibition of RAS activity by this AT1 receptor antagonist) and treatment with E2+ candesartan (A5) on the 6-OHDA-induced lesion relative to ovx rats treated with 6-OHDA alone (A2) and compared the corresponding controls injected with saline (group A1). A second series of experiments was used to investigate the effect of the presence (group-B ovx rats treated with E2) or absence (group-C ovx rats) of E2 (at doses that were neuroprotective in the experiments performed in series 1) on RAS activity. In experiments designed to study the effect of the presence/absence of E2 on the 6-OHDA-induced cell death or RAS activity, rats with stable high levels of E2 (i.e. similar to proestrus: ovx rats that received implants of E2 as described below; groups A3, A5, B) were compared with rats with stable low levels of E2 (i.e. ovariectomized rats with empty implants; A1, A2, A4, C). Intact females have a 4-day estrous cycle, with a very short proestrus period (i.e. only 12 hours with high levels of E2). It is therefore expected that most of these rats will have levels of E2 similar to ovx rats when killed, and during most of the 6-OHDA lesion period (two weeks), and are thus not suitable for comparison with ovx rats. Furthermore, ovx also removes other sex steroids (in addition to E2). However, only E2 was reintroduced in the ovx + E2 groups to confirm that the observed effects were due to E2 and not to other sex steroids. Rats in the first series of experiments (group A; see Table 1) were injected intrastriatally with 6-OHDA or saline (controls) and treated with E2 or candesartan or saline (controls), then killed for immunohistochemical studies (i.e. location of RAS components in dopaminergic neurons and glial cells, and quantification of dopaminergic cell death), as described below. Rats in the second series of experiments (groups B, C) were killed by decapitation three weeks after ovariectomy. The brains were rapidly removed and the mesencephalon sliced coronally using a tissue chopper set to 1 mm. To obtain SNc, the individual 1 mm tissue slides were dissected on a pre-cooled glass plate under a stereoscopic microscope (Leica M220). The SNc was dissected according to Paxinos and Watson (1986), frozen on dry ice, and stored at -80 °C until processed for investigation of RAS activity by western blot, ACE and NADPH activity, and RT-PCR studies (see below).

Table 1
Experimental Design.

Group	n	S_C implant	i.c.i.	s.c.i.	To Study
A1	5	Empty	Saline	Saline	IH, Control
A2	6	Empty	6-OHDA	Saline	Cell death
A3	6	E2	6-OHDA	Saline	Cell death (E2)
A4	6	Empty	6-OHDA	CAND	Cell death (CAND)
A5	6	E2	6-OHDA	CAND	Cell death (E2+CAND)
B	18	E2	-	-	RAS Activity
C	18	Empty	-	-	RAS Activity

CAND = Candesartan; i.c.i. = Intracerebral injection; IH = Immunohistochemistry; s.c.i. = Subcutaneous injection; THir = Tyrosine hydroxylase immunoreactivity; RAS = Renin-angiotensin system.

Estrogen and Candesartan administration

Female rats were bilaterally ovariectomized through a dorsal incision and received Silastic implants placed subcutaneously in the midscapular region (Dziuk and Cook, 1966; Febo et al., 2005). Silastic implants were prepared with Silastic® tubing (1.47 mm ID × 1.95 mm OD, Dow Corning 508-006; VWR Scientific, Bridgeport, NJ) as described by Febo et al. (2005). Briefly, 5-mm-long sections of tubing were sealed at one end with Silastic silicone sealant (Dow Corning 732; VWR) and left to dry for 30 min. The implants were then either filled with crystalline 17-β- estradiol (17-β- estradiol benzoate; Sigma-Aldrich; groups A3, A5, B) or were left empty (groups A1, A2, A4, C); the open end was then sealed in the same way as the other end. Implants were air-dried and incubated in sterile saline for at least 12-16 h to allow the initial surge of high estradiol levels to be released before use. It has been reported that such implants achieve stable levels of plasma estradiol over 30 d, with a release rate of 75-100 pg/ml per 24 h (see Febo et al., 2005). However, stable levels of E2 have also been found to persist for only 7-24 days (Mannino et al., 2005). Therefore, rats were killed 3 weeks after E2 implantation (i.e. 2 weeks after 6-OHDA injection, see below).

Rats in groups A4 and A5 were injected subcutaneously with candesartan (Astra-Zeneca; 0.5 mg/kg/day) from 10 days before 6-OHDA injection until they were killed. It has been reported that candesartan is the most effective AT1 antagonist in crossing the blood-brain barrier, and that low doses have little effect on blood pressure and block brain AII effects (Unger, 2003). We used the regimen and dosage recommended in previous studies (0.5 mg/kg/day/2 weeks; Nishimura et al., 2000).

6-OHDA lesion

Ovx rats (group A; one week after ovariectomy) were injected intrastriatally with 6-OHDA (A2-5) or vehicle (A1). Thirty minutes prior to intrastriatal injection with 6-OHDA or vehicle, rats were treated with the selective inhibitor for the norepinephrine transporter desipramine (Sigma, 25 mg/kg i.p.) to prevent uptake of 6-OHDA by noradrenergic terminals. The rats were injected in the right striatum with 7.5 μg of 6-OHDA (in 3 μl of saline containing 0.2% ascorbic acid; Sigma, USA). Stereotaxic coordinates were 1.0 mm anterior to bregma, 3.0 mm right of midline, and 5.5 mm ventral to the dura; tooth bar at −3.3. Control animals were injected with 3 μl of sterile saline alone. Rats were killed 2 weeks post-lesion (i.e. 3 weeks post-ovariectomy). Previous studies on the time course of 6-OHDA lesions