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Review

Estradiol is a protective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies

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Abstract

We have shown that 17β-estradiol exerts profound protective effects against stroke-like ischemic injury in female rats. These effects are evident using physiological levels of estradiol replacement in ovariectomized rats and require hormone treatment prior to the time of injury. The protective actions of estradiol appear to be most prominent in the cerebral cortex, where cell death is not apparent until at least 4 h after the initiation of ischemic injury and where cell death is thought to be apoptotic in nature. Middle-aged rats remain equally responsive to the protective actions of estradiol. The maintenance of responsiveness of the cerebral cortex to the neuroprotective actions of estradiol was unexpected since responsiveness of the hypothalamus to estradiol decreases dramatically by the time animals are middle-aged. We believe that the protective actions of estradiol require the estrogen receptor-α, since estradiol does not protect in estrogen receptor-α knockout mice. We have also implemented a method of culturing cerebral cortical explants to assess the protective effects of estradiol in vitro. This model exhibits remarkable parallelisms with our in vivo model of brain injury. We have found that 17β-estradiol decreases the extent of cell death and that this protective effect requires hormone pretreatment. Finally, 17α-estradiol, which does not interact effectively with the estrogen receptor, does not protect; and addition of ICI 182,780, an estrogen receptor antagonist, blocks the protective actions of estradiol. We have begun to explore the molecular and cellular mechanisms of estradiol-mediated protection. In summary, our findings demonstrate that estradiol exerts powerful protective effects both in vivo and in vitro and suggest that these actions are mediated by estrogen receptors.

Keywords: Estradiol; Neuroprotection; Ischemia; In vitro; In vivo; Cell death

Contents

1. Introduction

Several clinical investigations demonstrate that post-menopausal women are more vulnerable than young women to neurodegenerative diseases, such as Alzheimer's

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and Parkinson's disease, ischemic brain injury, such as stroke, and memory or cognitive dysfunction. Furthermore, estrogen replacement therapy appears to decrease the risk and/or severity of neurodegenerative conditions and may also improve memory and cognition. During the past century, the average life span of women has increased dramatically; whereas, the age of the menopause has remained essentially fixed. Consequently, a large number of women will spend a significant proportion of their lives in a hypo-estrogenic postmenopausal state. Since estradiol influences so many aspects of central nervous system function, understanding the cellular and molecular mechanisms that underlie these protective actions is essential to preventing the deleterious consequences of prolonged hypoestrogenicity and to improving women's health.

Researchers have used in vivo and in vitro methods to study the mechanisms whereby estradiol protects against injury in both young and aging animals. Each approach has its advantages. The in vivo studies attempt to replicate clinical situations of brain injury in the context of the complexity of the whole animal. In vitro methods allow investigators to use more simple systems where direct and indirect actions of estradiol can be differentiated.

Multiple in vivo studies clearly show that estradiol exerts potent protective actions against brain injury. Females are less vulnerable to acute insults associated with cerebral ischemia, neurotrauma, hypoxia, and drug-induced toxicity (for reviews see Refs. [14,18,21,24,39]). Administration of estradiol or estrogenic compounds protects against stroke, like estrogen replacement therapy decreases the extent of injury, and may decrease mortality. Estrogens have also been shown to decrease cell death in numerous in vitro models of neural injury. Investigators have used different neurotoxic paradigms and culturing methods (reviewed in Refs. [14,18,28,39]) to evaluate the efficacy of estrogens to attenuate injury. The in vitro approach has helped us immensely to decipher the underlying molecular mechanisms by which estradiol attenuates the extent of injury. The approach has been essential to our understanding of the complex, interactive and multiple molecular mechanisms by which estradiol attenuates the extent of injury.

2. Estradiol protects against ischemic brain injury in vivo

We have found that administration of low and high physiological levels of 17β-estradiol, which mimic those that circulate in rats during the estrous cycle (low) or the peak levels observed on proestrus (high) [29], for 1 week prior to permanent occlusion of the middle cerebral artery leads to a dramatic decrease in the extent of the infarct compared to oil-treated controls [9]. Furthermore, we found that middle-aged rats remained equally responsive to the protective effects of estradiol compared to their young counterparts. Fig. 1 is a composite of representative coronal brain sections from oil- and estradiol-treated, young and middle-aged rats following cerebral ischemia. Moreover, both low and high physiological estradiol pretreatment afforded identical protection in both age groups (Fig. 2A,B). Analysis of treatment effects in the cerebral cortex and the striatum revealed that the neuroprotective effects of estradiol in young and middle-aged rats were region-specific: estradiol pretreatment dramatically and equivalently reduced the cortical infarct in both age groups (Fig. 3A,B). In contrast, estradiol did not decrease the extent of brain injury in the striatum in young or middle-aged rats (Fig. 3C,D).

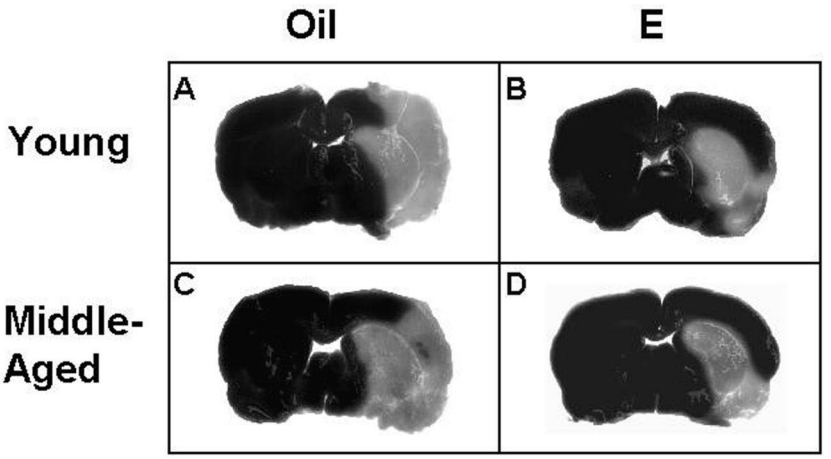

Fig. 1. Brain sections from a vehicle- (oil) and estradiol-treated, young and middle-aged rat 24 h following MCAO. Injured tissue is light and live tissue is darkly stained by TTC. In the absence of estradiol, brain injury was extensive in (A) young and (C) middle-aged rats. Physiological estradiol pretreatment (180 μg/ml) reduced the extent of infarct in both (B) young and (D) middle-aged rats. From Ref. [11] with permission.

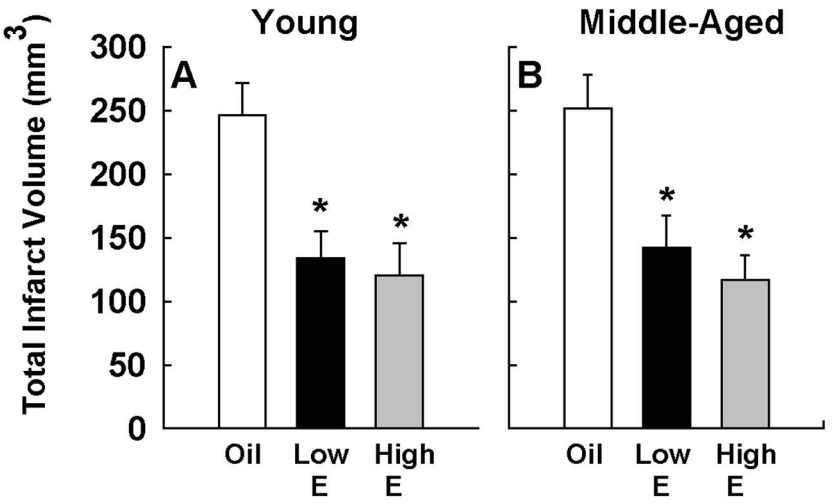

Fig. 2. Effects of estradiol on total ischemic brain injury in young and middle-aged rats. Low and high physiological levels of estradiol attenuate total infarct volume in (A) young (low E, $P<0.01$; high E, $P<0.03$) and (B) middle-aged (low E, $P<0.03$; high E, $P<0.01$) rats. Values represent mean±S.E. From Ref. [11] with permission.

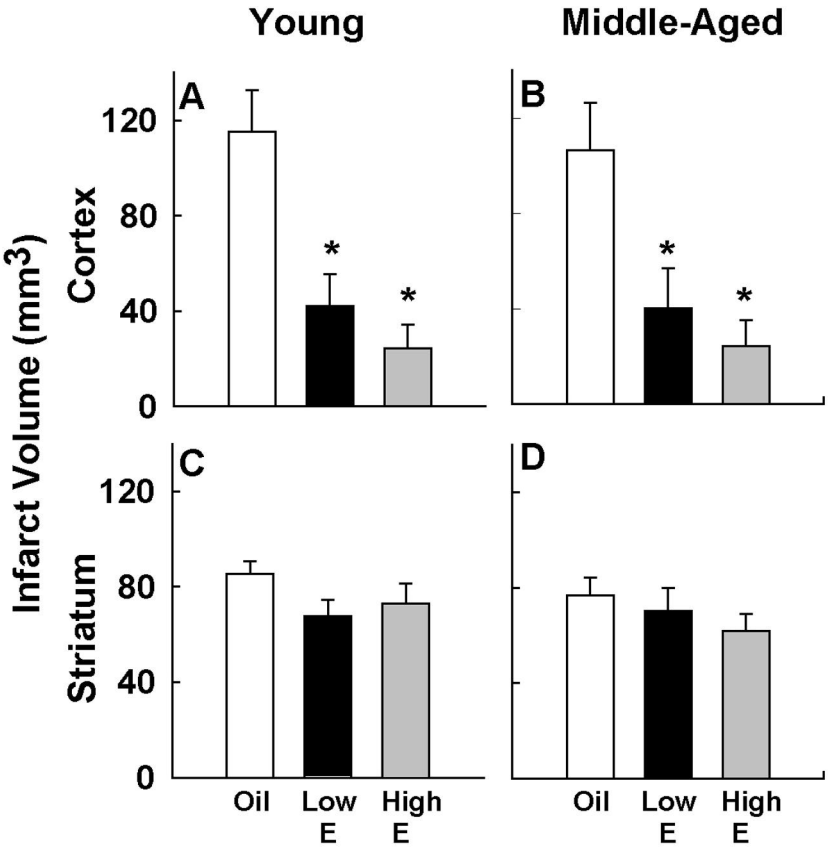

Fig. 3. The protective actions of estradiol are region specific in young and middle-aged rats. Neuroprotective effects of estradiol are confined to the cerebral cortex of (A) young (low E, $P<0.01$; high E, $P<0.01$) and (B) middle-aged (low E, $P<0.05$; high E, $P<0.03$) rats. Estradiol does not protect the striatum of either (C) young ($P=0.41$) or (D) middle-aged ($P=0.16$) female rats. Values represent mean±S.E. From Ref. [11] with permission.

3. Estradiol protects against ischemia/hypoglycemia in vitro

To determine whether estradiol protects against ischemic and metabolic injury in vitro, we performed parallel studies in explant cultures of the cerebral cortex obtained from neonatal rats. Explants were incubated in the presence or absence of vehicle or increasing concentrations of estradiol for 7 days. Low concentrations of estradiol (1, 10, and 30 nM) significantly protected cortical explants from KCN/2DG-induced cell death; however, higher concentrations of estradiol (≥60 nM) did not protect against injury-induced cell death (Fig. 4A). To determine how long estradiol must be present to exert its protective actions, estradiol (10 nM) was incubated with the explants for 7 days, 2 days, 24 h, 2 h prior to injury or was added at the time of injury. We found that estradiol did not protect against cell death if estradiol was present for less than 2 days (Fig. 4B). Finally, we tested whether the protective effects of estradiol require estrogen receptors by assessing whether 17α-estradiol, which does not efficiently activate the estrogen receptor, protects. We also assessed whether co-incubation of 17β-estradiol and 100-fold excess of an estrogen receptor antagonist, ICI 182,780, prevents the protective effects of 17β-estradiol. 17α-Estradiol did not exert any protective effect against cell death. Treatment with ICI 182,780 blocked the protective effects of 17β-estradiol (Fig. 4C).

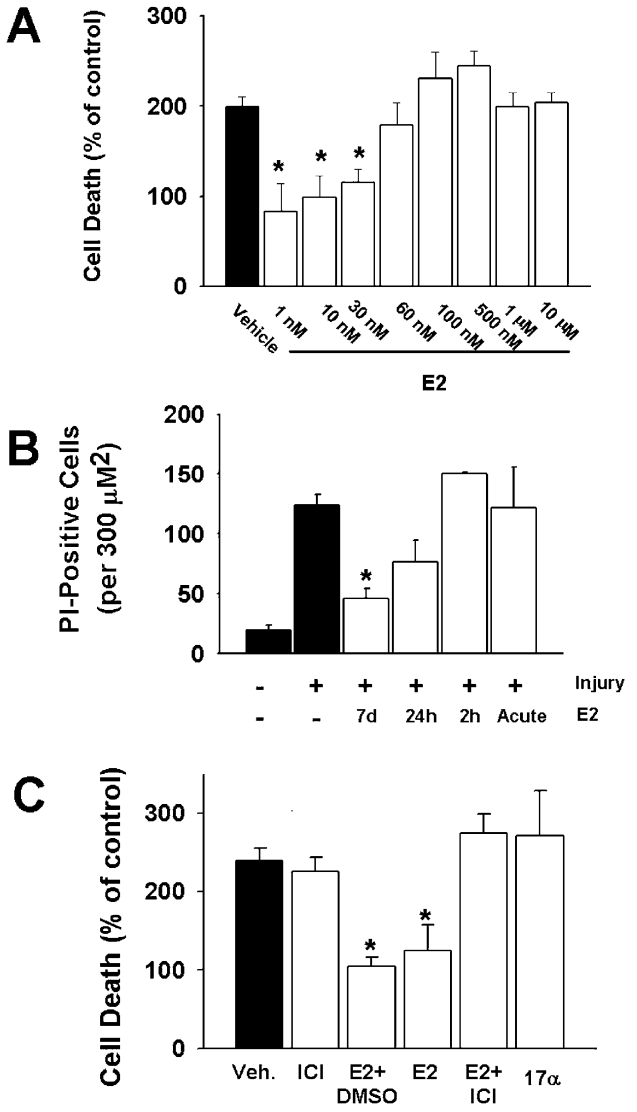

Fig. 4. (A) LDH release following ischemic injury in the presence of increasing amounts of estradiol. Bars represent mean of cell death±S.E. *Significantly different from the amount of cell death following injury in the absence of estradiol ($P<0.05$). (B) Cell death 24 h after ischemic injury as measured by propidium iodide uptake. Cultures were pretreated with 10 nM 2 for 7 days, 24 h, 2 h or at the time of injury. Bars represent the mean of dead cells±S.E.M. ($n=3–4$). *Significantly different from vehicle ($P<0.05$). (C) LDH release in controls and following ischemic injury following treatment with 17α- or 17β-estradiol in the presence or absence of ICI 182,780. Bars represent the mean of cell death expressed as a percent of control±S.E. ($n=3=6$). *Significantly different from vehicle ($P<0.05$). From Ref. [41] with permission.

4. Discussion

These results clearly establish that estradiol is a potent protective factor in the brain. Our studies have focused attention on the neuroprotective effects of physiological estradiol in the brain since we believe that these levels of hormone are highly relevant to replacement therapies that women will be using. We report four important findings. First, pretreatment with low or high physiological concentrations of estradiol following ovariectomy exerts striking and equivalent neuroprotection against stroke injury induced by permanent MCAO in young and middle-aged rats. Second, the effects of estradiol are region-specific and equivalently amplified in the cerebral cortex of young and middle-aged rats. Third, relatively low concentrations of estradiol exert significant protective effects in vitro explants of the cerebral cortex that are highly reminiscent of the effects observed in vivo. Fourth, the neuroprotective effects that we detected in vitro appear to be estrogen-receptor-dependent.

Our in vivo estrogen replacement paradigms produced serum levels that are equivalent to those achieved during the estrous cycle in rats: the low dose produces basal levels that circulate in blood on all days of the cycle except late on diestrous day 2 and proestrus; the higher dose is similar to that achieved transiently on the morning of proestrus [29]. Our data clearly show that in middle-aged female rats, the cerebral cortex remains responsive to the neuroprotective effects of physiological levels of estradiol, compared to young rats. We did not assess whether older animals remain responsive to estradiol since this question is impossible to answer using the current methods of artery occlusion: arteries become less elastic and, consequently, occlusion using the suture method becomes less reliable [32]. Thus, other models of experimental brain injury have

been used in aging studies [1,43]. For example, older male animals exhibited greater cerebral infarction in response to focal ischemia [8,13,30]. However, global ischemia has resulted in inconsistent effects in older animals and this may be related to several variables including strain [5,6], sex [2,30,43], experimental conditions [2] and location of the infarct [30].

We were surprised that estradiol treatment following ovariectomy exerted the same degree of neuroprotection in young and middle-aged female rats because responsiveness to estradiol is diminished during middle age when one measures a variety of other endpoints. Specifically we have found that hypothalamic responses to estradiol that are involved in the regulation of estrous cyclicity are attenuated in middle-aged rats, including (1) activation of GnRH neurons that leads to LH surges [23,37,38], (2) organization of diurnal rhythmicity in the hypothalamic neurotransmitter activity [7,37] or gene expression [22,34], and (3) stimulation of progesterone receptor binding [40]. For these reasons, we predicted that estradiol would be less able to protect the brains of older animals against ischemic brain injury. Since our data demonstrate that middle-aged rats remain equally responsive to the neuroprotective actions of estradiol compared to young animals, our findings imply that, despite changes in hypothalamic responsiveness, middle-aged women may continue to remain responsive to the non-reproductive, neuroprotective actions of estradiol in the cerebral cortex.

Interestingly, in this in vivo model of stroke, the neuroprotective actions of estradiol in young and aging female rats are confined to the cerebral cortex, and are not significant in the striatum. The region-specific effect may result from differential vascularization from the middle cerebral artery to the cortex compared to the striatum [31]. If blood flow to the striatum is more compromised compared to the cortex, then we would expect that cell death may result more from necrotic than apoptotic mechanisms of cell death, which may not be amenable to protection by estradiol. Data from our laboratory [10] and Dubal et al. (unpublished) and others [15,26] suggest that physiological concentrations of estradiol protect through mechanisms that attenuate apoptosis, but do not influence necrosis. Thus, if cell death in the striatum is predominantly rapid and through necrotic mechanisms, we would not expect estradiol to be able to protect this region of the brain.

Several lines of evidence demonstrate that the cerebral cortex is an important target for estradiol-mediated neuroprotection via estrogen receptor (ER)-mediated mechanisms [10,12]. We discovered that though ERα mRNA is not normally expressed in the cerebral cortex or the striatum of adult rats [25], cerebral ischemia dramatically and selectively up-regulates ERα, but not ERβ, gene expression in the cortex [10]. Furthermore, we recently investigated the roles of ERα and ERβ in mediating the protective effects of estradiol by utilizing ERα and ERβ-knockout mice in an animal model of stroke. Our results demonstrate that deletion of ERα completely abolishes the protective actions of estradiol in all regions of the brain, whereas the ability of estradiol to protect against brain injury is totally preserved in the absence of ERβ [12]. Thus, these results clearly establish that the estrogen receptor subtype, ERα, is a critical mechanistic link in mediating the protective effects of physiological levels of estradiol in brain injury. These results carry far-reaching implications for the selective targeting of estrogen receptors in the treatment and prevention of neural dysfunction associated with normal aging or brain injury.

Using explant cultures of the cerebral cortex, we show that the presence of low doses of estradiol protected against cell death, whereas higher levels were not protective. In addition, as in our in vivo experiments, pretreatment was required for estradiol to protect. Indeed, at least 2 days was required: treatment for 1 day or less did not protect against injury. Furthermore, our findings strongly suggest that estrogen receptors mediate the protective effects of estradiol in vitro, at least under our experimental conditions, since 17α-estradiol failed to protect against cell death, and the estrogen receptor antagonist, ICI 182,780, prevented estradiol from protecting against cell death.

We found that in vitro low doses (1, 10, 30 nM) of estradiol protected against ischemia-induced cell death; however, higher doses (60 nM and greater) did not. This narrow window of concentrations that protect is similar to that found by Singer et al. [27]. It is possible that estradiol affects neuronal excitability in cortical explants as it does in the arcuate nucleus [4], hippocampus [20,42] and nucleus accumbens [16]. It is possible that higher levels of estradiol that are not toxic in the basal state, may become toxic following injury. Thus, neurons that are in a hypersensitive state, may be more vulnerable to cell death after an neurodegenerative insult. Several investigators have demonstrated that pharmacological doses (10–50 μM) of estradiol are neuroprotective against neuronal cell death caused by β-amyloid toxicity, oxidative stress, and excitotoxicity [3,17,33], while in our studies the high levels of estradiol were not protective. Several possible reasons may underlie this apparent discrepancy: culture conditions vary widely, neuronal cell lines or primary cultures may respond differently to different concentrations of estradiol, and/or the duration of estradiol administration may influence the effects of varying doses of estradiol.

The majority of studies that have examined the neuroprotective actions of estradiol in vitro have used hippocampal explant or dispersed cells, or immortalized neuronal cell lines. In these experimental paradigms, a wide variety of neurotoxic stimuli including oxidative stress, excitotoxic insults and β-amyloid toxicity induce significant neuronal cell death that can be attenuated by estrogenic compounds [3,17,19,27]. Interestingly, cortical explants were much less vulnerable to selected neurotoxic stimuli than dispersed cortical or hippocampal neurons, immortalized

neuronal cell lines. Previous reports have shown that short exposure to excitatory amino acids causes significant cell death in dispersed hippocampal neurons [17]: a 5-min exposure to excitatory amino acids dramatically increases hippocampal cell death in dispersed cells [27] and a 30-min exposure to kainic acid or glutamate significantly increases hippocampal cell death in explant cultures [36]. In marked contrast, cortical explants were insensitive to either a short exposure to kainic acid or glutamate [35].

In summary, our results clearly demonstrate that estradiol plays a neuroprotective role in the injured brain in both young and middle-aged rats. Further, our in vitro studies demonstrate that estradiol protects against cell death in explant cultures of the cerebral cortex and that these methods may help to decipher the mechanisms of actions of estradiol. These data imply that older women may benefit from the protective effects of estrogen replacement therapy that utilizes relatively low concentrations of hormone.

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