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Mini Review

Steroid protection in aging and age-associated diseases

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ABSTRACT

Neuroactive steroids are secretory products of peripheral endocrine glands that modulate a variety of brain functions. A close relationship between neuroactive steroid structure and function becomes most evident under pathological circumstances. On one side, overproduction of glucocorticoid and mineralocorticoid neuroactive steroids may be detrimental to the hippocampus, which is enriched in glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). Thus, a dysfunction of the adrenocortical system in aging and age-associated diseases (diabetes, hypertension) is able to cause hippocampal damage. Whereas aging and uncontrolled diabetes show a predominant GR overdrive, a MR overdrive characterizes hypertensive animals. Some abnormalities commonly found in the hippocampus of aging, diabetic and hypertensive animals include decreased neurogenesis, astrogliosis and neuronal loss in the hilus of the dentate gyrus (DG). On the other side, and in contrast to adrenal gland-derived steroids, estrogens qualify as hippocampal neuroprotectants. Given to middle-age mice, estrogens stimulated proliferation and differentiation of newborn cells in the DG, decreased astrogliosis and increased hilar neuronal number. Similar estrogen effects were obtained in mice with streptozotocin-induced diabetes and in spontaneously hypertensive rats (SHR). The results suggest that in aging and age-associated diseases, adrenocortical steroid overdrive sensitizes the hippocampus to the pathological milieu imposed by a pre-existing degeneration or illness. In this setting, estradiol neuroprotection rescues hippocampal parameters previously altered by the pathological environment.

1. Neuroactive steroids, hippocampus and aging

Aging induces significant changes in the endocrinology and chemistry of the brain that involves the synthesis and signalling pathways of neurotransmitters, growth factors, neuropeptides and steroids (Smith et al., 2005). In the last case, the brain receives the impact of neuroactive steroids derived from peripheral endocrine glands and of the neurosteroids locally synthesized by steroidogenic enzymes of neurons and glial cells (Baulieu et al., 2001). Neuroactive steroids and neurosteroids regulate reproductive and neuroendocrine events; besides, they display beneficial effects concerning neuroprotection, myelination, behaviour, mood, learning and memory. On the contrary, under circumstances of high circulating levels of adrenal-derived glucorticoids or mineralocorticoids, harmful neurotoxic effects appear (De Kloet et al., 2007).

Neuroactive steroids of adrenal, ovarian, testicular or placental origin are lipophilic molecules that easily traverse the blood–brain barrier. Once in the brain, they modulate the function of neuronal and glial cells by means of receptor -dependent or -independent actions. In the first mechanism, steroids bind to intracellular receptors that act as nuclear transcription factors. In addition, non-genomic mechanisms follow steroid interaction with steroid membrane receptors, ion channels and neurotransmitter receptors; besides, some steroids such as estrogens and progesterone have direct antioxidant effects (Behl, 2002; De Kloet et al., 2007; McEwen, 2002). In the adult brain, steroids show activation effects involving the structure, function and neurochemistry of specific brain regions including the hippocampus. This brain area becomes highly vulnerable during aging. Experimental evidences have shown that the aging hippocampus is characterized by deficits in learning, memory and neurogenesis, changes of neurotransmission (mostly cholinergic), ion channels and electrical activity, altered expression of neuropeptides, growth factors and their receptors, changes in the expression of genes, transcription factors and steroid receptors, increased vasculopathy, high nitrergic activity, increased oxidative stress, neuronal loss and atrophy, astrogliosis demyelination and increased production of inflammatory mediators (rev. in Miller and O'Callaghan, 2005). In healthy elderly human subjects, MRI studies have revealed a reduction of hippocampal volume which becomes most marked in Alzheimer's patients (Petersen et al., 2000).

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2. Harmful effects of adrenal gland-derived steroids

There are divergent effects of adrenal steroids and sex steroids on the brain and specifically the hippocampus. The hippocampus is highly enriched in both glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which renders this region extremely sensitive to steroid ligands or to receptor hyperfunction. Fig. 1 shows that most pyramidal cells of the CA1 region of the rat hippocampus express nuclear MR (middle graph) and nuclear GR (lower graph), using the antibodies specified in the legend to Fig. 1. Interestingly, both cortisol in humans and corticosterone in rats and mice exert their effects on the hippocampus through binding to MR and GR. This is possible because the hippocampus cannot metabolize cortisol or corticosterone to inactive metabolites, due to the lack of 11β-hydroxysteroid dehydrogenase type 2. Since the concentration of cortisol or corticosterone is higher than that of aldosterone (the natural MR ligand), MR in hippocampus becomes occupied and saturated by the "glucocorticoids". Thus, both receptor types are involved in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. MR is a high affinity receptor that determines the sensitivity of the stress response, whereas GR is a low affinity receptor that promotes the termination of the stress response (De Kloet et al., 2007). Excess glucocorticoid secretion due to disturbances of the HPA axis has negative consequences upon the hippocampus, as shown in Cushing's disease, stress-related diseases, aging, diabetes mellitus, depression and neurodegeneration of the Alzheimer's type (De Kloet et al., 2007; Sapolsky et al., 1986). These diseases present increased levels of circulating glucocorticoids, a sustained response to stress, flattening of the diurnal rhythm of plasma corticosteroids and ACTH, decreased glucocorticoid negative feedback and hippocampal neuropathology. Signs of hippocampal vulnerability include atrophy of apical dendrites of pyramidal neurons in the CA3 region, astrogliosis, reduced neurogenesis in the DG, neuronal loss and reduced synthesis of brain-derived neurotrophic factor (BDNF). Inappropriate activation of GR by high levels of circulating glucocorticoids induces receptor down-regulation, because GR acts as a natural repressor at the promoter of the GR gene. This event contributes to further dysfunction of the HPA axis.

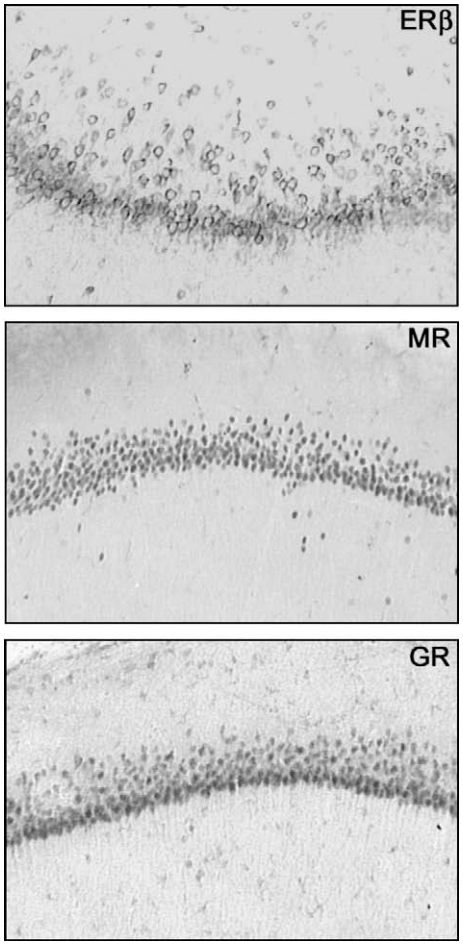

Fig. 1. Expression of neuroactive steroid receptors in the pyramidal cells of the CA1 region and adjacent regions of the dorsal hippocampus in rats. Immunocytochemistry was carried out on vibratome sections according to Pietranera et al. (2008). Photomicrographs represent from top to bottom the estrogen receptor isoform β (ERβ 503, chicken antibody raised against the whole human protein obtained from J-Å Gustafsson, Karolinska Institute, Huddinge, Sweden), the mineralocorticoid receptor (MR 365-4D6 antibody obtained from C. Gomez-Sanchez, University of Mississippi Medical Center, Jackson, MS, USA) and the glucocorticoid receptor (GR, MAB7 antibody, a kind gift of Dr. A. Wikstrom, Karolinska Institute, Huddinge, Sweden). Magnification: 200×.

Adrenal steroid overdrive also affects neurogenesis, a typical hippocampus-dependent function. In adult rodents, this process is restricted to the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG). Progenitors in the DG proliferate, migrate into the granular cell layer (GCL) and differentiate into mature granule cells (Kempermann et al., 2000; Tanapat et al., 2005). In aging animals, proliferation and migration of newborn cells in the DG is strongly reduced. It has been suggested that an adrenal corticosteroid overdrive is responsible in part for the deficient neurogenesis of stress and aging (De Nicola et al., 2006; Oomen et al., 2007). Functionally, neurogenesis is associated with learning and memory and acquisition of a fear-conditioned response (Tanapat et al., 2005). Since newly-formed neurons rapidly extend their projections to the CA3 pyramidal subfield of the hippocampus, changes of neurogenesis extend beyond the DG, according to Kempermann et al. (2000). Thus, the deficient neurogenesis characteristic of aging may upset the whole hippocampal function.

It is known that neural progenitors in the DG express GR and MR (Wong and Herbert, 2005), suggesting that the low hippocampal neurogenesis of senescent animals may be an index of glucocorticoid-mediated neurotoxicity. That glucocorticoids negative modulate neurogenesis is also supported by experiments showing that in adult or senescent rats, adrenalectomy stimulates cell proliferation in the DG (Cameron and McKay, 1999). In rodents with high levels of glucocorticoids caused by stress or diabetes mellitus, administration of the GR antagonist RU486 restores neurogenesis in the DG (Oomen et al., 2007; and unpublished results from our laboratory).

3. Estrogen neuroprotection

In contrast to the neurotoxic effects caused by a mineralo or glucocorticoid overdrive, estrogens qualify as 'neuroprotectants'. Estrogens prevent cell death, increase neuronal survival and neurite outgrowth, stimulate synaptogenesis and regulate cholinergic neurotransmission in different experimental situations. McEwen (2002) first reported that estrogens increase dendritic spine formation and synaptic density in CA1 pyramidal cells, an effect probably mediated by estrogen receptors (ER). In hippocampal neurons in culture, estrogens protect against glutamate toxicity, glucose deprivation, FeSO toxicity and amyloid-β peptide toxicity. Estrogens also inhibit β-amyloid deposits in some but not all transgenic mouse models of Alzheimer's disease (Goodman et al., 1996). Some of the estrogen effects could be genomically mediated, after interaction with estrogen receptors (ER). Estrogen binding has been reported in the hippocampal pyramidal cells and the hilus of the DG.

Of the two isoforms of the estrogen receptor, ERβ is abundantly expressed in pyramidal cells of the hippocampus, whereas ERα is found in CA1 interneurons and a subset of pyramidal and granule cells (Shughrue et al., 1997). Fig. 1 (upper graph) shows the immunocytochemical detection of ERβ in the rat hippocampus. In our hands, the ERβ 503 antibody shows an extranuclear localization of this receptor type in a majority of cells of the CA1 stratum pyramidale and in stratum oriens. ERβ activation by natural or isoform-specific ligands seems important for aging and neurodegeneration, because this isoform regulates hippocampal synaptic plasticity and improves cognition (Liu et al., 2008). Studies in KO mice reveal that ERβKO but not ERαKO show deficits in learning behaviour and chemical signalling of hippocampus after treatment of the animals with E_2 (Liu et al., 2008).

At the molecular level, estradiol neuroprotection is multifaceted. It has been suggested that brain effects of E_2 are mediated in part by up-regulation of the anti-apoptotic gene bcl-2 but also by interaction with growth factors such as IGF-1 (insulin-like growth factor 1) and BDNF (brain-derived neurotrophic factor) (Garcia-Segura et al., 2006; Scharfman and Maclusky, 2005). Similar to growth factors, estrogens activate the MAPK (mitogen-activated protein kinase), ERK (extracellular-regulated kinase), PI_3K (phosphatidylinositol-3-kinase), increase CREB (cyclic AMP-response element binding protein) phosphorylation and the Src pathway (Scharfman and Maclusky, 2005). An effect of E_2 related to memory formation in the hippocampus includes the enhancement of NMDA receptor activity and long-term potentiation (Liu et al., 2008). ERα and ERβ are expressed by neurons, astrocytes and oligodendrocytes, and the last cells are protected from cytotoxicity if E_2 is added to the culture medium.

Therefore, far beyond their reproductive function, estrogens are important neuroprotective and myelinating factors when damage, degeneration, ischemia or aging damages the CNS. According to the hypothesis of Behl (2002), estrogen may provide neuroprotection in Alzheimer's disease, Parkinson's disease, schizophrenia, depression, stroke, cerebral ischemia and changes of memory and cognition. In the hippocampus, a part of the brain related to limbic-associated functions and learning and memory, estrogens play an important role. In postmenopausal women in current use of estrogens, there is increased cerebral blood flow, increased glucose metabolism, larger hippocampal volume and enhance cognition (rev. in Sherwin and Henry, 2008). One way estrogens modulate hippocampal functions is by enhancement of the different steps of neurogenesis: proliferation, migration and differentiation. For instance, uptake of the thymidine analog bromodeoxyuridine (BrdU) by proliferating cells of the DG is higher in adult proestrus than in estrus rats, suggesting the participation of endogenous hormones, whereas BrdU+ cells are more abundant in ovariectomized-estradiol (E_2) replaced rats than in ovariectomized-vehicle-treated rats (Tanapat et al., 2005). The increase in proliferation is transient, and diminishes in animals subjected to prolonged ovariectomy or chronically overloaded with estrogens. A gender difference has been suggested, because in males the response of hippocampal neurogenesis to E_2 is attenuated. The effect on neurogenesis seems ER-mediated, since both ERα and ERβ mRNA are found in ≈80% of proliferating cells of the DG labelled with the Ki67 antibody, and in an important proportion of cells showing a more mature phenotype (Isgor and Watson, 2005). A sign of estrogen neuroprotection may be the positive control of neurogenesis. This possibility receives support from published data showing that E_2 enhances neurogenesis under situations presenting a deficient cell proliferation, such as ischemic stroke, diabetes mellitus and aging (Pietranera et al., 2006; Saravia et al., 2004, 2007).

4. Estrogen reversal of aging effects in the hippocampus: effects on the stress response and GR of old rats

As already mentioned aging disturbs the regulation of the HPA axis and leads to reduced levels of GR in the hippocampus. To compensate for these effects, we have investigated whether estrogen therapy normalized the HPA response to stress and GR in hippocampus and paraventricular (PVN) nucleus, two sites that mediate glucocorticoid negative feedback (Ferrini et al., 1999). First, we studied the level of circulating corticosterone—the rat natural glucocorticoid in young (3–4 months) and old (20 months) male Sprague–Dawley rats in the basal state and following ether stress. While basal and ether-stimulated levels of plasma corticosterone were similar in the two groups, old animals presented a delayed termination of the response to ether stress. This effect correlated with a reduced protein expression for GR in the hippocampus of old rats, in consonance with the concept that GR is required for the termination of the response to stress (De Kloet et al., 2007). A dexamethasone inhibition test carried out in old animals, showed a failure to completely block plasma corticosterone after ether stimulation. Furthermore, in old rats GR-immunoreactive levels were reduced in CA1–CA2 hippocampal subfields and subiculum, while normal levels were obtained in CA3–CA4 and PVN. Half of the animals received a single E_2 benzoate pellet sc for 6 weeks. Plasma E_2 in these animals reached 430 pg/ml, representing a 13.5-fold increase over levels in old, untreated rats. Furthermore, prolonged E_2 treatment of old rats normalized the termination of the stress response, restored dexamethasone inhibition of plasma CORT and increased GR immunoreactivity in CA1 and CA2 hippocampal subfields and subiculum (Ferrini et al., 1999). The reappearance of GR after estrogen treatment supports the theory that GR is important for the termination of the stress response (De Kloet et al., 2007). These data are in agreement with other groups, who demonstrated that estrogen and estrogenic-like compounds increase hippocampal GR (Lephart et al., 2003). In conclusion, chronic estrogen exposure normalized the response to stress, restored feedback inhibition and increased hippocampal GR immunoreactivity in old rats. Thus, estrogens behave as glucocorticoid antagonists, in the sense that under pathological circumstances, estrogens and adrenal corticosteroids may exert opposite effects in the brain. Estrogens and phytoestrogens not only enhance GR abundance in hippocampus and GR mRNA in the amygdala and hypothalamus (Lephart et al., 2003) but also down-regulate transcription of the ACTH secretagogues CRH and AVP genes in the PVN and the proopiomelanocortin (POMC) gene in the pituitary corticotroph. Altogether, many reports raise the possibility that positive modulation of GR by estrogens could reinforce the glucocorticoid feedback mechanism. In human studies, stress-induced glucocorticoid elevations in postmenopausal women are blunted by estrogen replacement.

5. Estrogen reversal of aging effect in the hippocampus: effects on neurogenesis and other faulty hippocampal parameters of middle-age mice

The positive influence of estrogens on neurogenesis is mostly based on data from young animals, but data on older animals have recently appeared. In one study (Perez-Martin et al., 2005) 22-month-old ovariectomized rats received prolonged treatment with E_2 valerianate or phytoestrogens from soy bean extract. The authors concluded that cell proliferation in the old brain remains responsive to natural estrogens and phytoestrogens. Although in an indirect manner, a second report demonstrated that infusion of IGF-1 to 22-month-old rats increases both neurogenesis and blood levels of E_2, suggesting steroid participation in the cell pro-

liferation of this age group (Darnaudery et al., 2006). Interestingly, hippocampal neurogenesis starts to decline well before old age. For instance, cell proliferation and migration through the GCL is high in 2 week-old rats, it weakens at 1.5 month of age, and is drastically inhibited in 12-month (middle age) and 24-month (old age) rats. Considering this early decline, studies to elucidate estrogenic effects on the steps leading to neuronal maturation in the DG and on other hippocampal indicators of aging remain an important subject. This issue has clinical relevance, because the Women Health Initiative (WHI) randomized clinical trial claimed that estrogen alone increases the risk of developing mild cognitive impairment (Resnick et al., 2006), a process considered hippocampal-dependent. The WHI trial has been criticized on the grounds that the recruited women were several years past menopause, and at an age when estrogen responsiveness diminishes. Clinical trials support the effectiveness of hormone-replacement therapy in prevention rather than improvement of mental deterioration (Sherwin and Henry, 2008). Since middle age animals are fully responsive to E_2, this age period seems appropriate to counteract the development of age-associated neuropathology. Thus, middle age provides an attractive window of time to explore potential modulation of changes associated with aging, but paradoxically data are lacking during this time frame.

To fully appreciate hormonal effects in the middle age hippocampus, it seems important to expand the study to other age-sensitive parameters besides neurogenesis. A typical biomarker of the aging brain is the astrocyte hypertrophy, with increased expression of glial fibrillary acidic protein (GFAP) (Miller and O'Callaghan, 2005). Estrogens produce a down-regulatory effect on the astrocytosis with high GFAP expression of the brain of very old rats (22–26 months at the time of killing) and young rats receiving castration, traumatic or excitotoxic lesions. Another aging marker is the neuronal population of cells in the hilar region, which are markedly lost in old animals. Hilar neurons are partly derived from cells proliferating in the SGZ that later migrate into the hilar region. These hilar cells are extremely vulnerable to excitotoxic or ischemic hippocampal injury (for references: Saravia et al., 2007). It is unknown if estrogens prevent the hilar neuronal loss during aging, although a rescue effect of acute or chronic E_2 administration follows toxin-induced degeneration of neurons in this region.

Since aging defects are already pronounced at middle age, this period of rodent life seems an appropriate window to test the efficacy of neuroprotective steroids. In our laboratory (Saravia et al., 2007), we studied E_2 modulation of some hippocampal parameters—neurogenesis, astrogliosis, hilar cell number—at this age period. Middle age (10–12-month-old) male C57Bl/6 mice were implanted sc with E_2 (15 μg) or cholesterol pellets. Plasma E_2 in steroid-treated middle-age mice reached 550 pg/ml, representing a 36-fold increase over levels in middle-age steroid-naïve mice. Ten days after pellet implantation, mice received BrdU 4 h and 2 h before killing to study cell proliferation in the dentate gyrus (DG). A pronounced depletion of BrdU+ cells in the DG was found in cholesterol-treated middle-age mice, accompanied by hippocampal astrocytosis, neuronal loss in the hilus and lipofucsine deposits. Lipofucsine is an aging pigment that indicates increased oxidative stress. Middle-age mice receiving E_2 showed increased number of BrdU+ cells while other parameters, such as increased number of GFAP-immunopositive astrocytes and low number of hilar cells, were remarkably attenuated. When steroid treatment was prolonged for 2 months to study migration of cells into the granular layer of the DG, cell migration was unaffected by E_2. However, E_2-treated middle-age mice presented higher cell density and increased staining for doublecortin (DCX), a marker for differentiating neurons. Fig. 2 compares the results of DCX staining in young (top), steroid-naïve middle age (middle) and middle age estradiol-treated mice (lower graph). Young animals showed many DCX-positive progenitors with immunopositive long process, as opposed to middle-age mice receiving cholesterol vehicle which showed sparse, atrophic DCX-positive profiles. In contrast, middle-age mice treated with a single 150 μg E_2 pellet for 60 days presented better defined DCX-positive cell bodies with staining of processes. Thus, from the three basic steps of adult neurogenesis (proliferation, migration and differentiation), E_2 stimulated progenitor proliferation – even after long exposure to E_2 studied by Ki67 immunocytochemistry – and differentiation towards a neuronal lineage. This result, in conjunction with recovery from aging indicators such as increased lipofucsine deposits, loss of hilar neurons and astrocytosis, supports a wide range protection of the hippocampus of middle-age mice by estrogenic hormones.

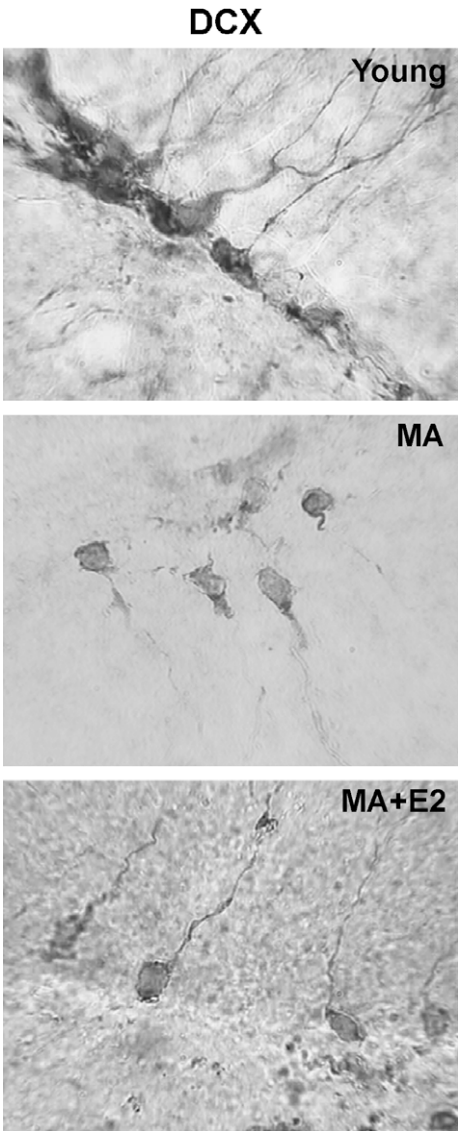

Fig. 2. Doublecortin (DCX)-immunopositive cells in the dentate gyrus from young (top), vehicle-treated middle-age (MA, middle) and estradiol (E_2)-treated middle-age mice (MA + E_2, bottom). E_2 treatment lasted for 60 days. Numerous DCX-immunopositive cells (differentiating progenitors) with profuse stained process are observed in a representative young mouse, in contrast to the atrophic phenotype observed in untreated MA mouse. The MA + E_2 mouse shows increased number of DCX-positive cells containing visible stained cell processes. Magnification: 1000×. (Reprinted from Psychoneuroendocrinology, vol. 32, Saravia et al. Neuroprotective effects of estradiol in hippocampal neurons and glia of middle-age mice. pp. 480–492. Copyright 2007, with permission from Elsevier).

6. E2 protective effects in the brain of diabetic and hypertensive animals

Chronic type I diabetes mellitus, as an age-related disease, presents a pronounced encephalopathy. Type 1 diabetes correlates with several brain disturbances, including hypersensitivity to stress, cognitive impairment, increased risk of stroke and dementia. In humans, long type I diabetes duration is a strong predictor of cognitive impairment (Brismar et al., 2007). Diabetic and aging animals show in common increased glucocorticoid overdrive, high levels of plasma CORT and decreased level of GR in hippocampus (De Nicola et al., 1991). Within the CNS, the hippocampus is considered a special target for alterations associated to diabetes. With these data on hand, we studied the ability for neurogenesis in the DG and SVZ of C57Bl/6 mice with one-month-old diabetes induced by administration of streptozotocin (STZ) (Saravia et al., 2004). Using BrdU labelling of cells in the S phase, we observed a strong reduction of cell proliferation rate in both brain regions of diabetic mice sacrificed 20 days after STZ administration. Second, since estrogens are active neuroprotective agents, we investigated whether E_2 (200 μg pellet implant in cholesterol during 10 days) restored brain cell proliferation in the diabetic mouse brain. Determination of plasma E_2 demonstrated a 33-fold increase in diabetic mice receiving hormone in comparison to controls (497.3 vs. 14.9 pg/ml), respectively). Our results demonstrated a complete reversibility of DG cell proliferation in E_2-treated diabetic mice. This plasticity change was not exclusive of the hippocampus, since estrogen treatment restored BrdU incorporation into newborn cells of the SVZ region of diabetic animals. Most interestingly estrogen treatment did not alter the hyperglycemic status of STZ-diabetic mice, suggesting a direct estrogen effect on the brain. Moreover, E_2 did not modify BrdU incorporation in control animals. Diabetic mice also showed abnormal expression of astrocyte markers in hippocampus. Thus, increased number of GFAP+ cells, indicative of astrogliosis, and increased number of apolipoprotein-E (Apo-E)+ astrocytes, a marker of ongoing neuronal dysfunction, were found in stratum radiatum below the CA1 hippocampal subfield of diabetic mice. Both parameters were reverted to normal by the same E_2 regime that up-regulated cell proliferation. These studies demonstrated that hippocampal neuropathology of uncontrolled diabetes is a plastic event sensitive to estrogen treatment. New experiments are needed to disclose if reversal of the diabetic encephalopathy with neuroprotective steroids could play a therapeutic role for hippocampal cognitive functions.

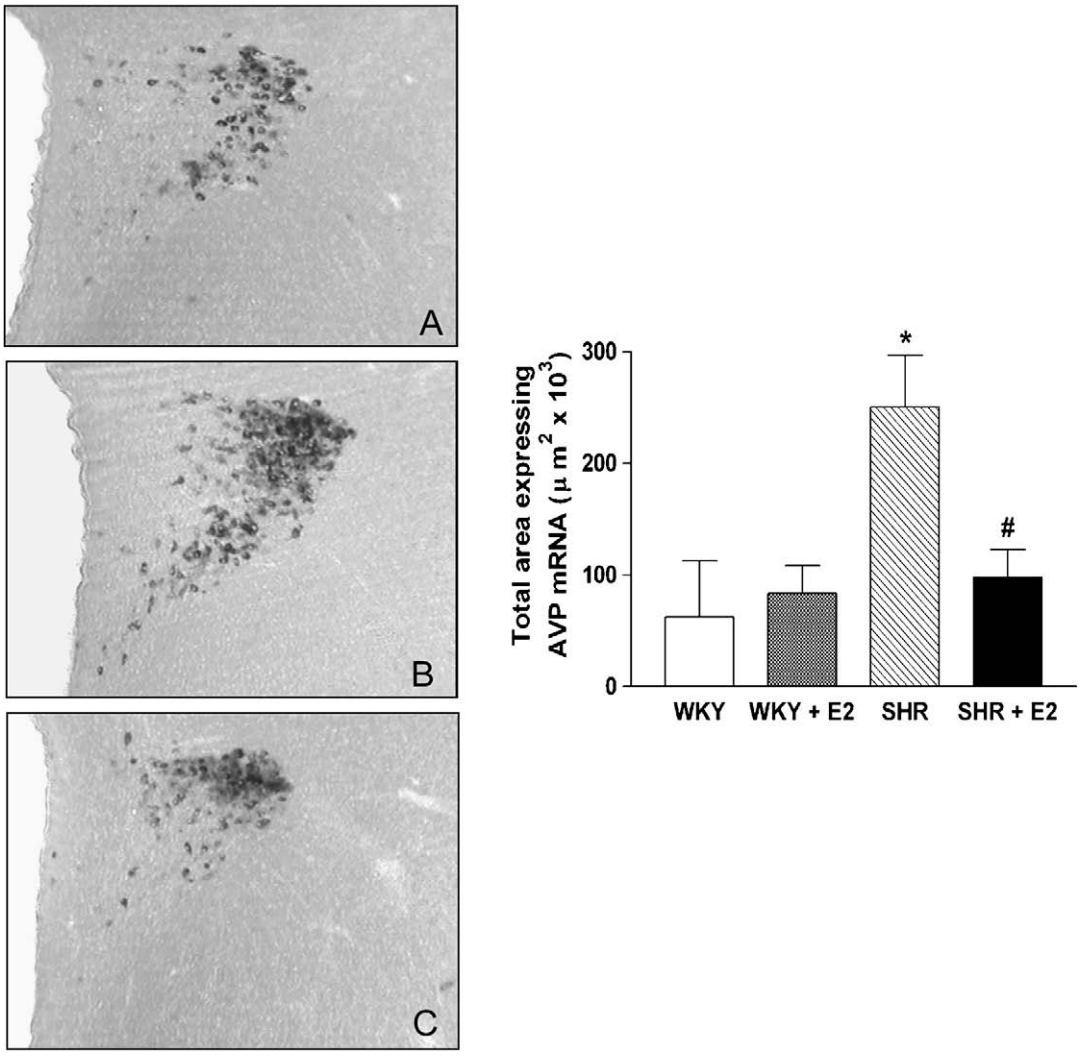

Fig. 3. Analysis of AVP mRNA in the paraventricular nucleus (PVN) labelled by non-isotopic in situ hybridization. In normotensive Wistar Kyoto (WKY) rat, expression was restricted to dorsal magnocellular cells (A), whereas SHR showed higher AVP mRNA in dorsal and medial magnocellular cells (B). The pattern in SHR + E_2 reverted to levels of normotensive rat (C). Magnification 200×. Quantitative analysis: $^{*}p < 0.05$ vs. WKY; $^{\#}p < 0.05$ vs. SHR. (Reprinted from Psychoneuroendocrinology, vol. 33, Pietranera et al. Protective effects of estradiol in the brain of rats with genetic or mineralocorticoid-induced hypertension. pp. 270–281. Copyright 2008, with permission from Elsevier.

Steroid neuroprotection was also studied in hypertensive animals. Because the incidence of hypertension increases with age, hypertensive encephalopathy is categorized as an age-associated disease. Brain abnormalities of hypertension may be linked to vascular remodelling, microglial activation and vascular inflammation. In addition, the MR overdrive prevailing in hypertension could play an etiopathogenic role, increasing hippocampal vulnerability to hypertensinogenic factors. The spontaneously hypertensive rat (SHR), which bears a form of endocrine hypertension with an hyperactive MR (Rahmouni et al., 2001), shows hippocampal abnormalities which are remarkably similar to those present in middle age animals and in animals with induced diabetes mellitus (Pietranera et al., 2008; Saravia et al., 2007). Thus, decreased cell proliferation in the DG, hippocampal astrogliosis and decreased neuronal density in the hilus are found in SHR. In addition, there is hypothalamic involvement, since the expression of the hypertensinogenic peptide arginine vasopressin (AVP) is markedly elevated (Pietranera et al., 2008). We found that E_2 treatment of SHR revert these abnormalities. The experiments were carried out in 16-week-old male SHR with blood pressure (BP) $\approx$ 190 mm Hg and their normotensive Wistar Kyoto (WKY) controls. Half of the animals in each group were implanted sc with a single E_2 benzoate pellet weighing 14 mg for 2 weeks. Plasma E_2 values were highly elevated in hormone-treated rats (2500 pg/ml) respect of hormone-free rats (35 pg/ml). E_2-treated SHR showed, in comparison to its respective steroid-free group: (a) enhanced proliferation in the DG measured by BrdU incorporation; (b) decreased number of GFAP-immunopositive astrocytes; (c) increased density of neurons in the hilus of the DG and (d) decreased hypothalamic AVP mRNA expression. The last effect may contribute to lower the BP, which is crucial to diminish the adverse consequences of the hypertensive encephalopathy of SHR. Fig. 3 shows the analysis of AVP mRNA by in situ hybridization in the PVN of the hypothalamus of a control normotensive rat (upper graph, A), SHR (middle, B) and SHR receiving E_2 treatment for 2 weeks. In quantitative terms, the high expression level of AVP mRNA in SHR was significantly decreased by E_2. Cells intensely positive for ER_2 are found in the magnocellular subdivision of the PVN (Shughrue et al., 1997), supporting a direct steroid effect upon the AVP-producing cells of the PVN. These results indicate that alterations in the hippocampus and hypothalamus of the SHR model are also plastic events reversible by steroid treatment (Pietranera et al., 2008). It is likely, although not yet proven, that E_2 effects in SHR involve changes of the expression or function of MR, which if unopposed becomes a "death receptor", according to Funder (2004). The E_2 protective effects reported in our studies may be of clinical interest to attenuate the consequences of hypertensive encephalopathy, because in women loss of estrogens in the menopause is a risk factor for development of hypertension and stroke.

7. Concluding remarks

Estrogens control non-reproductive events in the brain which bear important consequences for the treatment of aging and age-associated diseases. The intervening mechanism that accounts for estrogen effects may involve the intracellular ERα and ERβ, second messenger systems, membrane steroid receptors, antioxidant effects or several of these effects acting simultaneously (Behl, 2002; Goodman et al., 1996; McEwen, 2002.). However, it seems that the ERβ isoform is more likely to mediate neuroprotective and beneficial effects of estrogens on hippocampal function (Liu et al., 2008) and also in the hypothalamus (Pietranera et al., 2008). While the experiments here reported did not explore the molecular mechanism(s) of estrogen action, they uncovered important biological consequences of estrogen effects in the brain in the course of aging, diabetes and hypertension.

Thus, evidences in middle age, diabetic and hypertensive animals have confirmed reports showing that estrogens exert beneficial effects for the hippocampus. Several lessons remain from the use of these models. First, that hippocampal neuropathology is remarkably similar in aging, diabetes and hypertension. In this sense, the brain of diabetic and hypertensive animals present an "accelerated" aging. Second, that excess activity of the glucocorticoid or mineralocorticoid systems may increase hippocampal vulnerability. Third, that the damaging effect of GR/MR overdrive rather than being a permanent condition maintains a plastic time frame that is partially or totally reversible with the treatment of neuroprotective hormones, i.e., estrogens. Fourth, there is a dual role for neuroactive steroids (protection vs. toxicity) in neurodegenerative diseases. Our data demonstrate that estrogen treatment, albeit in pharmacological doses, is able to protect the hippocampus and overcomes the undesirable overdrive of GR/MR. Likewise, E_2 treatment alleviated the abnormal expression of the hypertensinogenic peptide AVP in the hypothalamus of SHR. Therefore, E_2 also acts in the brain to decrease BP, suggesting additional mechanisms of neuroprotection.

Lastly, results from middle-age mice are particularly relevant for comparison with humans and other species. In rodents, there is increased recognition that young or middle age rodents are highly sensitive to estrogen, whereas hormone responsiveness is attenuated in very old animals (Sherwin and Henry, 2008). Old monkeys, however, remain receptive to estrogens even after prolonged ovariectomy (Lacreuse, 2006). In humans, there is a "window of opportunity" for hormone-replacement therapy, because it has been reported that estrogen therapy prevents the deleterious effects of brain aging if given at the perimenopause, whereas they are inactive or may even exacerbate neurodegeneration when given late in life (Sherwin and Henry, 2008). Therefore, our studies provide a preclinical background to answer questions raised by degenerative diseases in humans. The effect of E_2 on neurogenesis in the animal models is strongly relevant, since stimulation of endogenous progenitors to repair cellular damage would be an alternative method to more costly or aggressive therapies, such as transplants of fetal neurons, stem cells or injection of viral vectors (Verret et al., 2007).

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References

Baulieu, E.E., Robel, P., Schumacher, M., 2001. Neurosteroids: beginning of the story. Int. Rev. Neurobiol. 46, 1–32.

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

Brismar, T., Maurex, L., Cooray, G., Juntti-Berggren, L., Lindström, P., Ekberg, K., Adner, N., Andersson, S., 2007. Predictors of cognitive impairment in type 1 diabetes. Psychoneuroendocrinology 32, 1041–1051.

Cameron, H.A., McKay, R.D., 1999. Restoring production of hippocampal neurons in old age. Nat. Neurosci. 2, 894–897.

Darnaudery, M., Perez-Martin, M., Belizaire, G., Maccari, S., Garcia-Segura, L.M., 2006. Insulin-like growth factor 1 reduces age-related disorders induced by prenatal stress in female rats. Neurobiol. Aging 27, 119–127.

De Kloet, E.R., Derijk, R.H., Meijer, O.C., 2007. Therapy Insight: is there an imbalanced response of mineralocorticoid and glucocorticoid receptors in depression? Nat. Clin. Pract. Endocrinol. Metab. 3, 168–179.

De Nicola, A.F., Magariños, A.M., Foglia, V.G., 1991. Neuroendocrine regulation in experimental diabetes (Houssay Lecture). In: Rifkin, H., Colwell, J.A., Taylor, S.I. (Eds.), Diabetes. Elsevier Science Publishers, pp. 3–8.

De Nicola, A.F., Saravia, F.E., Beauquis, J., Pietranera, L., Ferrini, M.G., 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, A.F., 1999. Estrogens normalize the hypothalamic–pituitary–adrenal axis response to stress and increase glucocorticoid receptor immuno-reactivity in hippocampus of aging male rats. Neuroendocrinology 69, 129–137.

Funder, J.W., 2004. Aldosterone, mineralocorticoid receptors and vascular inflammation. Mol. Cell. Endocrinol. 217, 263–269.

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

Goodman, Y., Bruce, A.J., Cheng, B., Mattson, M.P., 1996. Estrogens attenuate and CORT exacerbates excitotoxicity, oxidative injury, and amyloid β-peptide toxicity in hippocampal neurons. J. Neurochem. 66, 1836–1844.

Isgor, C., Watson, S.J., 2005. Estrogen receptor alpha and beta mRNA expressions by proliferating and differentiating cells in the adult rat dentate gyrus and subventricular zone. Neuroscience 134, 847–856.

Kempermann, G., Gast, D., Gage, F.H., 2000. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann. Neurol. 52, 135–143.

Lacreuse, A., 2006. Effects of ovarian hormones on cognitive function in nonhuman primates. Neuroscience 138, 859–867.

Lephart, E.D., Galindo, E., Bu, L.H., 2003. Stress (hypothalamic–pituitary–adrenal axis) and pain response in male rats exposed lifelong to high vs. low phytoestrogen diets. Neurosci. Lett. 342, 65–68.

Liu, F., Day, M., Muñiz, L.C., Bitran, D., Arias, R., Revilla-Sanchez, R., Grauer, S., Zhang, G., Kelley, C., Pulito, V., Sung, A., Mervis, R.F., Navarra, R., Hirst, W.D., Reinhart, P.H., Marquis, K.L., Moss, S.J., Pangalos, M.N., Brandon, N.J., 2008. Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. Nat. Neurosci. 11, 334–343.

McEwen, B.S., 2002. Estrogen actions throughout the brain. Rec. Progr. Horm. Res. 57, 357–370.

Miller, D.B., O'Callaghan, J.P., 2005. Aging, stress and the hippocampus. Ageing Res. Rev. 4, 123–140.

Oomen, C.A., Mayer, J.L., de Kloet, E.R., Joëls, M., Lucassen, P.J., 2007. Brief treatment with the glucocorticoid receptor antagonist mifepristone normalizes the reduction in neurogenesis after chronic stress. Eur. J. Neurosci. 26, 3395–3401.

Perez-Martin, M., Salazar, V., Castillo, C., Ariznavarreta, C., Azcoitia, I., Garcia-Segura, L.M., Tresguerres, J.A., 2005. Estradiol and soy extract increase the production of new cells in the dentate gyrus of old rats. Exp. Gerontol. 40, 450–453.

Petersen, R.C., Jack Jr., C.R., Xu, Y.C., Waring, S.C., O'Brien, P.C., Smith, G.E., Ivnik, R.J., Tangalos, E.G., Boeve, B.F., Kokmen, E., 2000. Memory and MRI-based hippocampal volumes in aging and AD. Neurology 54, 581–587.

Pietranera, L., Saravia, F., Gonzalez Deniselle, M.C., Roig, P., Lima, A., De Nicola, A.F., 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.E., Roig, P., Lima, A., De Nicola, A.F., 2008. Protective effects of estradiol in the brain of rats with genetic or mineralocorticoid-induced hypertension. Psychoneuroendocrinology 33, 270–281.

Rahmouni, K., Barthelmebs, M., Grima, M., Imbs, J.L., De Jong, W., 2001. Involvement of brain mineralocorticoid receptor in salt-enhanced hypertension in spontaneously hypertensive rats. Hypertension 38, 902–906.

Resnick, S.M., Maki, P.M., Rapp, S.R., Espeland, M.A., Brunner, R., Coker, L.H., Granek, I.A., Hogan, P., Ockene, J.K., Shumaker, S.A., 2006. Women's health initiative study of cognitive aging investigators. effects of combination estrogen plus progestin hormone treatment on cognition and affect. J. Clin. Endocrinol. Metab. 91, 1802–1810.

Sapolsky, R., Krey, L.C., McEwen, B.S., 1986. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr. Rev. 7, 284–304.

Saravia, F.E., Beauquis, J., Pietranera, L., De Nicola, A.F., 2007. Neuroprotective effects of estradiol in the hippocampus of middle age mice: involvement of neurons and glial cells. Psychoneuroendocrinology 32, 480–492.

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

Scharfman, H.E., Maclusky, N.J., 2005. Similarities between actions of estrogen and BDNF in the hippocampus: coincidence or clue? Trends Neurosci. 28, 79–85.

Sherwin, B.B., Henry, J.F., 2008. Brain aging modulates the neuroprotective effects of estrogen on selective aspects of cognition in women: a critical review. Front. Neuroendocrinol. 29, 88–113.

Shughrue, P.J., Lane, M.V., Merchentaler, I., 1997. Comparative distribution of estrogen receptor α and β mRNA in the rat central nervous system. J. Comp. Neurol. 388, 507–525.

Smith, R.G., Betancourt, L., Sun, Y., 2005. Molecular endocrinology and physiology of the aging central nervous system. Endocr. Rev. 26, 203–250.

Tanapat, P., Hastings, N.B., Gould, E., 2005. Ovarian steroids influence cell proliferation in the dentate gyrus of the adult female rat in a dose- and time-dependent manner. J. Comp. Neurol. 481, 252–265.

Verret, L., Trouche, S., Zerwas, M., Rampon, C., 2007. Hippocampal neurogenesis during normal and pathological aging. Psychoneuroendocrinology 32 (Suppl. 1), S26–S30.

Wong, E.Y.H., Herbert, J., 2005. Roles of mineralocorticoid and glucocorticoid receptors in the regulation of progenitor proliferation in the adult hippocampus. Eur. J. Neurosci. 22, 785–792.