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Neuroprotective effects of estradiol in hippocampal neurons and glia of middle age mice

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Lipofuscin;
doublecortin;
Ki67

Summary
During aging the hippocampus experiences structural, molecular, and functional alterations. Protection from age-related disorders is provided by several factors, including estrogens. Since aging defects start at middle age, we studied if 17 β-estradiol (E_2) protected the hippocampus 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. Ten days afterwards they received bromodeoxyuridine (BrdU) 4 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 astrocytosis, and by neuronal loss in the hilus. Middle age mice receiving E_2 showed increased number of BrdU+cells while the other parameters were remarkably attenuated. When steroid treatment was prolonged for 2 months to study migration of cells in 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, a marker for differentiating neurons. 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 other aging indicators as increased deposits of the aging pigment lipofuscin in DG cells, loss of hilar neurons and astrocytosis supports a wide range protection of hippocampal function of middle age mice by estrogenic hormones.

Abbreviations: E_2, 17β estradiol; BrdU, bromodeoxyuridine; DG, dentate gyrus; SGZ, subgranular zone; DCX, doublecortin; GCL, granular cell layer; HPA, hypothalamic–pituitary–adrenal; GFAP, glial fibrillary acidic protein; MA, middle age; chol, cholesterol

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1. Introduction

Aging is accompanied by pathological changes that preferentially target the hippocampus (Smith et al., 2005). Thus, deficits in learning, memory and its associated neurogenesis, changes of neurotransmission, ion channels and electrical activity, altered expression of neuropeptides, growth factors and their receptors, anomalous expression of genes and transcription factors, changes of steroid receptors, vasculopathy, high nitrergic activity and increased oxidative stress, neuronal loss and atrophy, astrogliosis and demyelination account for most hippocampal abnormalities of aging (Ferrini et al., 1999; McEwen, 1999; Driscoll and Sutherland, 2005; Miller and O'Callaghan, 2005).

A typical feature of aging is the reduction of hippocampal neurogenesis (Cameron and McKay, 1999; Kuhn et al., 1996; Kempermann et al., 2002; Heine et al., 2004). In the adult, this process is restricted to the subventricular zone 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 (Cameron and McKay, 1999; Cameron et al., 1993; Heine et al., 2004). Functionally, neurogenesis is associated with learning and memory and acquisition of a fear-conditioned response (Gould et al., 2000; Shors et al., 2002). Since newly-formed neurons may optimize interconnections between the DG and the CA3 pyramidal subfield, the whole hippocampal function may be influenced by changes of neurogenesis (Kempermann et al., 2002).

Steroid hormones strongly influence neurogenesis besides other hippocampal parameters. Estrogens are neuroprotective hormones (Behl, 2002; García-Segura et al., 2001; McEwen et al., 2001) and they positively control neurogenesis. For instance, uptake of the thymidine analog bromodeoxyuridine (BrdU) by proliferating cells of the DG is higher in 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., 1999). The increase in proliferation is transient, and diminishes in animals subjected to prolonged ovariectomy or chronically overloaded with estrogen (Ormerod et al., 2003). A gender difference has been suggested, because in males the response of hippocampal neurogenesis to E_2 is attenuated (Galea et al., 2006). In our experience, a ceiling effect probably accounts for the failure of E_2 to increase granule cell proliferation in normal male mice. In contrast, the response to E_2 is noticeable in diabetic male mice, which show extremely low cell proliferation rates under basal conditions (Saravia et al., 2004, 2006; Beauquis et al., 2006).

It is well accepted that in aging animals, proliferation and migration of newborn cells in the DG is strongly reduced (Heine et al., 2004). In part, this reduction may be exacerbated by a dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis (van Eekelen et al., 1991; Ferrini et al., 1999; Sapolsky, 1999; Dalm et al., 2005). Hyperfunction of the HPA axis increases the secretion of adrenal steroids, which negatively impact on hippocampal integrity and on cell proliferation and differentiation in the DG (Miller and O'Callaghan, 2005; Wong and Herbert, 2005). Progenitors in the DG express glucocorticoid and mineralocorticoid receptors (Wong and Herbert, 2005). Therefore, hippocampal neurogenesis and HPA axis activity are inversely correlated, suggesting that the low hippocampal neurogenesis of senescent animals may be an index of glucocorticoid-mediated neurotoxicity (Cameron and McKay, 1999; Kempermann et al., 2002).

That estrogens positively control neurogenesis has been mostly informed for young animals (Tanapat et al., 1999, 2005; Ormerod et al., 2003), while few studies evaluated a similar role in old animals. In one study (Perez-Martin et al., 2005) 22-month-old ovariectomized rats received prolonged treatment with estradiol 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 insulin growth factor type I (IGF-1) to 22-month-old rats increases both neurogenesis and blood levels of E_2, suggesting steroid participation in the cell proliferation 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 (Heine et al., 2004). 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 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 it recruited old postmenopausal women (65–79 years old), an age span when estrogen responsiveness diminishes (Foster, 2005). Clinical trials support the effectiveness of hormone-replacement therapy in prevention rather than improvement of mental deterioration (Henderson et al., 1994; Wang et al., 2000). Since middle age animals are fully responsive to E_2 (Brewer et al., 2006; Wise, 2006), 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, and paradoxically, the literature is not abundant in this period of life.

To fully appreciate hormonal effects on 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 the glial fibrillary acidic protein (GFAP) (Goss et al., 1991; Nichols et al., 1993; David et al., 1994). 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 (Day et al.,1993; Garcia-Ovejero et al., 2002; Lei et al., 2003). Another hallmark of aging is lipofuscin, an autofluorescent pigment that accumulates inside neurons due to increased oxidative stress (Keller et al., 2004). E_2 treatment decreases brain lipofuscin in 12-, 18- and 24-month-old rats, according to one study that included