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Estradiol therapy in adulthood reverses glial and neuronal alterations caused by perinatal asphyxia

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ABSTRACT

The capacity of the ovarian hormone 17β-estradiol to prevent neurodegeneration has been characterized in several animal models of brain and spinal cord pathology. However, the potential reparative activity of the hormone under chronic neurodegenerative conditions has received less attention. In this study we have assessed the effect of estradiol therapy in adulthood on chronic glial and neuronal alterations caused by perinatal asphyxia (PA) in rats. Four-month-old male Sprague–Dawley rats submitted to PA just after delivery, and their control littermates, were injected for 3 consecutive days with 17β estradiol or vehicle. Animals subjected to PA and treated with vehicle showed an increased astrogliosis, focal swelling and fragmented appearance of MAP-2 immunoreactive dendrites, decreased MAP-2 immunoreactivity and decreased phosphorylation of high and medium molecular weight neurofilaments in the hippocampus, compared to control animals. Estradiol therapy reversed these alterations. These findings indicate that estradiol is able to reduce, in adult animals, chronic reactive astrogliosis and neuronal alterations caused by an early developmental neurodegenerative event, suggesting that the hormone might induce reparative actions in the Central Nervous System (CNS).

Introduction

The neuroprotective effects of the ovarian hormone 17β-estradiol have been widely described against a great variety of neuropathological conditions in animal neuroprotective models. These include experimental models of stroke, Parkinson's disease, Alzheimer's disease, multiple sclerosis and excitotoxicity, among others (Bourque et al., 2009; Garcia-Segura and Balthazart, 2009; Kipp and Beyer, 2009; Kruse et al., 2009; Lebesgue et al., 2009; Pike et al., 2009; Suzuki et al., 2009). In most of these studies, estradiol has been shown to prevent neuronal loss and gliosis when animals were treated with the hormone either before or shortly after the initiation of brain damage. There is little evidence that estradiol may repair brain tissue after a chronic process of neurodegeneration, in particular when this process has been initiated at early life stages.

Perinatal asphyxia is still an obstetric problem that might induce several alterations in the full-term births. Of the affected newborns, 15–20% die in the postnatal period, and 25% will remain with permanent neurobiological deficits like spasticity, epilepsy, mental retardation (Hill and Volpe, 1981; Amiel-Tison and Ellison, 1986; Vannucci and Perlman, 1997; Gunn, 2000; Shankaran, 2009) and visual alteration in adult life (Osborne et al., 2004). Although the immature brain is relatively protected from hypoxia by adaptive mechanisms, severe insults can trigger self-sustaining damaging cascades lasting for days or weeks and result in prominent injury (Ferriero, 2004). The neurons that are most sensitive and vulnerable to PA are located in the CA1, CA3 and CA4 regions of the hippocampus, in the cerebellum, in layers III, V and VI of the neocortex and in the neostriatum (Kirino, 1982; Petito and Pulsinelli, 1984; Capani et al., 2009). Some of the processes associated with asphyxia occur at synapses site, including nitric oxide release (Capani et al., 1997, 2009; Loidl et al., 1997), elevation of glutamate in the extracellular space (Busto et al., 1989; Choi and Rothman, 1990), aberrant cell signaling (Kamme and Wieloch, 1996), phospholipidic degradation and lipooxidation (Farooqui et al., 1994), and finally excessive release of free radicals (Capani et al., 2001). In previous works, we have shown long term biochemical, cellular and subcellular alterations, including reactive astrogliosis, increase in the immunoreactivity for high weigh neurofilaments (200 kDa NF) (Cebral et al., 2006), and high level of ubi-proteins and free ubiquitin at the post synaptic density (PSD) in neostriatum (Capani et al., 2009). Taken together, this data suggests that PA could lead to neurodegenerative alterations (Capani et al., 2009). These events are well correlated with behavioral alterations (Loidl et al., 2000).

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We have extensively worked on experimental hypothermia as therapeutic tool to reduce the damage produce by hypoxia (Capani et al., 1997, 2003, 2009). Experimental hypothermia has shown to block most of the processes that trigger the mechanisms for the generation of the cell death (Capani et al., 2009). Although hypothermia has been already used in clinical trials, it has been shown to be effective only in very restrictive neurological pathologies produced by PA (Gonzalez and Ferriero, 2008; Shankaran, 2009).

According to our knowledge (Capani et al., 2009) there is not a proper therapeutic tool to treat the neurological diseases produced by PA. Therefore, in this study we test if estrogens are effective to reduce the experimental chronic cytological alterations induced by PA in rats. Our main aims were to determine: (i) whether alterations in the CA1 area of hippocampus were still detectable in four-month-old animals submitted to perinatal asphyxia, and (ii) whether these alterations could be reverted in adult rats by the treatment with 17β-estradiol.

Materials and methods

Animals

All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of Buenos Aires (School of Medicine) and conducted according to the principles of the Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). Sprague–Dawley female rats in the fifteenth day of pregnancy were placed in individual cages and maintained on a 12:12 h light/dark cycle in a controlled temperature (21 ± 2 °C) and humidity ($65 \pm 5\%$) environment. The animals had access to food (Purina chow) and tap water *ad libitum*. One group of animals ($n = 4$) were used as surrogate mothers, another group ($n = 5$) were assigned to perinatal asphyxia procedures, and the remaining animals ($n = 5$) were the mothers of the control pups.

Induction of asphyxia

Five full-term pregnant rats on gestational day 22, were anesthetized (Dorfman et al., 2006), rapidly decapitated and the uterus horns were isolated through an abdominal incision and placed in a water bath at 37 °C for 19 min (sub-severe perinatal asphyxia) (Bjelke et al., 1991; Van de Berg et al., 2003; Capani et al., 2009). We used 19 min as the maximum time of PA because 21 or more minutes in this condition result in a survival rate lower than 3% (Capani et al., 2009). Following asphyxia, the uterus horns were rapidly opened, the pups were removed, the amniotic fluid was cleaned and the pups were stimulated to breathe by performing tactile intermittent stimulation with pieces of medical wipes for a few minutes until regular breathing was established. The umbilical cord was ligated and the animals were left to recover for 1 h under a heating lamp. When their physiological conditions improved, they were given to surrogate mothers who had delivered normally within the last 24 h. The different groups of pups were marked and mixed with the surrogate mothers' normal litters (control animals that were left undisturbed). We maintained litters of 10 pups with each surrogate mother.

17β estradiol treatment

Sixteen adult male rats were injected i.p, 117 days after perinatal asphyxia, with 17β estradiol (water soluble, E4389, Sigma, St. Louis, Mo; 250 μg/kg) or vehicle (0.9% saline solution). Previous studies have shown that doses of estradiol not very different from the one selected for this study, reduce gliosis in male rats after a penetrating brain injury or after immune challenge (Tapia-Gonzalez et al., 2008; Barreto et al., 2009). The injections were repeated daily for 3 days consecutively (up to 119 days after perinatal asphyxia). Animals were distributed in 4 experimental groups: (i) control animals injected with vehicle, (ii) control animals injected with 17β estradiol, (iii) animals submitted to perinatal asphyxia and injected with vehicle and, (iv) animals submitted to perinatal asphyxia and treated with 17β estradiol.

Tissue fixation and immunohistochemistry

At postnatal day 120, sixteen animals were anesthetized with chloral hydrate (28% w/v; 0.1 ml/100 g body weight), and perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were removed and post-fixed in the same fixative solution for 2 h at room temperature, and then immersed overnight at 4 °C in 0.1 M phosphate buffer, pH 7.4. Coronal hippocampal sections (40 μm thickness) were obtained using a Vibratome (VT 1000 S, Leica Microsystems, Wetzlar, Germany).

Immunohistochemistry was performed on free floating sections under moderate shaking. Endogenous peroxidase was quenched (3% H_2O_2, 30% methanol, 70% PBS 0.1 M) and non-specific labeling was blocked using 5% normal goat serum. Sections were incubated overnight at 4 °C with a mouse primary antibody that recognizes 120 kDa neurofilaments (120 NF, 1:500, Chemicon International, Temecula, CA, USA) or a mouse primary antibody that recognizes phosphorylated high and medium molecular weight neurofilament proteins (pH/M NF, 1:250, Chemicon International, Temecula, CA, USA) overnight at 4 °C. After several washes, the sections were incubated for 2 h at room temperature with secondary antibodies (Biotinylated anti mouse IgG, diluted 1:300, Vector Laboratories Inc., Burlingame, CA, USA). Labeling was revealed using the ABC kit (Vector Laboratories Inc., Burlingame, CA, USA). Peroxidase activity was revealed with 0.01% hydrogen peroxide, using 3,3′-diaminobenzidine as the chromogen (DAB, Sigma). Immunostaining was absent when the first antibody was omitted.

For immunofluorescence, tissue sections were blocked for 30 min in phosphate-buffered saline (PBS) containing 0.3% Bovine Serum Albumin (BSA; Sigma, St. Louis, MO, USA) and 0.3% Triton X-100. Sections were incubated overnight at 4 °C with a rabbit polyclonal anti GFAP antibody (1:2000, Sigma St. Louis, MO, USA) or a mouse monoclonal anti MAP-2 antibody (1:500, Sigma St. Louis, MO, USA). After washing in buffer, tissue sections were incubated for 2 h at room temperature with Alexa 488 goat anti-rabbit IgG (1:200, Molecular Probes) or Alexa 594 goat anti-mouse IgG (1:200, Molecular Probes). Sections were counterstained with DAPI (Vector Laboratories Inc., Burlingame, CA, USA) to label cell nuclei and mounted with Vectashield mounting medium. Immunostaining was absent when the first antibody was omitted. In order to minimize inter-assay variations, samples from all experimental groups were processed in parallel.

Morphometric analysis

The volume fraction of immunoreactive material for MAP-2, 120 NF and pH/M NF was estimated using the point-counting method of Weibel (1979) and a grid delimiting 5000 μm^2 in the *striatum radiatum* of CA1. A total area of 75,000 μm^2 was evaluated in each animal. Percentage of reactive area was estimated using Image J Program (Image J 1.41o, NIH, USA). The number of GFAP immunoreactive astrocytes was estimated in the *striatum radiatum* of CA1 by the optical dissector method (Howard and Reed, 1998) using total section thickness for dissector height (Hatton and von Bartheld, 1999) and a counting frame of 55×55 μm. A total of 78 counting frames were assessed per animal. Section thickness was measured using a digital length gauge device (Heidenhain-Metro MT 12/ND221; Traunreut, Germany) attached to the stage of a Leitz microscope. Cell nuclei from GFAP immunoreactive cells that came into focus while focusing down through the dissector height were counted. All counts were performed on coded sections. The volume of the *striatum radiatum* of CA1 was estimated using the point-counting method of Weibel (1979). Since no significant differences in

this parameter were observed among the different experimental groups, the changes in the number of GFAP immunoreactive cells per unit volume with the optical dissector method are assumed to reflect changes in the total number of GFAP immunoreactive cells.

Statistical analysis

Material from four rats was analyzed for each experimental group and for each parameter studied ($n=16$). All statistical analyses were performed by two-way analysis of variances (ANOVAs) with birth condition (CTL and PA) and treatment (Vhi and 17β) as the main factors. When interaction effects were significant, analyses of the simple effects were carried out by post hoc comparisons using Student's *t*-test (two-tailed) adjusted by Bonferroni correction. In any case neither the assumption of normal distribution (Shapiro–Wilk test) nor equality of variances (Levene's test) was rejected. Results were expressed as the mean ± SEM. Differences with a probability of 5% or less were considered to be significant ($P<0.05$). All statistical analyses were done using the PASW Statistics 18 software (SPSS Inc, Chicago, IL, USA).

Results

GFAP immunostaining in the CA1 hippocampal area. Effects of PA and 17β treatment

Hippocampal sections from PA animals treated with vehicle, stained with GFAP and observed at confocal microscopy showed a marked astrogliosis in *striatum radiatum* of CA1 hippocampal area, which is a characteristic feature for chronic process of neurodegeneration (Fig. 1C). The morphometric analysis in the *striatum radiatum* of CA1 confirmed this data. The two-way analysis of variance indicated that the main factors of birth condition and treatment were both significant ($F=26.76$, $P<0.001$; $F=8.27$, $P<0.01$, respectively), and the interaction was also significant ($F=23.44$, $P<0.01$). Post hoc analysis of the simple effects revealed that treatment with 17β estradiol in CTL rats had not significant effect on the number of GFAP immunoreactive astrocytes in comparison to CTL rats injected with vehicle ($P=$ n.s., see Figs. 1-B, 1-A and 2). PA rats injected with vehicle showed a markedly significant increase of the number of GFAP immunoreactive astrocytes in comparison to CTL rats treated with vehicle ($P<0.005$, see Figs. 1-C, 1-A and 2). Estradiol treatment in PA rats significantly reduced the number of GFAP immunoreactive astrocytes in comparison to PA rats treated with vehicle ($P<0.005$, see Figs. 1-D, 1-C and 2), and moreover they did not show significant differences with CTL rats injected with vehicle ($P=$ n.s., see Figs. 1-D, 1-A and 2), suggesting a reversion of astrogliosis associated with hypoxia at birth by 17β estradiol treatment in the adulthood.

Dendritic alterations induced by PA and reversion by 17β estradiol treatment

Confocal studies using MAP-2 were conducted to determine the dendritic changes after PA insult. Animals subjected to PA and treated with vehicle showed focal swelling and markedly fragmented appearance of MAP-2 immunoreactive apical dendrites in the CA1 hippocampal area, compared to control animals treated with vehicle (see Figs. 3-A and 3-C). In addition, the statistical analysis revealed

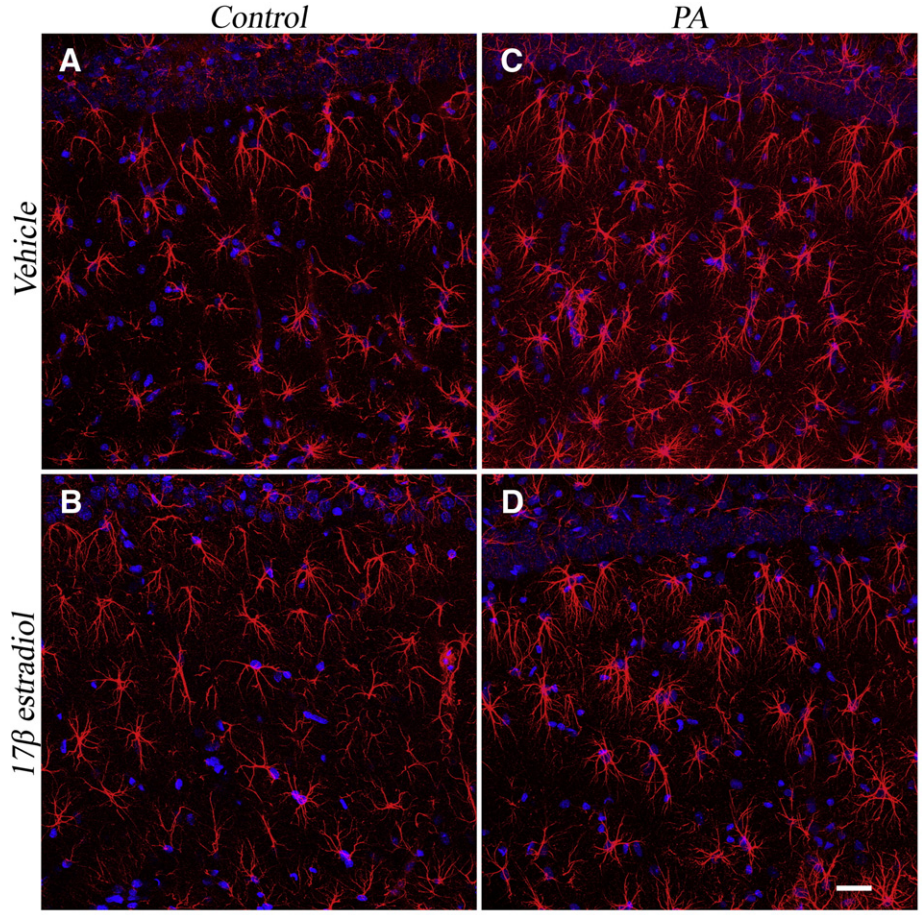

Fig. 1. Confocal microscope images of GFAP immunostaining from the *striatum radiatum* of CA1 hippocampal area. The hippocampus of PA animals treated with vehicle showed a marked reactive astrogliosis (panel C) respect to controls injected with vehicle and 17β estradiol (panels A and B). See in panel D that 17β estradiol treatment reduce the reactive astrogliosis in PA animals. Scale bar: 10 μm.

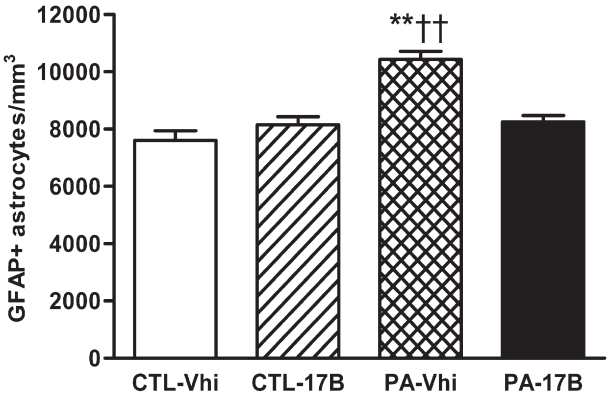

Fig. 2. Assessment of the number of GFAP immunoreactive astrocytes in the *striatum radiatum* of CA1. Estradiol by itself did not produce any statistical differences in the number of GFAP immunoreactive astrocytes, since CTL rats treated with estradiol (CTL-17β) did not show differences in the number of GFAP+ astrocytes in comparison to CTL rats treated with vehicle (CTL-Vhi). Animals that were submitted to PA and treated with vehicle (PA-Vhi) showed a significant increase of GFAP immunoreactive astrocytes in comparison to CTL-Vhi and PA rats treated with 17β estradiol (PA-17β). Moreover, number of GFAP immunoreactive astrocytes in PA-17β rats did not differ from those seen in CTL-Vhi rats, demonstrating that estradiol treatment in adult rats blocked the reactive astrogliosis associated with a PA event. Bars and error bars represent mean + SEM. $^{**}P<0.005$, PA-Vhi vs. CTL-Vhi; ††$P<0.005$, PA-Vhi vs. PA-17β.

that neither the main factor of birth condition nor the main factor of treatment was significant ($F=1.66$, $P=$ n.s.; $F=2,99$; $P=$ n.s, respectively), but the interaction was ($F=6.23$, $P=0.016$). Post hoc analysis indicated that 17β estradiol treatment in CTL rats did not produce any significant changes of the percentage of reactive area of MAP-2 positive dendrites with respect to CTL rats injected with vehicle ($P=$ n.s., see Figs. 3-B, 3-A and 4). Rats subjected to PA and treated with vehicle showed a significant reduction of the percentage of reactive area of MAP-2 positive dendrites in comparison to CTL rats injected with vehicle ($P<0.05$, see Figs. 3-C, 3-A and 4). Estradiol treatment in adults rats that suffered from PA significantly improved the mean percentage of reactive area of MAP-2 positive dendrites in comparison to PA rats treated with vehicle ($P<0.05$, see Figs. 3-D, 3-C and 4), while no differences were detected between PA rats treated with 17β estradiol and CTL rats injected with vehicle ($P=$ n.s., see Figs. 3-D, 3-A and 4).

Effects of PA and 17β estradiol treatment on axonal neurofilaments

Axonal alterations were studied using immunocytochemistry for medium molecular weight neurofilaments (120 NF) and phosphorylated high and medium molecular weight neurofilaments (pH/M NF). Fig. 5 shows a representative example of a hippocampal section immunostained for 120 NF. No obvious differences in 120 NF immunostaining were detected between animals subjected to PA and control animals. In agreement with the qualitative observations, the statistical analysis showed that neither the main factor of birth condition nor the main factor of treatment nor the interaction was significant ($F<1$; $F=2.3$, $P=$ n.s.; $P<1$, respectively. See Fig. 6). In contrast, noticeable changes were seen in the volume fraction of immunoreactive material for phosphorylated high and medium molecular weight neurofilaments after PA and 17β treatment. The two-way ANOVA revealed that the

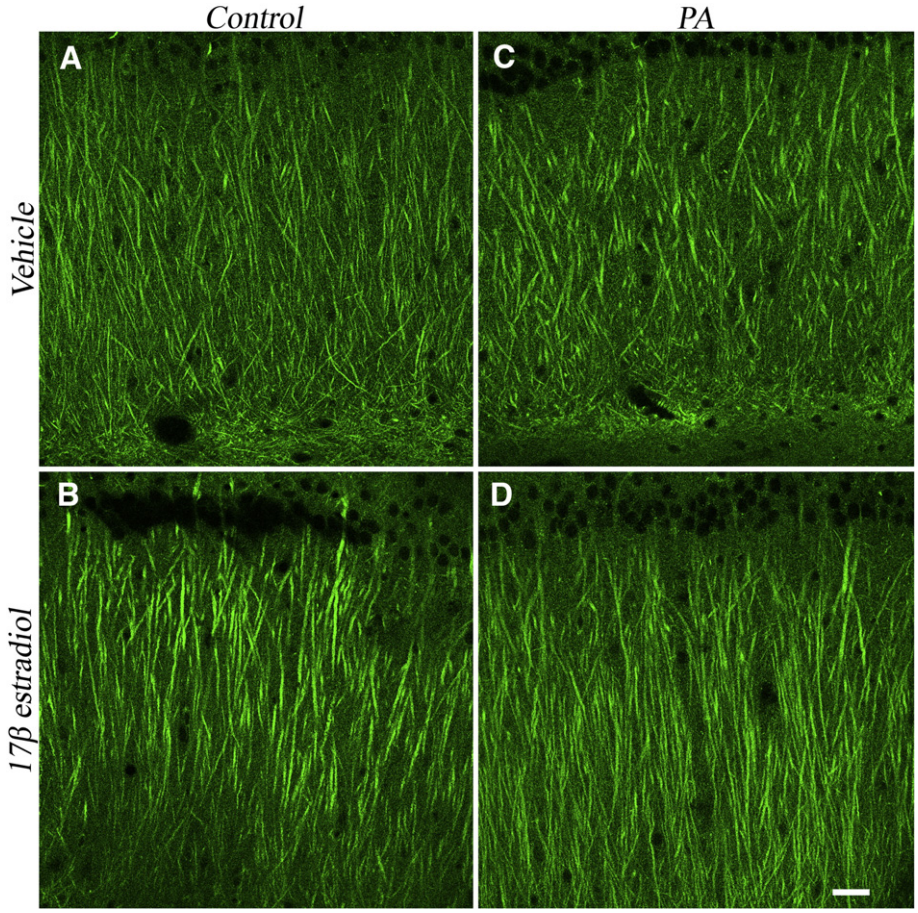

Fig. 3. Confocal microscope images of MAP-2 immunostaining from the *striatum radiatum* of CA1 hippocampal area. MAP-2 immunostaining revealed a focal swelling and a markedly fragmented appearance of distal dendrites in the CA1 region of PA animals injected with vehicle (panel C) respect to control groups treated with vehicle and 17β estradiol (panels A and B). Asphyctic animals injected with 17β estradiol (panel D) shows similar morphological characteristics to the control groups (panels A and B). Scale bar: 10 μm.

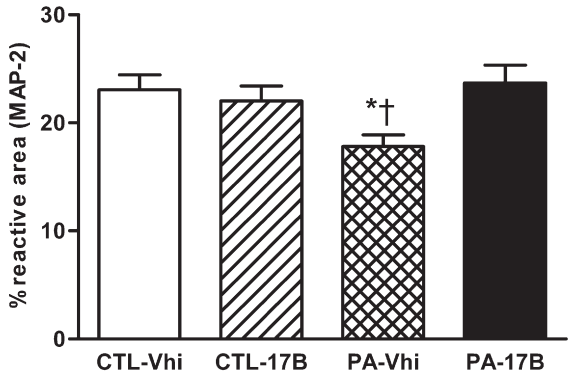

Fig. 4. Assessment of the percentage of reactive area of MAP-2 positive dendrites in the *striatum radiatum* of CA1. The statistical analysis revealed a significant decrease of the percentage of reactive area of MAP-2 positive dendrites in PA rats treated with vehicle (PA-Vhi) in comparison to CTL rats treated with vehicle (CTL-Vhi) and PA rats treated with 17β estradiol (PA-17β). In addition, PA-17β rats did not differ in the percentage of reactive area of MAP-2 positive dendrites in comparison to CTL-Vhi rats, suggesting a reversion of dendritic alterations associated with perinatal asphyxia. Estradiol by itself did not produce any differences in the percentage of reactive area of MAP-2 positive dendrites, since CTL-17β rats did not differ in the percentage of reactive area of MAP-2 positive dendrites from CTL-Vhi rats. Bars and error bars represent mean + SEM. *$P<0.05$, PA-Vhi vs. CTL-Vhi; †$P<0.05$, PA-Vhi vs. PA-17β.

main effects of birth condition and treatment and the interaction were all significant ($F=41.93$, $P<0.001$; $F=7.79$, $P<0.01$; $F=15.8$, $P<0.001$, respectively). Post hoc analysis showed that treatment with 17β Estradiol in adults CTL rats had not any effect in the percentage of the reactive area of pH/M NF (CTL-Vhi vs. CTL-17 β, $P=$ n.s., See Figs. 7-A, 7-B and 8). Rats subjected to PA and injected at adulthood with vehicle showed a significantly reduced percentage of the reactive area of pH/M NF than CTL rats treated with vehicle ($P<0.005$, see Figs. 7-C, 7-A and 8). The treatment with 17β Estradiol in PA rats enhanced the percentage of the reactive area of pH/M NF in comparison to PA rats treated with vehicle ($P<0.005$, see Figs. 7-D, 7-C and 8). Furthermore, 17β Estradiol treatment in PA rats increased the percentage of the reactive area of pH/M NF to a similar level to the one seen in CTL treated with vehicle ($P=$ n.s., CTL-Vhi vs. AP-17 β. See Figs. 7-A, 7-D and 8).

Discussion

In this work we demonstrate that PA in rats results in permanent changes in glial and neuronal cells in the hippocampus after four months of the induction of asphyctic insult. We have focus our study on CA1 area of hippocampus since it is one of the most affected areas by PA insult (Petito and Pulsinelli, 1984). We have detected increased astrogliosis, focal swelling and fragmentation of CA1 apical dendrites, decreased MAP-2 immunoreactivity, and decreased phosphorylated high and medium molecular weight neurofilaments in the hippocampus of adult rats subjected to PA. These alterations are well established markers of neurodegenerative damage in the central nervous system. Treatment with 17β estradiol has blocked these alterations in the hippocampal cytoskeleton. Thus, this data suggests that 17β estradiol could be a possible therapeutic tool for the neurological disorders produced by the asphyxia during birth.

Cytoskeletal alterations are blocked by estradiol treatment

Estradiol treatment of adult rats that were subjected to perinatal asphyxia resulted in a decrease in the number of GFAP immunoreactive astrocytes in the hippocampus to similar values to those seen in control animals. These findings are in agreement with previous reports that indicate that estradiol reduces reactive astrogliosis after different forms of brain injury in adult animals (Arevalo et al., in press;

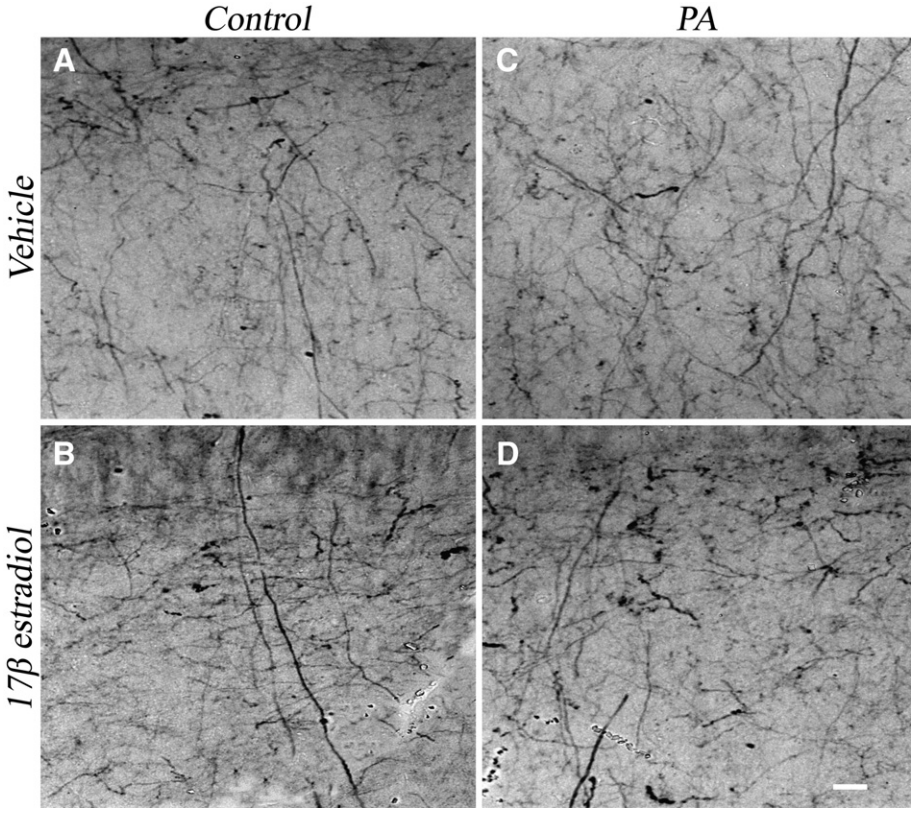

Fig. 5. Optical microscope images show a representative example of a *striatum radiatum* of CA1 hippocampal area immunostained for 120 NF. No obvious difference were observed in PA animals treated with vehicle (Panel C) respect to CTL injected with vehicle or 17β Estradiol (Panel A and B, respectively). Asphyctic animals treated with 17β Estradiol (Panel D) showed similar morphological characteristics to control groups (Panel A and B). Scale bar: 10 μm.

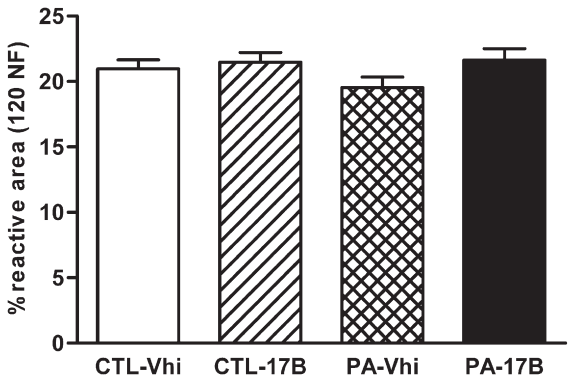

Fig. 6. Assessment of the percentage of reactive area of medium molecular weight neurofilaments (120 NF) in the *stratum radiatum* of CA1. No differences among any of the groups were detected by the statistical analysis in agreement with the qualitative observation. Bars and error bars represent mean + SEM.

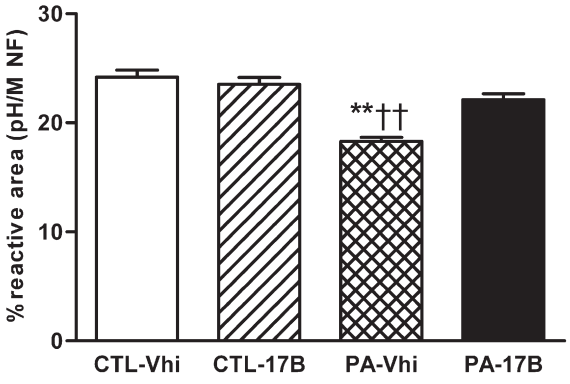

Fig. 8. Assessment of the percentage of reactive area of phosphorylated high and medium molecular weight neurofilament (pH/M NF) in the *stratum radiatum* of CA1. Estradiol by itself did not produce any statistical differences in the percentage of reactive area of pH/M NF, since CTL rats treated with estradiol (CTL-17β) did not show differences in the percentage of reactive area of pH/M NF in comparison to CTL rats treated with vehicle (CTL-Vhi). Animals that were submitted to PA and treated with vehicle (PA-Vhi) showed a significant decrease of the percentage of reactive area of pH/M NF in comparison to CTL-Vhi and PA rats treated with 17β estradiol (PA-17β). Moreover, the percentage of reactive area of pH/M NF in PA-17β rats did not differ from that seen in CTL-Vhi rats, demonstrating that estradiol treatment in adult rats blocked the axonal alterations associated with a PA event. Bars and error bars represent mean + SEM. $^{**}P<0.005$, PA-Vhi vs. CTL-Vhi; $\dagger\dagger P<0.005$, PA-Vhi vs. PA-17β.

Barreto et al., 2009). However, most studies have tested the antigliotic properties of estradiol when the hormone was administrated before or shortly after the induction of brain damage. Our findings indicate that the hormone is also able to reduce, in adult animals, chronic reactive astrogliosis caused by an early developmental neurodegenerative event.

Under neurodegenerative conditions astrocytes proliferate and the expression of GFAP is increased (Eng and Ghirnikar, 1994; Pekny and Nilsson, 2005). Alterations in dendrites are also a common finding under neurodegenerative conditions (Ikonomidou et al., 1989; Hsu and Buzsáki, 1993; Hori and Carpenter, 1994; Matesic and Lin, 1994; Ramón y Cajal, 1995) and the aberrant phosphorylation of neurofilaments is a hallmark of axonal degeneration (Grant and Pant, 2000; Sihag et al., 2007). Consistent with this data we have shown in previous work reactive astrogliosis in neostriatum of rat at six months after PA (Cebral et al., 2006). In addition, we have recently demonstrated that rats at six month after PA showed clear signs of synaptic neurodegeneration in the neostriatum and accumulation of ubi-proteins (Capani et al., 2009). Taken together, our findings indicate that the experimental model of perinatal asphyxia used in this study causes neurodegenerative alterations that are still detectable in adult life. The existence of permanent alterations in the hippocampus of rats subjected to perinatal asphyxia are in agreement with the permanent neurological deficits associated with this pathological condition in humans (Hill and Volpe, 1981; Amiel-Tison and Ellison, 1986; Osborne et al., 2004).

The alterations produced by perinatal asphyxia in the volume fraction of immunoreactivity material for phosphorylated high and

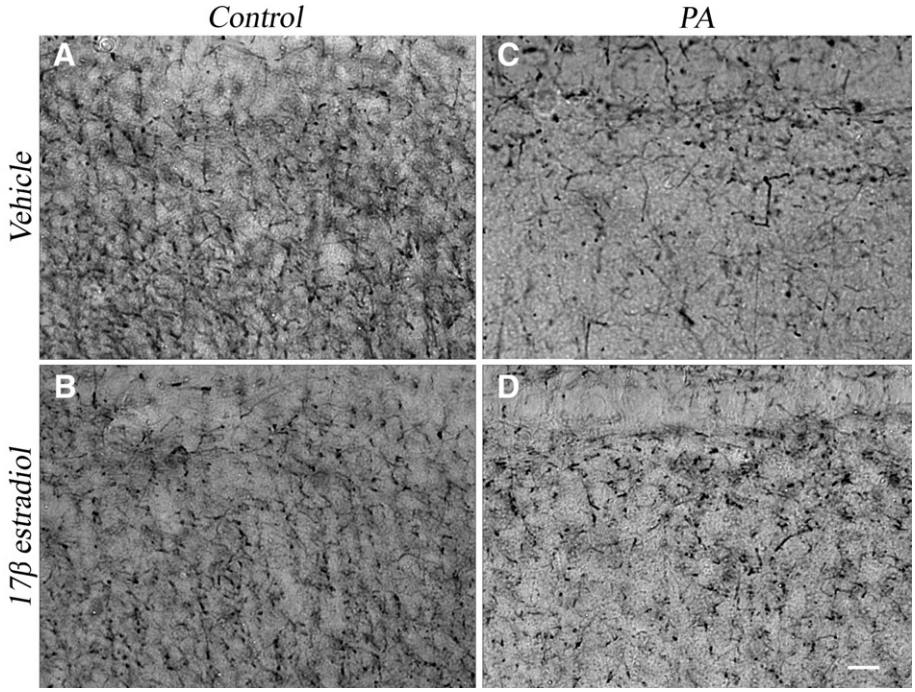

Fig. 7. Optical microscope images show a representative example of a *striatum radiatum* of CA1 hippocampal area immunostained for phosphorylated H/M NF. Obvious differences in pH/M NF immunostaining were detected between PA treated with vehicle (panel C) and control groups (panels A and B). Estradiol treatment reverts the decrease of staining seen in asphyctic animals (panel D). Scale bar: 10 μm.

medium molecular weight neurofilaments are in agreement with our previous finding of NF alterations in the neostriatum of six month-old rats (Cebral et al., 2006). This data suggested that NF alterations are a consistent modification induced by PA. In addition, abnormal NF accumulations are found in several human neurological diseases, such as amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, progressive supranuclear palsy, Charcot–Marie–Tooth disease, diabetic neuropathy, giant axonal neuropathy, and NF inclusion disease (Hirano, 1994; Mori et al., 1996; Bomont et al., 2000; Shepherd et al., 2002). Since NF proteins are preferential targets for oxidative stress (Gélinas et al., 2000; Hand and Rouleau, 2002) estrogens can induce its neuroprotective function modulating free radical production. Moreover, estrogen therapy was able to preserve the microtubule organization of the dendrite severely modified by perinatal asphyxia. A substantial body of evidences suggested that estrogens are related with the facilitation and preservation of neurite growth during the development of nervous system although the molecular mechanisms are not still fully understood (Dominguez et al., 2004). Recently, it was proposed that 17β-estradiol-mediated ERK activation is involved in the maintenance of neuritic arborisation and neuronal morphology in pro-apoptotic conditions (Miñano et al., 2008).

Conclusions

In conclusion, our findings suggest that estradiol therapy in adult life may reverse neural deficits caused by early brain damage, such as the ones caused by perinatal asphyxia. Therefore, in addition to its well characterized neuroprotective actions to prevent neuronal damage, estradiol may also have neuro-reparative properties, decreasing brain alterations caused by early life events. Since estradiol may have numerous undesirable peripheral effects, further studies should address the effect of selective estrogen receptor modulators or non-feminizing estrogens, which may represent a more adequate therapeutic approach.

Acknowledgments

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