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# INCREASED AROMATASE EXPRESSION IN THE HIPPOCAMPUS OF SPONTANEOUSLY HYPERTENSIVE RATS: EFFECTS OF ESTRADIOL ADMINISTRATION

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**Abstract—There is high incidence of hippocampal abnormalities in spontaneously hypertensive rats (SHR), including decreased neurogenesis in the dentate gyrus, astrogliosis, low expression of brain derived neurotrophic factor and decreased neuronal density in the hilar region, respect of normotensive Wistar Kyoto rats (WKY). Estradiol treatment given for 2 weeks normalized the faulty hippocampal parameters of SHR, without having effects on WKY rats. The present work studied the potential role of local estrogen biosynthesis in the hippocampus of SHR and WKY, by measuring the expression of aromatase, the key enzyme responsible for estrogen biosynthesis and involved in neuroprotection. We used 4 month old male SHR and WKY, half of which received a single sc pellet of 12 mg estradiol benzoate and the remaining half a cholesterol implant. Hippocampi were dissected and processed for aromatase mRNA expression using real time PCR. A second batch of animals was processed for aromatase and glial fibrillary acidic protein (GFAP) immunocytochemistry. Basal level of aromatase mRNA was higher in SHR respect of WKY. Following estradiol treatment, aromatase mRNA was further increased in the SHR group only. In the hilus of the dentate gyrus of cholesterol-implanted SHR, we found aromatase immunoreactive cell processes and fibers more strongly stained respect of WKY rats. Estradiol treatment of SHR further increased the length of immunoreactive processes and fibers in the hilar region and also increased aromatase immunoreactivity in the CA1 but not the CA3 pyramidal cell region. WKY rats were spared from the estradiol effect. Double-labelling experiments showed that aromatase+ processes and fibers of the hilus of SHR-treated rats did no colocalize with GFAP+ astrocyte cell bodies or processes. In conclusion, basal and estradiol-stimulated aromatase expression was enhanced in hypertensive rat hippocampus. A combination of exogenous estrogens and those locally synthesized may better alleviate hypertensive encephalopathy.** 

**Key words: spontaneously hypertensive rats, aromatase, estradiol, neuroprotection, hippocampus.**

Estrogens function as hippocampal neuroprotectants for pathological conditions, including trauma, aging, neurodegeneration, excitotoxicity, oxidative stress, hypoglycemia, amyloid-$\beta$ peptide exposure and ischemia. In these cases, hippocampal pathology is prevented in part or totally by treatment with estrogens (Goodman et al., 1996; McEwen, 2002; McCullough and Hum, 2003; Garcia-Segura et al., 2006; Wise, 2006; Suzuki et al., 2007). Estrogen effects could be mediated by interaction with intracellular receptors of the ER$\alpha$ or ER$\beta$ subtype although nonclassical, non-genomic mechanisms may also be involved in estrogen effects on the brain (Shughrue et al., 1997; Barha et al., 2009). At the cellular and molecular level, estrogens control adult neurogenesis in the dentate gyrus, prevent cell death, increase neuronal survival and neurite outgrowth, show anti-apoptotic actions, stimulate synaptogenesis, regulate neurotransmission, interact with neurotrophic factors, activate several kinases and increase CREB (cyclic AMP-response element binding protein) phosphorylation (Gould et al., 2000; McEwen et al., 2001; Behl, 2002; Scharfman and Maclusky, 2005; De Nicola et al., 2006; Garcia-Segura et al., 2006; Barha et al., 2009). Therefore, a multiplicity of mechanisms mediates estrogen neuroprotective effects.

Spontaneously hypertensive rats (SHR) are animal models of essential (or primary) hypertension ideally suited to study estrogen neuroprotection. This is due to the fact that besides myocardial fibrosis, abnormalities of microvessels and kidney damage, a pronounced hippocampal pathology distinguishes SHR. Hippocampal changes include neuronal loss, gliosis with increased expression of the astrocyte marker glial fibrillary acidic protein (GFAP), decreased growth factor expression and abnormal neurogenesis (Sabbatini et al., 2000, 2002; Tomassoni et al., 2004; Ueno et al., 2004; Pietranera et al., 2006, 2008; Kronenberg et al., 2007; Hennigan et al., 2009). SHR also present additional brain abnormalities, consisting of blood–brain barrier disruption, hydrocephalus, decrease white matter volume in the forebrain and increases in the synthesis of the vasoactive peptides angiotensin II and arginine vasopressin (Sabbatini et al., 2000; Ueno et al., 2004;

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*Abbreviations:* BDNF, brain-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; ILIGV/area, inverse logarithm of grain intensity per area; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats.

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Pietranera et al., 2004; Saavedra et al., 2006). Behaviorally, SHR have been considered models for dementia, brain aging and the attention deficit hyperactivity syndrome (Jesmin et al., 2004; Paglieri et al., 2008). However, whether hippocampal neurochemical and behavioral changes depend on a pre-existing hypertension or whether hypertension and brain pathology reflect a central defect of this strain is still undecided (Imaki et al., 1998; Rahmouni et al., 2001; Miyakubo et al., 2002; Sabbatini et al., 2002).

Our laboratory has reported that hippocampal vulnerability of hypertensive rats is normalized by estrogens. Thus, estradiol administration to SHR increases neuronal precursor proliferation and differentiation in the subgranular zone of the dentate gyrus, increases the number of hilar neurons in the dentate gyrus, attenuates astrogliosis, and increases the expression of brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus (Pietranera et al., 2004, 2006, 2008, 2010). It is highly auspicious, then, that the hippocampus of hypertensive rats retains estrogen responsiveness. In humans, estrogens and the cytochrome P450 19 (CYP19) gene (aromatase) are thought to be susceptibility factors for essential hypertension (Shimodaira et al., 2008).

In addition to being secreted from peripheral endocrine glands and transported into the brain, there is local estradiol synthesis from cholesterol or androgen precursors by brain cells. Early studies by Naftolin et al. (1996) and Balthazart et al. (1992) have detected aromatase in the limbic and hypothalamic regions by immunocytochemistry. The hippocampus is endowed with all the enzymes necessary for estradiol biosynthesis, including the aromatization step converting C19 androgens into C18 estrogens. Aromatase activity and expression have been reported in the hippocampus of several species, including mice, rats, fish, birds, monkeys and humans (Balthazart et al., 1992; Naftolin et al., 1996; Strobl-Mazzulla et al., 2008; Yague et al., 2008, 2010; Zhao et al., 2008; Yilmaz et al., 2009). The enzyme seems highly active in the hippocampus, since in mice the content of estradiol in this tissue is six-fold higher than in plasma (Hojo et al., 2009). Light and electron microscopy examinations localized aromatase immunoreactivity in neuronal perikarya, dendrites, axonal processes and terminal boutons (Naftolin et al., 1996). A role for neuronal aromatase activity in the hippocampus may involve synaptic development and plasticity (Rune et al., 2006). In addition, reactive astrocytes strongly expressed aromatase following diverse forms of brain injury in several lesioned areas including the hippocampus (Garcia Segura et al., 1999; Azcoitia et al., 2003; Carswell et al., 2005; Saldanha et al., 2009).

Brain estradiol biosynthesis is under regulatory control by a number of factors as reported for human glioma cell cultures, hippocampal dispersion cultures, and different brain regions from rat, mouse, quail, songbirds and fish (Zhao et al., 2008; Munetsuna et al., 2009; Prange-Kiel et al., 2009; Yague et al., 2009; Yilmaz et al., 2009; Charlier et al., 2010). Both androgens and estrogens show modulatory effects on aromatase, explained by the presence of androgen and estrogen-responsive elements in the CYP19 gene (Harada et al., 1993; Lephart, 1996; Bourguiba et al., 2003; Strobl-Mazzulla et al., 2008; Zhao et al., 2008; Yilmaz et al., 2009). Interestingly, tonic estrogen treatment of ovariectomized mice increases by 69% aromatase gene expression in the hippocampus, whereas cyclic estradiol administration has the opposite effect (Iivonen et al., 2006).

Therefore, taken into consideration the reported estradiol regulation of brain aromatase and the effects of estrogens in the hippocampus of SHR, the present investigation pursued three main objectives. First, to identify possible differences between the hippocampus of SHR and the control normotensive Wistar Kyoto (WKY) rats in the basal expression of aromatase mRNA and protein. Second, to investigate if exogenously administered estradiol was able to regulate aromatase expression in the hippocampus of SHR and WKY rats, considering previous differences in hormonal responses between the two strains. The third objective intended to localize the enzyme at the cellular level using double immunofluorescence histochemistry for aromatase and the astrocyte marker GFAP.

## EXPERIMENTAL PROCEDURES

### Experimental animals

Four month old male WKY rats and SHR (≈ 250 g body weight) were purchased from Charles River, Madrid, Spain and the University of La Plata, Argentina. Rats were group-housed based on blood pressure values and steroid treatment. Animals were kept under conditions of controlled humidity, temperature (22 °C), a 12:12 h light/dark cycle (lights on at 07.00h) and given food and water *ad libitum*. At this age period, mean blood pressure (BP) measured by a tail cuff method measured 192±10 mm Hg in SHR and 113.0±5 mm Hg in WKY rats ($P<0.001$).

For steroid treatment, animals were anesthetized using isofluorane and implanted sc with a single 12 mg pellet of 17 $\beta$-estradiol benzoate (Sigma, St. Louis, MO, USA) during the last 2 weeks of the experiment. For sham-treatment, rats were also anesthetized and received a 12 mg pellet of cholesterol (Sigma, St. Louis, MO, USA) instead of estradiol. Previous work demonstrated that this estradiol regime normalizes neurogenesis, the number of hilar neurons, growth factor expression, choline-acetyltransferase immunoreactive neurons, glucocorticoid receptors and astrogliosis in the hippocampus and forebrain of SHR and Dexicorticosterone acetate (DOCA)-salt-treated rats and in aging rats (Ferrini et al., 1999; Pietranera et al., 2008). Circulating levels of 17 $\beta$-estradiol were measured at the time of sacrifice (i.e., after 2 weeks of pellet implantation) by radioimmunoassay (Pietranera et al., 2008). Estradiol levels in steroid-untreated rats were similar between WKY 26.7±0.04 and SHR 26.1±2.1 pg/ml serum. A substantial increase in estradiol levels were shown in estradiol-treated WKY 1201.7±192 and SHR 2028.6±297.5 pg/ml serum ($P<0.01$ vs. respective untreated group). Although mean serum estradiol levels appeared higher in the SHR + estradiol group respect of the WKY + estradiol group, differences were not statistically significant using ANOVA and Bonferroni's post hoc test or a non-parametric test followed by Dunn's multiple comparison test ($P>0.05$). Estrogenized and cholesterol-implanted SHR and normotensive control WKY rats were used to quantify aromatase mRNA using real time PCR and for the immunocytochemical detection of aromatase and GFAP. Animal handling followed the Guide for the Care and Use of Laboratory Animals (NIH Guide), and was approved by local Animal Care and Use Committees. Every effort was made to minimize animal suffering and to reduce

the number of animals used to the minimum required for statistical accuracy.

### Quantitative assay of aromatase mRNA by real time PCR

For real time PCR, four groups of animals were used: WKY (10 rats), SHR (10 rats), WKY plus estradiol (12 rats) and SHR plus estradiol (12 rats). Anesthetized rats were killed by decapitation and the whole brain removed from the skull. Bilateral hippocampi were dissected out from the brain and total RNA was then extracted using Trizol reagent (Life Technologies, Invitrogen) and RNeasy Lipid Tissue Mini Kit (Quiagen). Total RNA was subjected to Dnase 1 (Invitrogen) treatment (2 U for 10 min at room temperature) to remove residual contaminating genomic DNA. cDNA templates for PCR amplification were synthesized from 2 $\mu$g of total RNA using a SuperScript II Rnase H reverse transcriptase (Invitrogen) for 60 min at 42 °C in the presence of random hexamer primers. Primers for real-time RT-PCR were designed using Oligo Primer Analysis Software version 6.54 (Molecular Biology Insights Inc., Cascade, CO, USA). Sequences for aromatase gene amplification were sense: 5′-TATTGGAAATGCTGATTGCGG-3′ and antisense: 5′-TTGGGCTTGGGGAAATACTCG-3. Ribosomal 18s was chosen as a housekeeping gene based on the similarity of mRNA expression across all samples templates.

The relative gene expression for the aromatase mRNAs was determined using the ABI PRISM 7500 sequence Detection System (Applied Biosystems, Foster City, CA, USA). Relative gene expression data were calculated using the $2^{-\Delta\Delta ct.}$ method (Livak and Schmittgen, 2001) and it was determined as fold induction with respect to its respective control. PCR was performed in triplicate under optimized conditions: 95 °C at 10 min followed by 40 cycles at 95 °C for 0.15 s and 60 °C for 1 min. Primer concentrations were 0.2 $\mu$M for ribosomal 18s and 0.4 $\mu$M for aromatase.

### Immunocytochemical determination of aromatase and GFAP

For aromatase and GFAP immunocytochemistry, four groups of rats were used: WKY, SHR, WKY plus estradiol and SHR plus estradiol. Each group was comprised of $n=5$ rats. Anesthetized rats were transcardially perfused with 60 ml of 0.9% saline followed by 60 ml 3% paraformaldehyde (PFA) in phosphate buffer (PB), pH: 7.4. After overnight incubation in 3% PFA, brains were transferred to Tris-buffered saline (TBS), pH: 7.4, sectioned frontally at 50 $\mu$m using a vibrating microtome and processed for free-floating immunocytochemistry. Briefly, sections were first treated for 45 min with a solution of methanol (50%) and hydrogen peroxide (5%) in TBS to quench endogenous peroxidase activity. For single immunocytochemistry, sections were then incubated with a rabbit polyclonal anti-aromatase antibody (1/500 dilution) for 48 h at 4 °C. The aromatase antibody was generated at the Cajal Institute against a 15 amino acid peptide corresponding to residues 488–502 of mouse aromatase. Specificity of this antibody has been previously described and cross reacts with rat, human and monkey cytochrome P450 aromatase (Garcia Segura et al., 1999; Yague et al., 2008, 2010). After incubation with the primary antibody, sections were exposed to a biotinylated goat anti-rabbit IgG (dilution 1/1000 dilution, Pierce Meridian, IL, USA) and processed following the Vectastain ABC kit instructions (Vector, Burlingame, CA, USA). Immunoreactions were visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB) in 0.01% hydrogen peroxide. The same number of sections ($n=6$ per animal) were studied for each experimental group.

Double immunocytochemistry staining was employed to discern if aromatase colocalizes with GFAP, a marker of quiescent and reactive astrocytes. In this case, sections were incubated with the above-mentioned aromatase antibody and with a mouse monoclonal anti-GFAP antibody (1/1000 dilution, Sigma, St. Louis, MO, USA). The sections were then incubated with goat anti-rabbit IgG conjugated to Alexa Green 488 and with goat anti-mouse IgG conjugated to Alexa Red 568 both at 1/1000 dilution (Molecular Probes, Eugene, OR, USA). Immunofluorescent microscopic images were examined using a Leica confocal laser microscope (Heidelberg, Germany).

### Densitometric analysis of immunoreactive aromatase

Staining intensity and immunoreactive aromatase area ($\mu m^2$) were determined for the CA1 and CA3 hippocampal areas by computer-assisted image analysis (Labombarda et al., 2002). The Optimas program used in our analysis transforms differences in color intensity of immunopositive cells into gray differences, and results were expressed as the inverse logarithm of grain intensity per area (ILIGV/area; Ferrini et al., 1999; Labombarda et al., 2002). Digitalized images of tissue sections (six sections per rat, five rats in each group) were processed simultaneously under identical operating conditions, such as light beam, wavelength and gray-scale threshold throughout the experiment. Images were acquired at the same magnification using a digital camera Panasonic GP-KR222 connected to an Olympus BH2 microscope and the image analysis software Bioscan Optimas VI.

In addition, digital images from the hilus of the hippocampus were taken. After background substraction and gray-scale threshold determination, aromatase-immunopositive fiber density was quantified by image analysis (Optimas, Bioscan VI) according to previously reported protocols (Abrahám et al., 2000; González et al., 2009; Skup et al., 2002). Surface area covered by aromatase-positive fibers and the length of positive fibers were computed and expressed as aromatase-immunopositive fiber density ($\mu$m immunopositive fiber/0.02 $mm^2$) (González et al., 2009).

### Statistical analysis

Group differences in aromatase mRNA determined by real time PCR and differences in aromatase immunopositive fiber density and intensity was performed by one-way ANOVA followed by Newman–Keuls post hoc test. Results were expressed as mean arbitrary units ± SEM (mRNA), mean ILIGV/area ± SEM (immunoreaction intensity of CA1-CA3 subregions) or mean fiber length ($\mu$m) ± SEM per 0.02 $mm^2$ (immunoreactive fiber length of the hilus). A $P<0.05$ was considered to be significant.

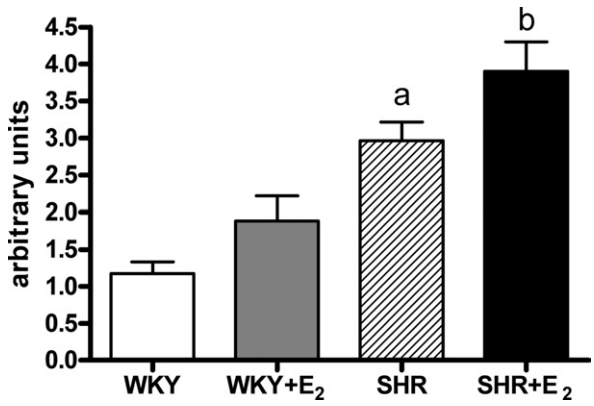


**Fig. 1.** Quantitative analysis of aromatase mRNA in whole hippocampus obtained by real time PCR of normotensive Wistar Kyoto (WKY) rats without or with estradiol treatment (WKY+$E_2$) and spontaneously hypertensive rats (SHR) without or with estradiol (SHR+$E_2$) treatment. Statistical analysis by ANOVA and post hoc test demonstrated: [a] $P<0.05$ vs. WKY; [b] $P<0.05$ vs. all other groups.

## RESULTS

Fig. 1 shows quantitative data obtained by real time PCR of aromatase mRNA levels in whole hippocampus of WKY rats and SHR with or without estradiol administration. A significantly higher basal level of aromatase mRNA was found in SHR (2.76±0.29 au) respect of WKY rats (1.17±0.15) ($F_{(3,40)}$=16.07; $P$<0.05). Estradiol treatment of SHR produced a further elevation of aromatase mRNA, surpassing the high basal levels of the same group implanted with cholesterol. In contrast, estradiol treatment was inactive regarding aromatase mRNA levels of WKY rats (Fig. 1, SHR+$E_2$: 3.36±0.46 au, $P$<0.05 vs. SHR, WKY and WKY+$E_2$ groups). Although real time PCR demonstrated group changes in aromatase gene expression, the neuroanatomical site(s) of the hippocampus responsible for the observed changes remained unknown.

To answer this question, we performed immunocytochemistry with a specific antibody to compare at the microanatomical level the distribution of aromatase protein expression in SHR and WKY rats. Results showed that aromatase immunoreactivity was localized in cell processes and cytoplasm of neurons of several hippocampal areas, such as the pyramidal CA1, CA2, and CA3 subregions (Fig. 2). In the CA pyramidal neurons, staining for aromatase was observed in perikarya, cell processes and occasional small varicosities. We found significant differences in aromatase immunoreactivity in the CA1 (Fig. 3A) but not in the CA3 pyramidal cell layer (Fig. 3B). In the CA1 area, the SHR plus estradiol group showed the highest staining intensity compared to all other groups ($F_{(3,16)}$= 44.28; $P$<0.001).

Weak aromatase immunostaining was found in the granule cell layer of the dentate gyrus. Statistical analysis of the immunoreaction intensity of the cell layer (ILIGV/area) did not reveal group differences (WKY: 0.25±0.006; WKY + estradiol: 0.246±0.001; SHR: 0.255±0.004; SHR + estradiol: 0.258±0.009; $n$=5 rats per group, $P$: NS). In contrast, strong aromatase immunolocalization was found in the hilar region of the dentate gyrus. An interesting finding was a stronger aromatase staining of fiber collaterals, axonal varicosities and occasional cell bodies in the hilar region of the dentate gyrus of SHR compared to WKY rats (Fig. 4A vs. C). The immunoreactive fibers of this hilus might correspond to mossy fibers arising from granule cells of the dentate gyrus (Claiborne et al., 1986). Computerized image analysis to determine the length of immunoreactive processes in this area showed significant group differences (Fig. 5). Thus, length of immunoreactive processes was significantly higher in steroid-naive SHR (1576±23 μm) respect of steroid-untreated WKY (742±66 μm) ($F$(3,15)=15.70; $P$<0.01). Furthermore, estradiol treatment did not change the length of aromatase immunoreactive fibers in the normotensive group, but significantly increased the length of fibers in the SHR group (2110±22 μm; $P$<0.01 vs. all other groups).

We also carried out double immunofluorescence staining in the hilar region of the dentate gyrus to elucidate if aromatase immunopositive fibers colocalize with GFAP+ astrocyte processes. This possibility was considered in view of the fact that a pronounced GFAP+ astrogliosis was present in SHR (Tomassoni et al., 2004; Pietranera et al., 2006). However, as shown in the representative image from an estradiol-treated SHR (Fig. 6), we discarded the

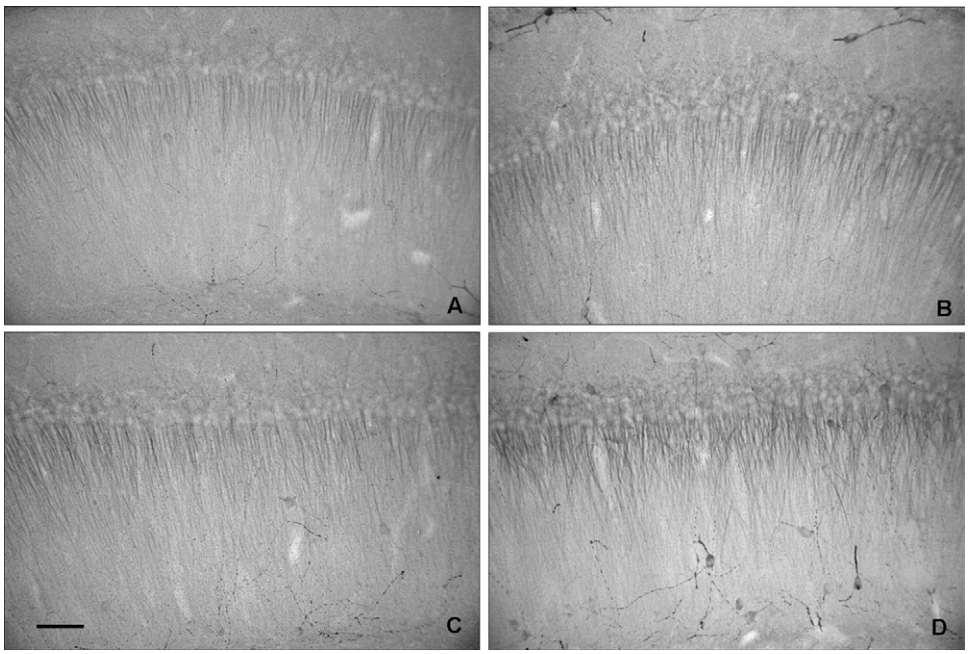


**Fig. 2.** Immunocytochemistry for aromatase in the CA1 pyramidal area of the hippocampus from WKY (A), WKY+$E_2$ (B), SHR (C) and SHR+$E_2$ (D) groups. Stronger immunostaining of CA1 pyramidal cell processes and the presence of scattered immunoreactive neuronal bodies and cell processes in the stratum radiatum, below the CA1 area, are observed in SHR+$E_2$ (D) respect of the other groups. Scale bar: 150 μm.

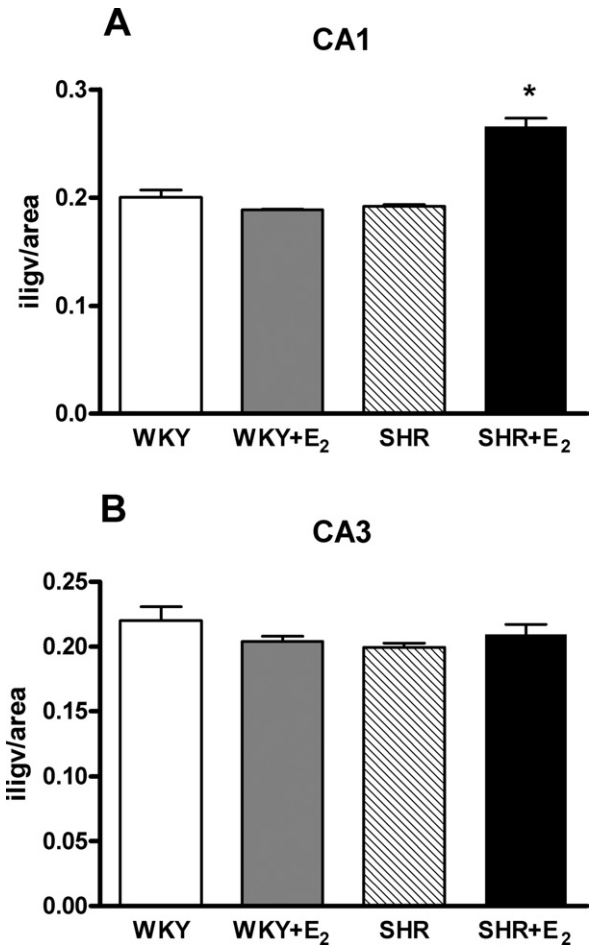


**Fig. 3.** Effects of estradiol on aromatase immunostaining intensity in the CA1 pyramidal cell layer (A) and CA3 pyramidal cell layer (B) in WKY, WKY+$E_2$, SHR and SHR+$E_2$ groups. Statistical analysis using ANOVA and post hoc test demonstrated that higher immunostaining intensity was restricted to the CA1 layer of the SHR+$E_2$ group (* $P<0.001$ vs. all other groups in CA1).

colocalization of GFAP+ astrocyte bodies or processes (red) with aromatase (green) in the hilar region. In some cases, aromatase immunopositive processes aroused from granule cells showing a patchy aromatase immunoreactive cytoplasmic profile (white arrows). Double-labeling for GFAP-aromatase was also studied in the CA1 and CA3 areas, but in agreement with results obtained in the hilar region of the dentate gyrus, aromatase staining was absent from GFAP+ astrocytes.

## DISCUSSION

A comparison basal and estradiol-stimulated aromatase expression between SHR and WKY rats produced three interesting sets of data. First, our results showed for the first time that basal and estradiol-stimulated aromatase mRNA levels were enhanced in hypertensive rat hippocampus, compared to the normotensive WKY rats. Second, our experiments also showed a higher basal and estradiol-stimulated aromatase protein expression in cell processes and fibers delimited by the upper and lower blades of the dentate gyrus and within the hilar region of SHR, respect of WKY rats. Finally, our data demonstrated that estradiol treatment preferentially increased aromatase immunoreaction intensity in the CA1 pyramidal cell region of SHR, respect of WKY, WKY+$E_2$ and SHR groups.

These findings add new information on the effects of hypertension and a sex steroid on aromatase expression in the hippocampus. Two important questions arise from the study of aromatase in SHR. First, why was the enzyme higher in steroid-untreated SHR than WKY? Second, why did estradiol preferentially stimulated aromatase mRNA and protein in SHR over WKY? It is possible that high basal aromatase levels of SHR constituted a reparative response to hippocampal damage. Ischemic damage increases aromatase in the peri-infart area of SHR (Carswell et al., 2005), whereas ischemic damage is greater in aromatase KO mice respect of controls (McCullough and Hurn, 2003), supporting a protective role for brain aromatase. Thus, vascular changes may partly account for the high basal expression of aromatase in SHR. Additionally, several factors including neurotransmitters, neuropeptides, changes of cell signaling and steroids modulate aromatase expression and/or activity. The enzyme responds to several neurotransmitters including the glutamatergic neurotransmission, which is hyperactivated in SHR (Balthazart et al., 2006; Russell, 2001; Li and Pan, 2007). The work of Balthazart et al. has shown that in the quail, calcium-induced phosphorylation rapidly decreases enzyme activity (Balthazart et al., 2003), although participation of this mechanism in SHR is unknown. Among the neuropetides, vasopressin levels are higher in SHR (Imaki et al., 1998; Pietranera et al., 2008); this could lead to modulation of corticosterone levels, and hence to modification of aromatase levels. Glucocorticoids can increase aromatase levels, at least in cultured glioma cells (Yague et al., 2009). In turn, the response to estradiol is in line with previous demonstrations that other faulty hippocampal parameters are normalized by estrogen treatment of SHR without effect on WKY rats (Pietranera et al., 2008, 2010). Along the same direction, animal models of aging and type I diabetes that show hippocampal deficits resembling SHR, are sensitive to estradiol stimulation in contrast to young animals or non-diabetic controls that are not affected by estrogenization (Saravia et al., 2004, 2007). Therefore, it is no unreasonable to hypothesize that estradiol neuroprotection is more easily unveiled under conditions of increased oxidative stress, hypertension, ischemia, aging or neurodegeneration (De Nicola et al., 2009).

There are many reports that testify the positive, negative or lack of hormonal regulation of aromatase in several species. Early work has shown that brain aromatase is regulated by androgens in mammals and by estrogens in birds and fish (Roselli et al., 1985; Balthazart et al., 1992). In monkeys aromatase immunoreactive protein has been found in CA1-CA3 pyramidal neurons, granule cells of the dentate gryus and interneurons but not in astrocytes, without evidences for estrogen sensitivity (Yague et al., 2008). However, hippocampal aromatase expression is raised in hypoestrogenic postmenopausal women (Ishunina et al.,

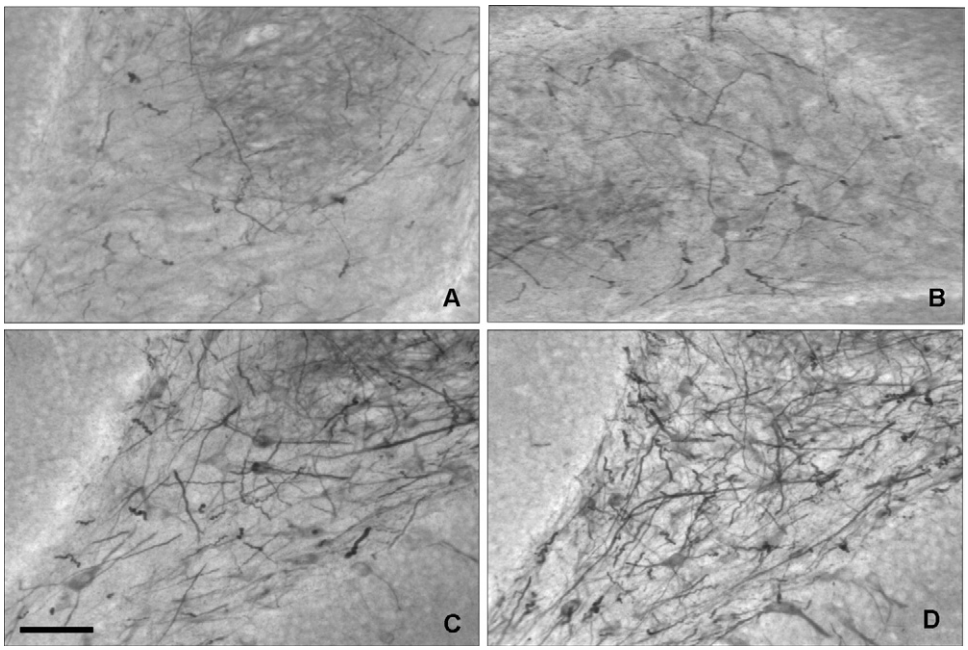


**Fig. 4.** Aromatase protein expression in the dentate gyrus of normotensive Wistar Kyoto rat without (A) and with estrogen treatment (B), SHR (C) and SHR receiving estradiol treatment for 2 wks (D). Immunostaining was more intense in fibers and cell processes in steroid-naive SHR respect of WKY. Estradiol treatment further increased immunostaining for aromatase in cell processes and in sparse neuronal bodies of the dentate gyrus of SHR only. Scale bar: 100 $\mu$m.

2007; Luchetti et al., in press). Alzheimer's neurodegeneration is also associated with several aromatase gene polymorphisms (Butler et al., 2010). In type I diabetic rats, aromatase levels are unchanged in hippocampus at 4 weeks but increased after 12 weeks of hyperglycemia (Burul-Bozkurt et al., 2010). In rat hippocampus cell cultures, retinoic acid shows positive effects on the enzyme (Munetsuna et al., 2009). Recent work has shown that estradiol regulation of aromatase mRNA in mouse hippocampus seems highly dependent on administration protocols, showing increases after tonic steroid exposure and decreases following phasic steroid treatment (Iivonen et al., 2006). The strong stimulation by estradiol of aromatase

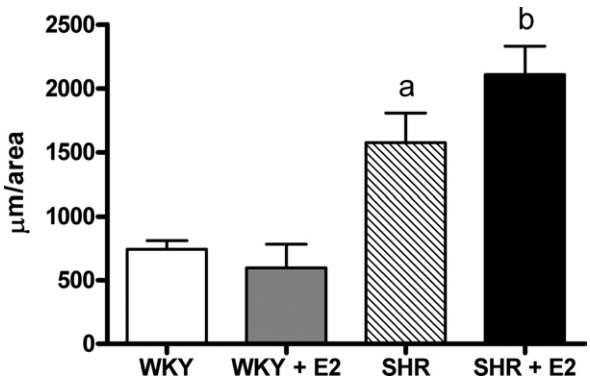


**Fig. 5.** Statistical analysis of the length of aromatase immunoreactive cell processes in WKY, WKY+$E_2$, SHR and SHR+$E_2$ groups. Results were expressed as length in $\mu$m per 21064 $\mu m^2$ area of the hilus of the dentate gyrus. [a] $P<0.01$ vs. WKY and WKY+$E_2$; [b] $P<0.001$ vs. WKY and WKY+$E_2$.

in the central nervous system of fish and mouse hypothalamus (Strobl-Mazzulla et al., 2008; Yilmaz et al., 2009) is explained by the presence of an estrogen-responsive element on the CYP 19 gene (Harada et al., 1993; Lephart, 1996). Other studies have linked estrogen potentiation of

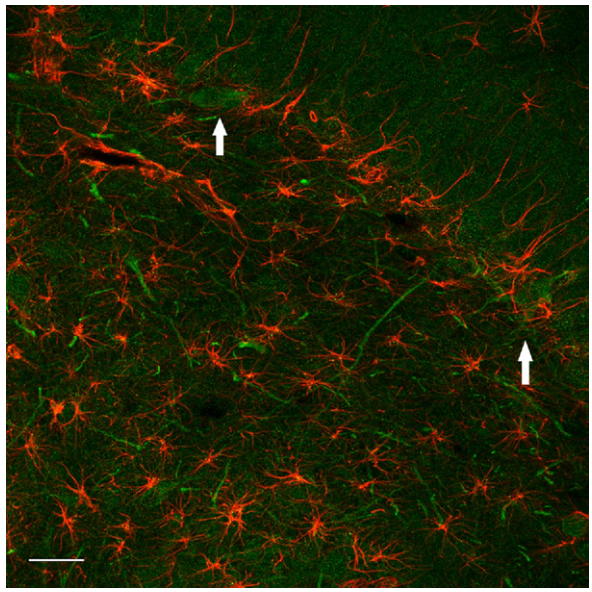

**Fig. 6.** Double immunofluorescence staining for aromatase (green) and glial fibrillary acidic protein (GFAP, red) in the hilus of the dentate gyrus of an SHR receiving estradiol. Aromatase immnunofluorescence is present in fibers and processes but not in GFAP+ astrocytes. White arrows point to aromatase + processes emanating from granule neurons of the dentate gyrus. Scale bar: 50 $\mu$m.

calcium influx via neuronal L-type calcium channels to modulation of hippocampal neuroprotection in an estrogen-receptor independent manner (Sarkar et al., 2008). Of interest to the present work, aromatase gene polymorphisms are linked with essential hypertension in human patients (Shimodaira et al., 2008): Altogether, these reports support the high plasticity but also a complex regulation of brain aromatase, due to its differential modulation by hormones and environmental situations.

It is likely that the increased levels of aromatase and its stimulation by estradiol provide beneficial effects to the hippocampus of SHR. The neuroprotective role of brain aromatase has been thoroughly reviewed (Garcia Segura, 2008; Saldanha et al., 2009), and as shown by Rune et al. (2006), it is involved in hippocampus synaptogenesis and synaptic plasticity. In this trend, our previous work has shown that 2 weeks of exposure of SHR to estradiol enhances the expression of BDNF mRNA in the dentate gyrus, increases BDNF protein levels in whole hippocampal tissue, increases the density of doublecortin-positive immature neurons in the granule cell layer of the dentate gyrus, increases neuronal density in the hilar region and attenuates hippocampal astrogliosis (Pietranera et al., 2006, 2008, 2010). Altogether, previous data in conjunction with the present investigation, suggest that a combination of exogenous estrogens and those locally synthesized by the enhanced aromatase expression may increase local estrogen levels sufficiently to alleviate the encephalopathy of SHR. A question remains, however, regarding the neuroanatomical site where systemic estradiol administration regulates hippocampal aromatase expression. Direct estrogen effects on the hippocampus are well accepted. Nevertheless, additional evidence shows that afferent pathways arising from estrogen-sensitive subcortical regions regulate hippocampal plasticity (Prange-Kiel and Rune, 2006). This paracrine, indirect mechanism may be also involved in estradiol effects on aromatase expression.

Estrogens play important roles in the hippocampus, related to learning and memory, neurotransmission, synaptogenesis, neurogenesis, trophic factor expression, antiaging and antioxidant effects and the control of glial cell function (Goodman et al., 1996; Behl, 2002; McEwen, 2002; Garcia Segura et al., 2006; Manthey and Behl, 2006; Wise, 2006; Spencer et al., 2008; Barha et al., 2009). We and others have demonstrated beneficial effects of estrogens in SHR, and the present work showed that aromatase activation may be a potential parameter involved in this effect. An interesting clue arises from the increased aromatase immunoreactive fiber network and cell processes of the dentate gyrus of SHR. Some of these fibers might correspond to mossy fiber trajectories connecting granule cells of the dentate gyrus with the CA3 pyramidal cells, which in turn connect with CA1 neurons via the Schaffer collaterals (Swanson, 1982; Bayer, 1985). This possibility relies on an exclusive neuronal source of aromatase, discarding the contribution by astrocytes (this work), oligodendrocytes and microglia (Cesi et al., 1993; Yague et al., 2010). It is possible, then, that locally-synthesized neuronal estrogens, in addition to exogenously given estradiol, might influence the "trisynaptic" hippocampal circuit and neurons of the CA1 area involving higher hippocampal functions. The lack of estradiol effect on the CA3 region may represent a ceiling effect, in which under our experimental conditions does not allow additional aromatase stimulation. A ceiling effect has been previously postulated for estradiol modulation of hippocampus spine density (Prange-Kiel and Rune, 2006). Further investigations are warranted to understand the role of estrogen-stimulated aromatase expression in the hippocampal circuit with behavior, learning, memory and neuroendocrine responses of SHR.

## CONCLUSION

The present results show important changes of aromatase expression in the hippocampus from rats with essential hypertension (SHR) respect of normotensive WKY rats. Thus, basal expression levels of aromatase mRNA in whole hippocampus and of aromatase immunoreactive protein of cell processes in the hilus were increased in SHR respect of WKY rats. Tonic estradiol treatment of SHR via pellet implantation produced a further increase of aromatase mRNA in whole hippocampus and also increased immunoreactive aromatase in the CA1 pyramidal cell layer and of cell processes in the hilar region. The same parameters were unmodified in WKY receiving estradiol. Finally, aromatase immunoreactivity did not colocalize with the astrocyte marker GFAP, suggesting its neuronal origin. The current findings support that a combination of exogenous estrogens and those locally synthesized may better alleviate the hippocampal encephalopathy previously described in SHR.

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## REFERENCES

Abrahám I, Harkany T, Horvath KM, Veenema AH, Penke B, Nyakas C, Luiten PGM (2000) Chronic corticosterone administration dose-dependently modulates A$\beta_{(1-42)}$ and NMDA-induced neurodegeneration in rat magnocellular nucleus basalis. J Neuroendocrinol 12:486–494.

Azcoitia I, Sierra A, Veiga S, Garcia-Segura LM (2003) Aromatase expression by reactive astroglia is neuroprotective. Ann N Y Acad Sci 1007:298–305.

Balthazart J, Baillien M, Ball GF (2006) Rapid control of brain aromatase activity by glutamatergic inputs. Endocrinology 147: 359–366.

Balthazart J, Baillien M, Charlier TD, Ball GF (2003) Calcium-dependent phosphorylation processes control brain aromatase in quail. Eur J Neurosci 17:1591–1606.

Balthazart J, Foidart A, Surlemont C, Harada N, Naftolin F (1992) Neuroanatomical specificity in the autoregulation of aromatase-immunoreactive neurons by androgens and estrogens: an immunocytochemical study. Brain Res 574:280–290.

Barha CK, Lieblich SE, Galea LA (2009) Different forms of oestrogens rapidly upregulate cell proliferation in the dentate gyrus of adult female rats. J Neuroendocrinol 21:155–156.

Bayer S (1985) Hippocampal region. In: The rat nervous system Vol 1; forebrain and midbrain (Paxinos G, ed), pp 335–352. New York, NY: Academic Press.

Behl C (2002) Estrogen as a neuroprotective hormone. Nat Rev Neurosci 3:433–442.

Bourguiba S, Lambard S, Carreau S (2003) Steroids control the aromatase gene expression in purified germ cells from the adult male rat. J Mol Endocrinol 31:83–94.

Burul-Bozkurt N, Pekiner C, Kelicen P (2010) Diabetes alters aromatase enzyme levels in sciatic nerve and hippocampus tissues of rats. Cell Mol Neurobiol 30:445–451.

Butler HT, Warden DR, Hogervorst E, Ragoussis J, Smith AD, Lehmann DJ (2010) Association of the aromatase gene with Alzheimer's disease in women. Neurosci Lett 468:202–206.

Carswell HV, Dominiczak AF, Garcia-Segura LM, Harada N, Hutchison JB, Macrae IM (2005) Brain aromatase expression after experimental stroke: topography and time course. J Steroid Biochem Mol Biol 96:89–91.

Cesi PN, Melcangi RC, Celotti F, Martini L (1993) Distribution of aromatase activity in cultured neurons and glia cells. J Steroid Biochem Mol Biol 44:637–639.

Charlier TD, Cornil CA, Ball GF, Balthazart J (2010) Diversity of mechanisms involved in aromatase regulation and estrogen action in the brain. Biochim Biophys Acta 1800:1094–1105.

Claiborne BJ, Amaral DG, Cowan WM (1986) A light and electron microscopic analysis of the mossy fibers of the rat dentate gyrus. J Comp Neurol 246:435–458.

De Nicola AF, Pietranera L, Beauquis J, Ferrini MG, Saravia FE (2009) Steroid protection in aging and age-associated diseases. Exp Gerontol 44:34–40.

De Nicola AF, Saravia FE, Beauquis J, Pietranera L, Ferrini MG (2006) Estrogens and neuroendocrine hypothalamic-pituitary-adrenal axis function. Front Horm Res 35:157–168.

Ferrini M, Piroli G, Frontera M, Falbo A, Lima A, De Nicola AF (1999) Estrogens normalize the hypothalamic-pituitary-adrenal axis response to stress and increase glucocorticoid receptor immunoreactivity in hippocampus of aging male rats. Neuroendocrinology 69:129–137.

Garcia-Segura LM (2008) Aromatase in the brain: not just for reproduction anymore. J Neuroendocrinol 20:705–712.

Garcia-Segura LM, Sanz A, Mendez P (2006) Cross-talk between IGF-I and estradiol in the brain: focus on neuroprotection. Neuroendocrinology 84:275–279.

Garcia-Segura LM, Wozniak A, Azcoitia I, Rodriguez JR, Hutchison RE, Hutchison JB (1999) Aromatase expression by astrocytes after brain injury: implications for local estrogen formation in brain repair. Neuroscience 89:567–578.

González SL, López-Costa JJ, Labombarda F, Deniselle MC, Guennoun R, Schumacher M, De Nicola AF (2009) Progesterone effects on neuronal ultrastructure and expression of microtubule-associated protein 2 (MAP2) in rats with acute spinal cord injury. Cell Mol Neurobiol 29:27–39.

Goodman Y, Bruce AJ, Cheng B, Mattson MP (1996) Estrogens attenuate and CORT exacerbates excitotoxicity, oxidative injury, and amyloid -peptide toxicity in hippocampal neurons. J Neurochem 66:1836–1844.

Gould E, Tanapat P, Rydel T, Hastings N (2000) Regulation of hippocampal neurogenesis in adulthood. Biol Psychiatry 48:715–720.

Harada N, Abe-Dohmae S, Loeffen R, Foidart A, Balthazart J (1993) Synergism between androgens and estrogens in the induction of aromatase and its messenger RNA inthe brain. Brain Res 622:243–256.

Hennigan A, Callaghan CK, Kealy J, Rouine J, Kelly AM (2009) Deficits in LTP and recognition memory in the genetically hypertensive rat are associated with decreased expression of neurotrophic factors and their receptors in the dentate gyrus. Behav Brain Res 197:371–377.

Hojo Y, Higo S, Ishii H, Ooishi Y, Mukai H, Murakami G, Kominami T, Kimoto T, Honma S, Poirier D, Kawato S (2009) Comparison between hippocampus-synthesized andcirculation-derived sex steroids in the hippocampus. Endocrinology 150:5106–5112.

Iivonen S, Heikkinen T, Puolivali J, Helisalmi S, Hiltunen M, Soininen H, Tanila H (2006) Effects of estradiol on spatial learning, hippocampal cytochormome P450 19, and estrogen alpha and beta mRNA levels in ovariectomized female mice. Neuroscience 137:1143–1152.

Imaki T, Naruse M, Harada S, Chikada N, Nakahuma N, Yoshimoto T, Demura H (1998) Stress-induced changes of gene expression in the paraventricular nucleus is enhanced in spontaneously hypertensive rats. J Neuroendocrinol 10:633–643.

Ishunina TA, Fischer DF, Swaab DF (2007) Estrogen receptor alpha and its splice variants in the hippocampus in aging and Alzheimer's disease. Neurobiol Aging 28:1670–1681.

Jesmin S, Togashi H, Sakuma I, Mowa CN, Ueno K, Yamaguchi T, Yoshioka M, Kitabatake A (2004) Gonadal hormones and frontocortical expression of vascular endothelial growth factor in male stroke-prone, spontaneously hypertensive rats, a model for attention-deficit/hyperactivity disorder. Endocrinology 145:4330–4343.

Kronenberg G, Lippoldt A, Kempermann G (2007) Two genetic rat models of arterial hypertension show different mechanisms by which adult hippocampal neurogenesis is increased. Dev Neurosci 29:124–133.

Labombarda F, Gonzalez SL, Gonzalez DM, Guennoun R, Schumacher M, De Nicola AF (2002) Cellular basis for progesterone neuroprotection in the injured spinal cord. Neurotrauma 19:343–355.

Lephart ED (1996) A review of aromatase cytochrome P450. Brain Res Rev 22:1–26.

Li DP, Pan HL (2007) Glutamatergic inputs in the hypothalamic paraventricular nucleus maintain sympathetic vasomotor tone in hypertension. Hypertension 49:916–925.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408.

Luchetti S, Bossers K, Van de Bilt S, Agrapart V, Morales RR, Frajese GV, Swaab DF (in press) Neurosteroid biosynthetic pathways changes in prefrontal cortex in Alzheimer's disease. Neurobiol Aging, in press.

Manthey D, Behl C (2006) From structural biochemistry to expression profiling: neuroprotective activities of estrogen. Neuroscience 138:845–850.

McCullough LD, Hurn PD (2003) Estrogen and ischemic neuroprotection: an integrated view. Trends Endocrinol Metab 14:228–235.

McEwen BS (2002) Estrogen actions throughout the brain. Recent Prog Horm Res 57:357–384.

McEwen BS, Akama K, Alves S, Brake SG, Bulloch K, Lee S, Li C, Yuen G, Milner TA (2001) Tracking the estrogen receptor in neurons: implications for estrogen-induced synapse formation. Proc Natl Acad Sci U S A 98:7093–7100.

Miyakubo H, Hayashi Y, Tanaka J (2002) Enhanced response of subfornical organ neurons projecting to the hypothalamic paraventricular nucleus to angiotensin II in spontaneously hypertensive rats. Auton Neurosci 95:131–136.

Munetsuna E, Hojo Y, Hattori M, Ishii H, Kawato S, Ishida A, Kominami SA, Yamazaki T (2009) Retinoic acid stimulates 17beta-estradiol and testosterone synthesis in rat hippocampal slice cultures. Endocrinology 150:4260–4269.

Naftolin F, Horvath TL, Jakab RL, Leranth C, Harada N, Balthazart J (1996) Aromatase immunoreactivity in axon terminals of the vertebrate brain. An immunocytochemical study on quail, rat, monkey and human tissues. Neuroendocrinology 63:149–155.

Paglieri C, Bisbocci D, Caserta M, Rabbia F, Bertello C, Canade A, Veglio F (2008) Hypertension and cognitive function. Clin Exp Hypertens 30:701–710.

Pietranera L, Saravia F, Gonzalez Deniselle MC, Roig P, Lima A, De Nicola AF (2006) Abnormalities of the hippocampus are similar in deoxycorticosterone acetate-salt hypertensive rats and spontaneously hypertensive rats. J Neuroendocrinol 18:466–474.

Pietranera L, Saravia F, Roig P, Lima A, De Nicola AF (2004) Mineralocorticoid treatment upregulates the hypothalamic vasopressinergic system of spontaneously hypertensive rats. Neuroendocrinology 80:100–110.

Pietranera L, Saravia FE, Roig P, Lima A, De Nicola AF (2008) Protective effects of estradiol in the brain of rats with genetic or mineralocorticoid-induced hypertension. Psychoneuroendocrinology 33:270–281.

Pietranera L, Lima A, Roig P, De Nicola AF (2010) Involvement of brain-derived neurotrophic factor and neurogenesis in oestradiol neuroprotection of the hippocampus of hypertensive rats. J Neuroendocrinol 22:1082–1092.

Prange-Kiel J, Rune GM (2006) Direct and indirect effects of estrogen on rat hippocampus. Neuroscience 138:765–772.

Prange-Kiel J, Fester L, Zhou L, Jarry H, Rune GM (2009) Estrus cyclicity of spinogenesis: underlying mechanisms. J Neural Transm 116:1417–1425.

Rahmouni K, Barthelmebs M, Grima M, Imbs JL, deJong W (2001) Involvement of brain mineralocorticoid receptor in salt-enhanced hypertension in spontaneously hypertensive rats. Hypertension 38:902–906.

Roselli CE, Horton LE, Resko JA (1985) Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system. Endocrinology 117:2471–2477.

Rune GM, Lohse C, Prange-Kiel J, Fester L, Frotscher M (2006) Synaptic plasticity in the hippocampus: effects of estrogen from the gonads or hippocampus? Neurochem Res 31:145–155.

Russell VA (2001) Increased AMPA receptor function in slices containing the prefrontal cortex of spontaneously hypertensive rats. Metab Brain Dis 16:143–149.

Saavedra JM, Benicky J, Zhou J (2006) Angiotensin II: multitasking in the brain. J Hypertens Suppl 24:S131–S137.

Sabbatini M, Catalani A, Consoli C, Marletta N, Tomassoni D, Avola R (2002) The hippocampus in spontaneously hypertensive rats: an animal model of vascular dementia? Mech Ageing Dev 123:547–559.

Sabbatini M, Strocchi P, Vitaioli L, Amenta P (2000) The hippocampus in spontaneously hypertensive rats: a quantitative microanatomical study. Neuroscience 100:251–258.

Saldanha CJ, Duncan KA, Walters BJ (2009) Neuroprotective actions of brain aromatase. Front Neuroendocrinol 30:106–118.

Saravia F, Beauquis J, Pietranera L, De Nicola AF (2007) Neuroprotective effects of estradiol in hippocampal neurons and glia of middle age mice. Psychoneuroendocrinology 32:480–492.

Saravia F, Revsin Y, Lux-Lantos V, Beauquis J, Homo-Delarche F, De Nicola AF (2004) Oestradiol restores cell proliferation in dentate gyrus and subventricular zone of streptozotocin-diabetic mice. J Neuroendocrinol 16:704–710.

Sarkar SN, Huang RQ, Logan SM, Yi KD, Dillon GH, Simpkins JW (2008) Estrogens directly potentiate neuronal L-type Ca2+ channels. Proc Natl Acad Sci U S A 105:15148–151453.

Scharfman HE, Maclusky NJ (2005) Similarities between actions of estrogen and BDNF in the hippocampus: coincidence or clue? Trends Neurosci 28:79–85.

Shimodaira M, Nakayama T, Sato N, Saito K, Morita A, Sato I, Takahashi T, Soma M, Izumi Y (2008) Association study of aromatase gene (CYP19A1) in essential hypertension. Int J Med Sci 5:29–35.

Shughrue PJ, Lane MV, Merchentaler I (1997) Comparative distribution of estrogen receptor $\alpha$ and $\beta$ mRNA in the rat central nervous system. J Comp Neurol 388:507–525.

Skup M, Dwornik A, Macias M, Sulejczak D, Wiater M, Czarkowska-Bauch J (2002) Long-term locomotor training up-regulates TrkB(FL) receptor-like proteins, brain-derived neurotrophic factor, and neurotrophin 4 with different topographies of expression in oligodendroglia and neurons in the spinal cord. Exp Neurol 176: 289–307.

Spencer JL, Waters EM, Romeo RD, Wood GE, Milner TA, McEwen BS (2008) Uncovering the mechanisms of estrogen effects on hippocampal function. Front Neuroendocrinol 29:219–237.

Strobl-Mazzulla PH, Lethimonier C, Gueguen MM, Karube M, Fernandino JI, Yoshizaki G, Patiño R, Strüssmann CA, Kah O, Somoza GM (2008) Brain aromatase (Cyp19A2) and estrogen receptors, in larvae and adult pejerrey fish Odontesthes bonariensis: neuroanatomical and functional relations. Gen Comp Endocrinol 158: 191–201.

Suzuki S, Gerhold LM, Bottner M, Rau SW, Dela CC, Yang E, Zhu H, Yu J, Cashion AB, Kindy MS, Merchenthaler I, Gage FH, Wise PM (2007) Estradiol enhances neurogenesis following ischemic stroke through estrogen receptors alpha and beta. J Comp Neurol 500:1064–1075.

Swanson LW (1982) Normal hippocampal circuitry. Neurosci Res Prog Bull 20:624–637.

Tomassoni D, Avola R, Di Tullio A, Sabbatini M, Vitaioli L, Amenta F (2004) Increased expression of glial fibrillary acidic protein in the brain of spontaneously hypertensive rats. Clin Exp Hypertens 26:335–350.

Ueno M, Sakamoto H, Tomimoto H, Akiguchi I, Onodera M, Huang C-L, Kamenishi K (2004) Blood-brain barrier is impaired in the hippocampus of young adult spontaneously hypertensive rats. Acta Neuropathol 107:532–538.

Wise PM (2006) Estrogen therapy: does it help or hurt the adult and aging brain? Insights derived from animal models. Neuroscience 138:831–835.

Yague JG, Azcoitia I, DeFelipe J, Garcia-Segura LM, Muñoz A (2010) Aromatase expression in the normal and epileptic human hippocampus. Brain Res 1315:41–52.

Yague JG, Garcia-Segura LM, Azcoitia I (2009) Selective transcriptional regulation of aromatase gene by vitamin D, dexamethasone and mifepristone in human glioma cells. Endocrine 35:252–261.

Yague JG, Wang AC, Janssen WG, Hof PR, Garcia-Segura LM, Azcoitia I, Morrison JH (2008) Aromatase distribution in the monkey temporal neocortex and hippocampus. Brain Res 1209: 115–127.

Yilmaz MB, Wolfe A, Cheng YH, Glidewell-Kenney C, Jameson JL, Bulun SE (2009) Aromatase promoter I.f is regulated by estrogen receptor alpha (ESR1) in mouse hypothalamic neuronal cell lines. Biol Reprod 81:956–965.

Zhao C, Fujinaga R, Yanai A, Kokubu K, Takeshita Y, Watanabe Y, Shinoda K (2008) Sex-steroidal regulation of aromatase mRNA expression in adult male rat brain: a quantitative non-radioactive *in situ* hybridization study. Cell Tissue Res 332:381–391.

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