Experimental Neurology 247 (2013) 158–164

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

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

Estradiol increases dendritic length and spine density in CA1 neurons of the hippocampus of spontaneously hypertensive rats: A Golgi impregnation study

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ARTICLE INFO

Article history:
Received 6 February 2013
Revised 20 March 2013
Accepted 12 April 2013
Available online 27 April 2013

Keywords:
Estradiol
Spontaneously hypertensive rats
Hippocampus
Dendrites
Spines
Neuroprotection

ABSTRACT

Increased neuronal vulnerability has been described in the brain of spontaneously hypertensive rats (SHR), models of primary hypertension. Previous data indicate that estradiol treatment corrects several dysfunctions of the hippocampus and hypothalamus of SHR. Considering this evidence we analyzed the dendritic arborization and spine density of the CA1 subfield in SHR and Wistar–Kyoto (WKY) normotensive rats with and without estradiol treatment. Five month old male SHR and WKY rats received single estradiol or cholesterol pellets (sham treatment) for 2 weeks. A substantial rise of circulating estradiol (>25 fold) and testicular atrophy was present in all estradiol-receiving rats. In both SHR and WKY rats, estradiol decreased blood pressure by ~20 mm Hg; however, a moderate hypertension persisted in SHR (164 mm Hg). Using a modified Golgi impregnation technique, apical and basal dendrites of the CA1 subfield were subjected to Sholl analysis. Spine density was also statistically analyzed. Apical dendritic length was significantly lower in SHR compared to WKY rats ($p < 0.01$), whereas estradiol treatment increased dendritic length in the SHR group only (SHR vs SHR + estradiol; $p < 0.01$). Apical dendritic length plotted against the shell distances 20–100, 120–200 and 220–300 μm, revealed that changes were more pronounced in the range 120–200 μm between SHR vs. WKY rats ($p < 0.05$) and SHR vs. SHR + estradiol ($p < 0.05$). Instead, basal dendrites were not significantly modified by hypertension or steroid treatment. Spine density of apical dendrites was lower in SHR than WKY ($p < 0.05$) and was up-regulated in the SHR + estradiol group compared to the SHR group ($p < 0.001$). Similar changes were obtained for basal dendritic spines. These data suggest that changes of neuronal processes in SHR are plastic events restorable by estradiol treatment. In conjunction with previous results, the present data reveal new targets of estradiol neuroprotection in the brain of hypertensive rats.

Introduction

Chronic elevation of blood pressure leads to severe brain damage. Neuropathology of hypertension includes remodeling of the microvascular wall, ventricular dilation, vasoconstriction, ischemia, cytotoxic edema, demyelination and micro infarcts (Mulvany, 2002; Rigsby et al., 2005). Inside the brain, the hippocampus is highly vulnerable to the effects of hypertension, as demonstrated by the atrophy of the hippocampus and temporal lobe, cognitive decline and risk of Alzheimer's disease found in hypertensive subjects (Korf et al., 2004; Paglieri et al., 2008; Petrovitch et al., 2000; Skoog et al., 1996).

A pronounced encephalopathy also characterizes the spontaneously hypertensive rats (SHR), a genetic model of essential hypertension commonly employed in morphological, neurochemical and pharmacological studies. Neuropathology of SHR includes hydrocephalus, increased expression of the astrocytic marker glial fibrillary acidic protein (GFAP), blood–brain barrier disruption, cytoskeletal breakdown, decreased growth factor expression, decreased forebrain white matter volume, abnormal neurogenesis and hyperstimulation of the vasopressinergic and angiotensinergic systems (Pietranera et al., 2004, 2006; Ritter et al., 1988; Saavedra, 2009; Tomassoni et al., 2004). Changes in learning and memory displayed by SHR made them models of dementia and the attention-deficit hyperactivity syndrome (Paglieri et al., 2008).

Among the hippocampus subfields, CA1 pyramidal neurons show high vulnerability associated to excitotoxic hyperactivation of n-methyl-d-aspartate (NMDA) receptors (Butler et al., 2010). In this regard, NMDA agonists added to organotypic cultures of hippocampus causes a larger increase of intracellular calcium in the CA1 rather than in the CA3 region (Stanika et al., 2010). This susceptibility explains why a selective death of hippocampus CA1 neurons occurs after ischemia

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http://dx.doi.org/10.1016/j.expneurol.2013.04.007

(Inagaki et al., 2012). Increased vulnerability of CA1 neurons has also been reported in stroke-prone spontaneously hypertensive rat (SHRSP) (Yasui and Kawasaki, 1994). In a recent study, decreased spine density from hippocampal pyramidal neurons of 8 months old SHR have been demonstrated (Sánchez et al., 2011), expanding the vulnerability hypothesis to the effects of hypertension.

It is widely accepted that estrogens exert powerful neuroprotective effects on hippocampal functions. Investigations on the mechanisms of estradiol neuroprotection have included the stimulation of insulin-like growth factor type I (IGF-1) and brain-derived neurotrophic factor (BDNF) signaling cascades, increased phosphorylation of CREB (cyclic AMP-response element binding protein), anti-oxidant effects, mitochondrial protection and stimulation of anti-apoptotic molecules (Azcoitia et al., 2011; Behl, 2002; McEwen, 2002). Estrogen action is due to activation of estrogen receptor α or β subtype, to the rapid activation of mitogen activated protein kinase (MAPK/ERK) pathway or to the membrane G-protein coupled receptor 30 (GPR30) (Azcoitia et al., 2011).

Estrogen treatment prevents the development of hippocampal damage that follows trauma, aging, neurodegeneration, excitotoxicity, oxidative stress, hypoglycemia, amyloid-β peptide exposure and ischemia (Azcoitia et al., 2011; Behl, 2002; Goodman et al., 1996; McCullough and Hurn, 2003; McEwen, 2002; Wise, 2006). Previous results from our laboratory have shown that estradiol is also neuroprotective in the brain of SHR. Treatment of male SHR with estradiol for 2 weeks enhances cell proliferation, doublecortin + neuronal progenitors, density of hilar neurons and mRNAs for BDNF and aromatase in the hippocampus, whereas it decreases hippocampal astrogliosis and arginine vasopressin mRNA in the hypothalamus (Pietranera et al., 2004, 2006, 2008, 2010, 2011). Estradiol treatment also decreases blood pressure of SHR (Belo et al., 2004), which may also help in the recovery of the brain abnormalities caused by hypertension.

In the present study, we assessed if defects of dendritic arbor and spine density of SHR (Sánchez et al., 2011) could be normalized by estradiol treatment. These parameters were investigated in the CA1 pyramidal subfield, an area vulnerable to the effects of hypertension but also showing a privileged sensitivity to natural and synthetic estrogens compared to other subregions (González-Burgos et al., 2012; Gould et al., 1990; Inagaki et al., 2012). The results indicated that dendrite and spine abnormalities of SHR are plastic events recovered by sex steroid treatment.

Materials and methods

Experimental animals and treatments

Five month old male spontaneously hypertensive rats (SHR) and normotensive Wistar–Kioto (WKY) rats were obtained from the Institute of Biology and Experimental Medicine Animal facility. All rats were housed under controlled conditions of temperature (22 °C) and lighting conditions (lights on 07:00–19.00 h) with free access to food and water.

Mean blood pressure was measured by an indirect tail-cuff method (Blood pressure system, Kent Scientific Corporation). Before experimentation, mean blood pressure was 187.7 ± 7.6 mm Hg in SHR and 125.5 ± 5.0 mm Hg in WKY rats ($p < 0.001$). Only SHR showing the highest blood pressure were included in the study. For steroid treatment, a group of hypertensive and normotensive rats were anesthetized using a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) given ip and implanted sc with a single 32 mg cholesterol pellet containing 12 mg of estradiol benzoate (Sigma) during the last 2 weeks of the experiment. A group of sham-treated rats were implanted with cholesterol pellets only. Previous reports have demonstrated that this estradiol regime decreases astrogliosis, and increases neurogenesis, neuronal density of the hilar region, expression of brain-derived neurotrophic factor (BDNF) and aromatase in SHR (Pietranera et al., 2008, 2010, 2011). A similar estradiol dosage also increases forebrain choline-acetyltransferase immunoreactive neurons of aging rats (Ferrini et al., 1999).

At the time of killing, blood and testis were collected for determination of serum levels of 17 β-estradiol and weight of the testis. Animal experiments followed the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethical Committee of the Institute of Biology and Experimental Medicine. Efforts were made to minimize animal suffering and to reduce the number of animals used in the different experiments.

Golgi impregnation technique

Brains were removed from anesthetized rats after intracardiac perfusion with 3% paraformaldehyde and processed by an osmium-free staining, a variant of the Golgi technique (González-Burgos et al., 1992) previously used in our laboratory (Beauquis et al., 2010). Brains were postfixed by inmersion in the same fixative solution for 24 h. The right and left hemispheres were separated and cut into 5 mm thick blocks. Blocks were kept in a solution of 3.5% potassium dichromate −40% formaldehyde for 48 h, then immersed in 3.5% potassium dichromate −10% formaldehyde overnight, and finally in a solution of 3.5% potassium dichromate in distilled water for the next five nights. After these procedures, tissue blocks were impregnated with a 0.75% silver nitrate solution for 48 h and stored in 30% sucrose at 4 °C. All preceding steps were done in the dark at room temperature. Coronal 200 μm sections were obtained using a vibratome at the level of the dorsal hippocampus (Bregma −2.30 to −3.80 mm) (Paxinos and Watson, 1997). The sections were placed on 2% gelatin-coated microscope slides, flattened with filter paper, placed in a humidity box, dehydrated, cleared and coverslipped with canada balsam medium.

Morphologic criteria for analysis of CA1 pyramidal cells

Golgi impregnation was assessed in the pyramidal neurons of the CA1 dorsal hippocampus according to plates 27–33 of a rat brain atlas (Paxinos and Watson, 1997). The CA1 area was chosen because CA3–CA4 pyramidal cells are much less sensitive to the effects of estradiol treatment (Inagaki et al., 2012; Woolley et al., 1990). Dendrites spanning from pyramidal cells of both hemispheres were drawn using a camera lucida attached to an Olympus BH-2 microscope (magnification 250×).

Pyramidal neurons selected for reconstruction and measurement of the dendritic tree followed a four point morphological criteria: (a) neurons had to be well impregnated, without evidence of incomplete staining; (b) neurons in which bodies or branches were obscured by blood vessels, glia or heavy clusters of dendrites from neighboring impregnated cells were excluded; (c) the apical and basal arborization had to be fully impregnated and mainly intact, without the presence of truncated branches; d) to map out dendrites spanning up or down from the plane of the soma, the arbor was traced through the entire 200 μm width of the section containing the Golgi-stained neuron. Two dimensional reconstruction of the dendritic tree was produced for each neuron by focusing up or down the 200 μm span and drawing each section of the dendrite with the help of a camera lucida at 250× magnification. Although the Sholl method may underestimate changes in the Z axis, the four experimental groups were examined similarly, throughout the 200 μm coronal section. Drawings of the complete neuron and its neurites were scanned with a Hewlett-Packard Scanjet 3610 scanner and then scaled and analyzed with NIH software ImageJ running the Sholl Analysis Plugin v1.0 (written by Tom Maddock and available at http://biology.ucsd.edu/labs/ghosh/software/index.html). An average of 5 Golgi-impregnated neurons per brain was considered to meet the criteria mentioned above for dendrites. Both hemispheres of each animal were used for analysis. The number of rats used for determination of dendritic length was 15 for WKY and WKY + estradiol, 17 SHR and 10 SHR + estradiol.

The Sholl analysis used in the present study (Sholl, 1953) provided a quantitative description of the dendritic tree by evaluating the number of dendrites that crossed through virtual concentric circles or shells distributed at equal distances d, centered in the soma of a neuron. The number of dendritic intersections i per shell was computed and branching complexity was evaluated. Total dendritic length (TDL), estimated by the sum of the products of d by i for each ring, was measured at three different ranges from the center of the neuronal soma (ranges: 0–100 μm, 120–200 μm and 220–300 μm).

The density of dendritic spines was also measured in pyramidal neurons of the CA1 region using an Olympus BH-2 microscope (magnification 1250×). The number of dendritic spines was estimated by a slight modification of the method of Woolley and McEwen (1994). Sampling of dendritic spines followed a four point morphological criteria: (a) belong to clearly distinguishable neurons found in the CA1 subfield; (b) location in the *stratum radiatum* 100 to 300 μm from the soma for apical spines or in the *stratum oriens* 50–80 μm from the soma for basal spines; (c) location in segments of apical or basal second order dendrites at least 20 μm long, with a distance of 20 μm (apical) or 10 μm (basal) from the primary dendrite (d) to remain in the plane of focus. Additionally, sampling of spines across experimental groups took place from one branch location only, in terminal secondary branches that did not bifurcate, and from dendrites of similar diameters. Spines were counted in 15 segments per animal. This number resulted from the analysis of spine density in neurons of the CA1 region showing full impregnation of soma and the dendritic arbor. Thereafter, spines were averaged per animal and results were expressed as the number of spines per μm dendritic length (mean ± S.E.M.). Number of rats in each experimental group was detailed in the determination of dendritic length.

Statistical analysis

Data were expressed as the mean ± S.E.M. Results were analyzed by two-way ANOVA followed by the *post-hoc* Bonferroni test. Statistical analyses were performed with Prism 4 GraphPad software (San Diego, CA, USA). Significance was set at $p < 0.05$.

Results

Serum estradiol levels

Measurements of serum 17 β-estradiol 2 weeks after pellet implantation showed substantial elevations of 17 β-estradiol in the steroid-treated groups compared to the cholesterol implanted rats. A two-way ANOVA analysis demonstrated a significant effect of steroid treatment ($F_{(1,16)} = 152.6$; $p < 0.001$). Post-hoc analysis showed that basal 17 β-estradiol levels of steroid-untreated male rats were similar between WKY (39.8 ± 11.6) and SHR (35.2 ± 2.8) pg/ml serum. A large increase in 17 β-estradiol levels was present in steroid-treated rats, without significant differences between WKY rats (1501 ± 171.8) and SHR (983.4 ± 91.6) pg/ml serum ($p < 0,001$ vs. respective untreated group).

Estradiol effects on mean blood pressure and testis weight

As expected, estradiol treatment for 2 weeks decreased blood pressure in normotensive and hypertensive rats (Belo et al., 2004; Xue et al., 2009). In both cases, blood pressure was decreased by 20 mm Hg although SHR remained moderately hypertensive (164 mm Hg) (Fig. 1). A two-way ANOVA of blood pressure levels revealed a main effect of strain ($F_{(1,16)} = 115.27$; $p < 0.0001$) and steroid treatment ($F_{(1,16)} = 14.97$, $p < 0.01$). Post-hoc tests showed that the estradiol-induced decrease of blood pressure was significant for WKY ($p < 0.05$) and for SHR ($p < 0.05$). The weight of the testis was determined in the four experimental groups as an index of estrogen action. A two-way ANOVA of testis weight indicated a main effect of steroid treatment ($F_{(1,26)} = 548.9$; $p < 0.001$). Post-hoc analysis revealed no differences in testis weight between WKY rats (1.3 ± 0.01 g) and SHR (1.5 ± 0.02 g). However, estradiol treatment produced a strong atrophy of the testis both in WKY rats (0.42 ± 0.06 g) and SHR (0.87 ± 0.02 g) ($p < 0.001$ vs. their respective steroid-untreated groups).

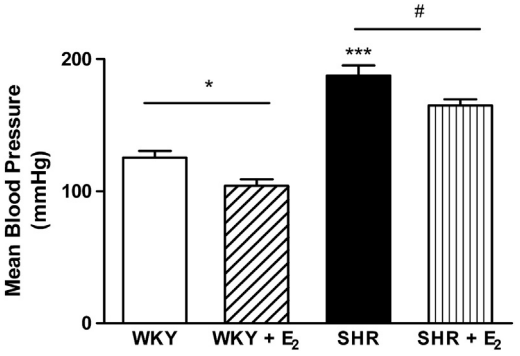

Fig. 1. Effects of estradiol (E_2) treatment on mean blood pressure of WKY rats and SHR. A two-way ANOVA followed by post-hoc comparisons, demonstrated that SHR showed higher blood pressure than WKY and WKY + E_2 rats (***$p < 0.001$), whereas the WKY + E_2 showed lower blood pressure than WKY (*$p < 0.05$). Estradiol treatment of hypertensive rats significantly decreased blood pressure (SHR vs. SHR + E_2, # $p < 0.05$). Results represent the mean ± S.E.M. ($n = 7$ rats per group).

Dendritic length and spine density in SHR and WKY rats with and without estradiol treatment

A two-way ANOVA of the apical dendritic length of the CA1 region revealed significant changes between WKY rats and SHR without and with estradiol treatment ($F_{(1,31)} = 5.283$; $p < 0.05$, Fig. 2A). Post-hoc analysis revealed that dendritic length was lower in SHR compared to WKY rats ($p < 0.01$). Whereas estradiol treatment significantly increased dendritic length in hypertensive rats (SHR vs. SHR + estradiol: $p < 0.01$), it was without effect in WKY rats. When dendritic length was plotted against the shell distances 20–100, 120–200 and 220–300 μm, differences between normotensive and hypertensive rats and the effect of estradiol treatment were most marked for the middle 120–200 μm range ($F\ treatment_{(1,31)} = 4.44$; $p < 0.05$; Fig. 2B) In the post-hoc analysis, significant differences were noted between WKY vs. SHR ($p < 0.05$) and SHR vs SHR + estradiol ($p < 0.05$). The apical dendritic length in the lower (20–120 μm) and higher (220–300 m) range of estradiol-treated SHR was not significant different from SHR in the ANOVA analysis. Quantitative analysis of the branching of the dendritic tree (Fig. 2C) revealed fewer dendritic intersections in steroid untreated SHR than in the estradiol-treated SHR group ($F_{(3, 434)} = 6.168$, $p < 0.01$)

Length of the basal dendritic arbor of the four experimental groups is shown in Fig. 3A, whereas dendritic length segregated into different shell distances is shown in Fig. 3B. ANOVA analysis did not reveal significant differences in basal dendritic length between steroid-untreated SHR and WKY rats or when both groups received estradiol treatment (Fig. 3A). Furthermore, basal dendrites traveled shorter distances (shell distances 20–200 μm) compared to apical dendrites of the same groups (shell distances 20–300 μm). However, in contrast to results of apical dendrites, estradiol did not modify the basal dendritic length at any shell distance from the pyramidal cell soma. No statistically significant differences were found in the number of basal dendritic intersections between the SHR vs. SHR + E_2 groups (Fig. 3C).

Fig. 4 shows examples of camera lucida drawings of the four experimental groups. Golgi impregnation of the WKY and WKY + estradiol neurons showed a strong arborization of the basal and apical dendritic trees. Fig. 4 also shows examples of Golgi staining of SHR neurons, one with diminished arborization (SHR-A) and a second with short, atrophic dendrites (SHR-B). Instead, drawing of CA1 neurons originated in the SHR plus estradiol group, showed a well developed dendritic arbor (SHR + E_2-A and SHR + E_2-B), resembling the WKY neurons.

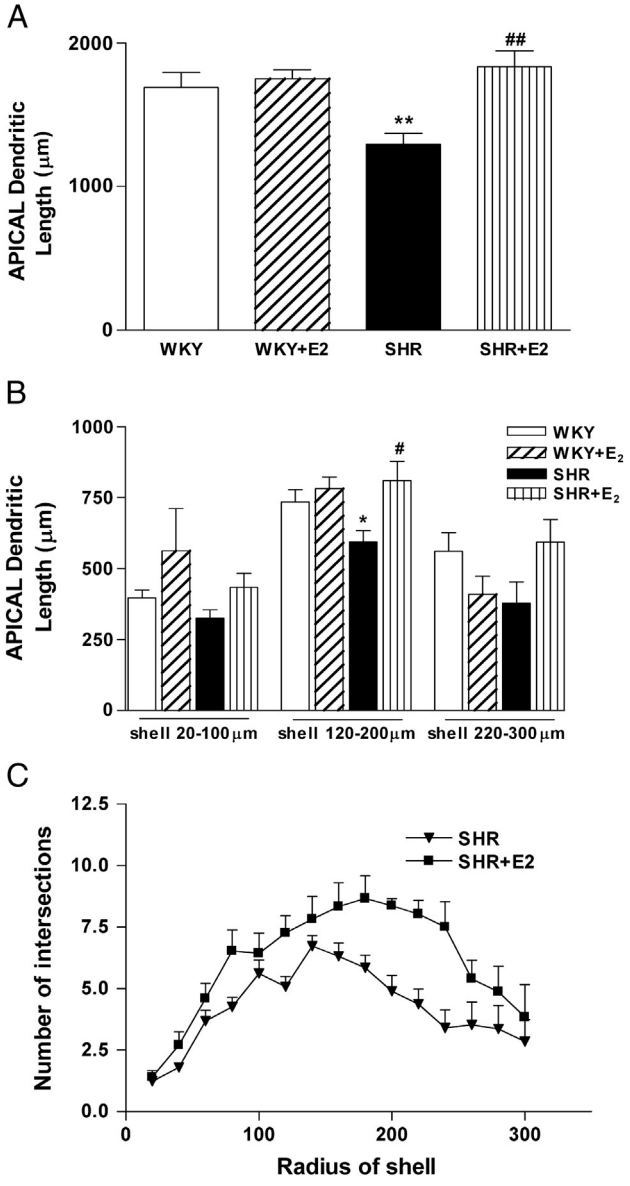

Fig. 2. A. Length of CA1 apical dendrites (μm) in the four experimental groups. Length was significantly decreased in SHR vs. WKY (**$p < 0.01$). Estradiol treatment significantly increased dendritic length in hypertensive rats (SHR vs. SHR + E_2: ##$p < 0.01$) but not in WKY rats. B. Analysis of apical dendritic length according to distances from soma (shells 20–100, 120–200 and 220–300 μm). The SHR group showed shorter dendrites compared to WKY rats in the distance range 120–200 μm (*$p < 0.05$). Estradiol treatment of SHR significantly increased dendritic length in the 120–200 μm range (SHR vs. SHR + E_2: #$p < 0.05$). C. The number of intersections plotted against radius of the shell for apical dendrites showed a significant effect of estradiol treatment for SHR compared to untreated SHR ($p < 0.01$).

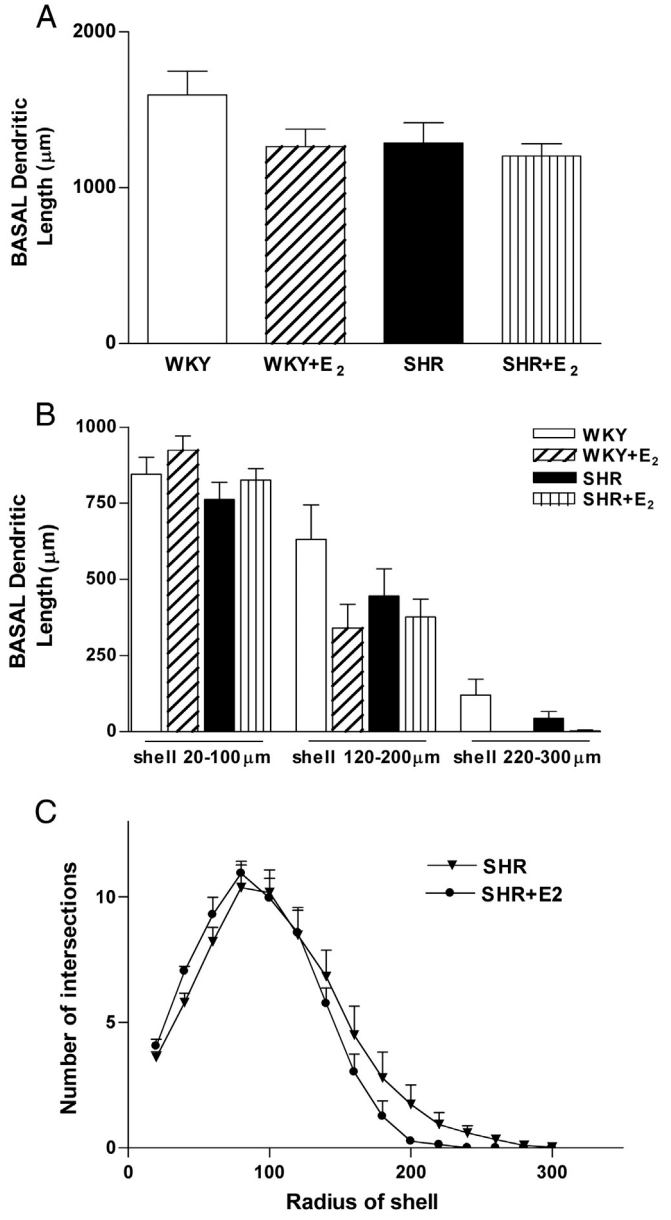

Fig. 3. A. Length of CA1 basal dendrites (μm) was similar in the four experimental groups. B. Analysis of basal dendritic length according to distances from soma (shells 20–100, 120–200 and 220–300 μm) showed no differences between groups. C. There were no differences in the number of intersections per shell between SHR and SHR + E_2 groups.

Spines protruding from second order dendrites were assessed separately for the apical and basal dendrites located in the stratum radiatum and oriens, respectively, and results expressed as the number of spines per μm dendritic length. A two-way ANOVA of the apical dendritic spines revealed significant changes between WKY rats and SHR without and with estradiol treatment ($F_{(1,44)} = 15.59$; $p < 0.001$. Post-hoc analysis demonstrated that SHR contained fewer apical dendritic spines than WKY (Fig. 5A, $p < 0.05$). Estradiol treatment significantly increased spine density of apical dendrites of SHR compared to steroid-untreated SHR ($p < 0.001$), but it was without effect on WKY rats. A two way ANOVA of spines of basal dendrites also showed significant changes between WKY rats and SHR with and without estradiol treatment ($F_{(1,39)} = 10.59$, $p < 0.01$). Post-hoc analysis revealed that spines of basal dendrites of SHR were lower than WKY rats ($p < 0.05$) and that estradiol treatment increased spine density of SHR ($p < 0.01$) but not in WKY rats (Fig. 5B). Finally, analysis of total spine density (apical plus basal) resembled the results of the separate determination of apical and basal dendritic spines. Thus, total spine density of the SHR group was lower than in WKY rats ($p < 0.01$) and in the SHR plus estradiol group ($p < 0.01$). Estradiol was inactive in the WKY rats (Fig. 5C). The photomicrographs of Fig. 6 provided examples of apical spine density in the WKY, WKY plus estradiol, SHR and SHR plus estradiol groups.

Discussion

The present investigation demonstrated that estrogens exerted a positive modulation of the dendritic arbor and spine density in the brain of SHR. In contrast, these parameters remained unaltered in WKY rats receiving similar treatment and showing comparable circulating steroid levels. Therefore, the most interesting results of the present data are that SHR have neuroanatomical responses to estradiol

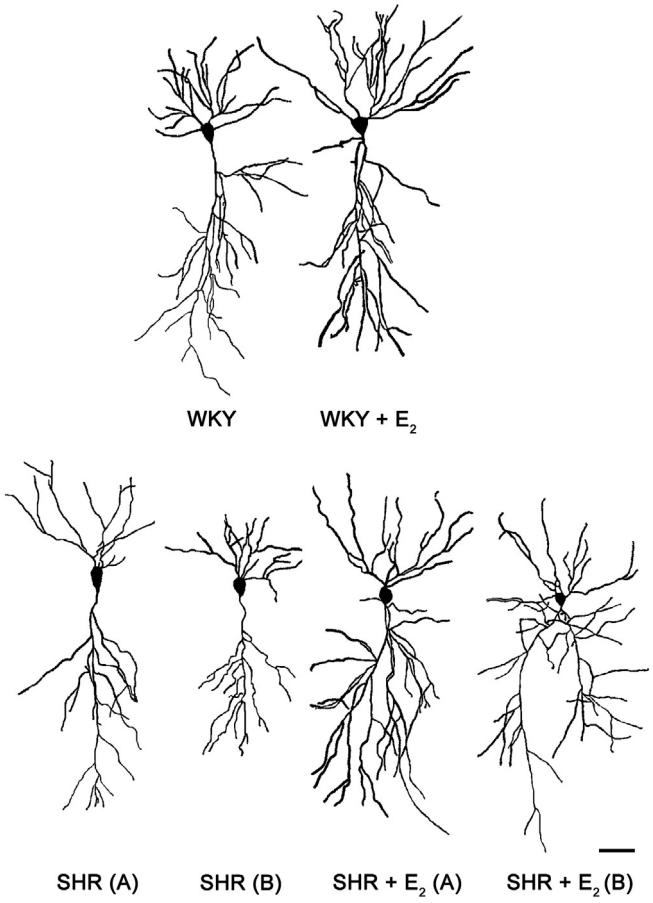

Fig. 4. Camera lucida drawings of apical dendritic arbor from WKY and WKY + E_2 group showed a well developed tree, with longer apical than basal dendrites. Two examples of apical arbor of hypertensive rats showed fewer dendrites (SHR A) and dendritic atrophy (SHR B). Instead, apical dendrites from estradiol-treated SHR (SHR + E_2 A and SHR + E_2 B) resembled the dendritic arbor from normotensive rats. Scale bar: 50 μm.

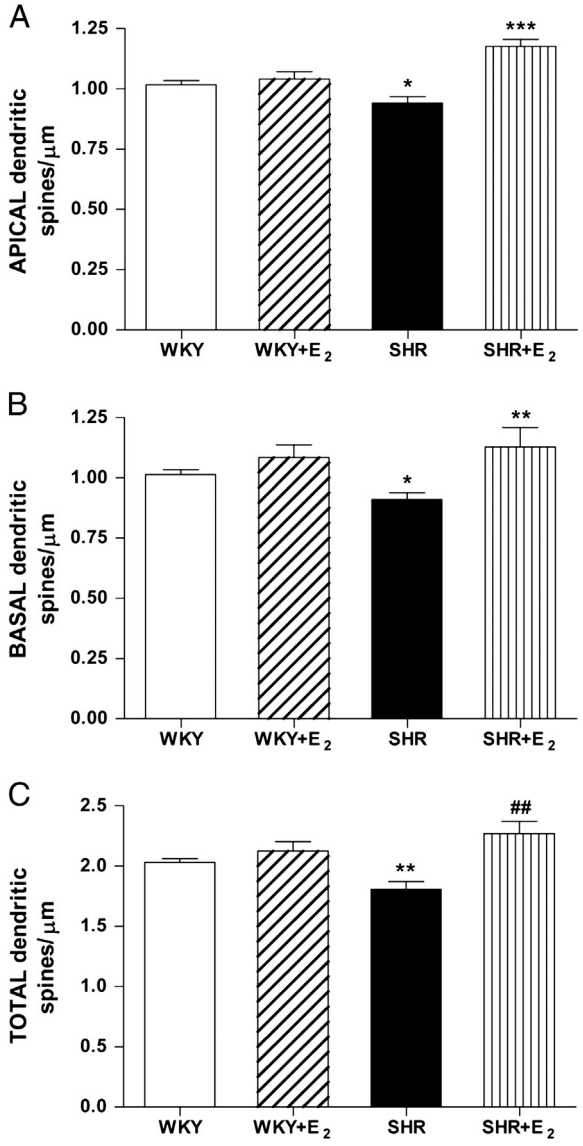

Fig. 5. Density of dendritic spines in the CA1 subfield. A: Fewer apical dendritic spines were present in SHR than in the WKY and WKY + E_2 groups (*$p < 0.05$). Following estradiol treatment, the SHR group showed significantly increased spine density compared to untreated SHR (***$p < 0.001$). B. Basal dendrite spine density was also lower in SHR vs. the WKY rats (*$p < 0.05$). Estradiol treatment increased basal spine density in SHR (SHR vs. SHR + E_2: **$p < 0.01$) but was without effect in the WKY rats C: Total density of spines resembled the results shown for apical and basal dendritic spines (**WKY vs. SHR, $p < 0.01$; ##SHR vs. SHR + E_2, $p < 0.01$).

while WKY normotensive rats do not. Previous work demonstrated that this paradox also applies to other parameters measured in SHR and WKY rats (Pietranera et al., 2010, 2011, 2012).

Sholl analysis of dendritic length from Golgi-impregnated CA1 pyramidal cells showed an atrophied apical, but not basal dendritic tree of SHR compared to normotensive WKY rats. Furthermore, atrophy was more pronounced in the shell distance 120 − 200 μm. A previously utilized estradiol protocol preventing hippocampal abnormalities of SHR (Pietranera et al., 2008, 2010, 2011), significantly increased apical dendritic length in SHR but not in WKY rats. The results also elucidated that spine density of apical dendrites was enhanced in the SHR + estradiol group compared to steroid-untreated SHR. Estradiol treatment also normalized the low density of basal dendritic spines of SHR. Therefore, measurement of total spine density (apical + basal) suggests that estradiol caused a remodeling of the whole cell connectivity, counteracting the damaging effect of hypertension on the CA1 subfield. However, it is recognized that one of the caveats of using the Golgi staining is that this method precludes the analysis of the different types of spines (thin, stubby and mushroom) present in dendrites from the CA1 region. In this regard, ultrastructural and confocal analyses are needed to define which types of spines are deficient in SHR and which are rescued by estradiol treatment in this group.

Neuroanatomically, CA1 pyramidal cells comprise the primary output cells of the hippocampus. Input to CA1 cells originates from glutamatergic Schaffer collaterals of the CA3 region. These collaterals contact apical dendrites in the stratum radiatum and basal dendrites in the stratum oriens (Amaral and Witter, 1989). So it is possible that glutamatergic excitotoxicity plays a role in the vulnerability of CA1 neurons. Expressions of this vulnerability include the reduced dendritic branching, dendritic arbor retraction, loss of dendrites and changes of spine density and morphology in the CA1 subfield caused by inflammatory factors (Sakić et al., 1998), hypobaric hypoxia (Titus et al., 2007), old age (Markham et al., 2005), global cerebral ischemia (Moralí et al., 2012), neurodegeneration of Alzheimer's transgenic mice (Merino-Serrais et al., 2011), hypertension (Sánchez et al., 2011) and type I diabetes mellitus (Beauquis et al., 2010). It has been also recognized that the strong dendritic remodeling and atrophy caused in the CA3 region by chronic stress and glucocorticoid treatment may be also present in rat CA1 pyramidal cells (Christian et al., 2011; Donohue et al., 2006; Lambert et al., 1998). In addition to these damaging factors, it is also recognized that physiological ups and downs of excitatory spines in the CA1 pyramidal region occur in normally cycling female rats (Gould et al., 1990). Estradiol increases

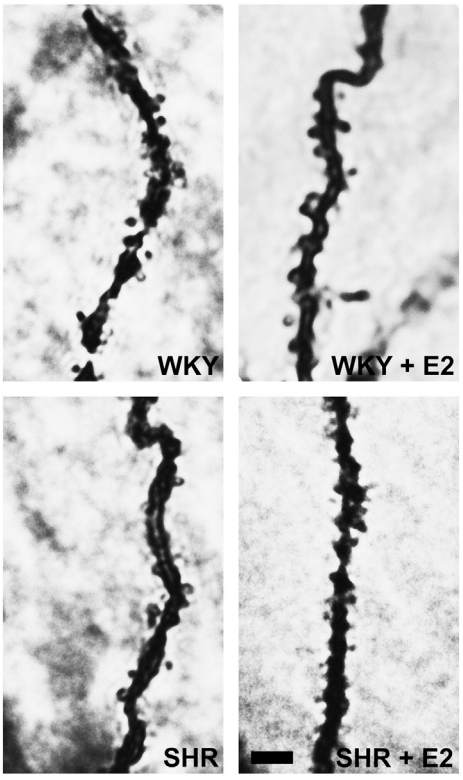

Fig. 6. Representative images of dendrites stained with the Golgi Technique. The microphotographs show abundant apical spines in WKY, WKY + E_2. and SHR + E_2 rats; in contrast fewer spines are observed in SHR. Scale bar: 5 μm.

the density of excitatory synapses in the CA1 region of female rats, although male rats are much less sensitive to estrogen-induced synapse formation in hippocampus (Woolley and McEwen, 1992). The latter finding is in agreement with the results obtained by us in WKY male rats, which remained unresponsive to estradiol treatment.

It is possible that the local microenvironment and the endocrine milieu contribute to the dendritic atrophy and low spine number of SHR. In this regard, adrenal steroids with mineralocorticoid and glucocorticoid activity play a pathogenic role in SHR. Intact adrenal glands or exogenous corticosterone are essential for the development of hypertension in young SHR, while alterations in corticosteroid negative feedback occur in adult animals (Gómez et al., 1996; Hashimoto et al., 1989). Mineralocorticoids also appear to maintain hypertension, since administration of the mineralocorticoid receptor (MR) antagonists RU28318 or spirolactone to SHR reduces blood pressure (BP), whereas infusion of aldosterone increases blood pressure of adrenalectomized SHR, but not WKY rats (Kenyon et al., 1981; Rahmouni et al., 2001). Binding assays have shown increased MR binding capacity in hippocampus and hypothalamus of SHR (Koch et al., 1982), enhanced activation of MR and increased MR mRNA in heart and the peripheral microcirculation of hypertensive rats (Konishi et al., 2003; Mirshahi et al., 1998). SHR respond to a single injection of a mineralocorticoid with an exaggerated response of the AVP system suggesting enhanced mineralocorticoid sensitivity (Pietranera et al., 2004). Thus, disturbances in the response to stress, high sensitivity to adrenal steroids and abnormalities of central and peripheral MR function may play a pivotal role in brain pathology of SHR. In agreement with the MR overdrive hypothesis, we have recently demonstrated hyperexpression of MR in hippocampus and hypothalamus of SHR (Pietranera et al., 2012). However, a relationship of MR overexpression with the decreased dendritic arborisation and low spine density of SHR is presently unknown but merits further appraisal.

Nevertheless, it is important to point out that hippocampal MR is an ambiguous receptor binding with high affinity both aldosterone and corticosterone (cortisol in humans). Whereas aldosterone levels in SHR are low due to down-regulation of the peripheral renin-angiotensin system (Watanabe et al., 1983), adrenal corticosterone is highly responsive to stress (Djordjevic et al., 2007). Over-expressed MR becomes damaging by the generation of reactive oxygen species, which facilitate the inappropriate activation of MR by glucocorticoids (Funder, 2007). A dysfunctional MR increases the synthesis of nitric oxide (NO), and increased activity of NO synthase leading to neurotoxic levels of NO have been reported in the brain of SHR (Ren, 2007). This mechanism could impair the mitochondrial respiratory chain in the vulnerable CA1 subfield neurons and be partly responsible for the dendritic atrophy of SHR.

In this scenario of uncontrolled severe hypertension, estrogens can bring protection to CA1 neurons by virtue of their strong anti-oxidant, anti-glutamatergic, neurotrophic and mitochondrial protective activities (Azcoitia et al., 2011; Behl, 2002; Brinton, 2008; McCullough and Hurn, 2003). An important question is why and how the differential sensitivity to estradiol may arise in SHR. In the hippocampus, an intermediate of estrogen neurotrophic effects is BDNF, which strongly stimulates the generation of dendritic spines (Murphy et al., 1998; Scharfman and MacLusky, 2006). In connection with this function, we have previously reported that a depletion of BDNF mRNA occurs in the dentate gyrus, but not the CA1 or CA3 areas of SHR. Estrogen treatment of SHR precludes BDNF mRNA depletion in the dentate gyrus, without affecting it in the CA1 area (Pietranera et al., 2010). However, input to CA1 neurons is processed at several levels, encompassing the entorhinal cortex, dentate gyrus, the mossy fibers, the CA3 neurons and the Schaffer collaterals. Therefore, up-regulation of BDNF by estradiol in the dentate gyrus may convey a trophic signal to the CA1 pyramidal neurons, explaining the improved response to estradiol treatment of SHR. This hippocampal anatomical circuit has been implicated in long-term potentiation, a cellular correlate of learning and memory involving BDNF (Lu and Chow, 1999; Scharfman and MacLusky, 2006).

However, alternative mechanisms may also explain the heightened sensitivity to estradiol in the SHR strain. For example, since estradiol lowers blood pressure in SHR (Belo et al., 2004; present study) and other models of hypertension (Xue et al., 2009), hypotensive effects may contribute to ameliorate brain dysfunctions of estrogenized SHR. The positive control of estradiol on dendritic spine density in the hippocampus occurs after activation of N-methyl-d-aspartate receptors (Woolley and McEwen, 1994), implying that changes of neurotransmitters may associate with estradiol effects in SHR. Additionally, enhanced aromatase expression in the CA1 pyramidal cell layer occurs following estradiol treatment of SHR, in contrast to the lack of estrogen effects in WKY normotensive rats (Pietranera et al., 2011). Therefore, a combination of exogenous estrogens and those locally synthesized may bolster the hormonal effects on dendritic length and spine density of hypertensive animals.

Acknowledgments

This work was supported by grants from FONCYT (PICT 2007-01044), CONICET (PIP 00542) and the University of Buenos Aires (20020100100089).

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