

Estrogen receptor α , not β , is a critical link in estradiol-mediated protection against brain injury

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Estradiol protects against brain injury, neurodegeneration, and cognitive decline. Our previous work demonstrates that physiological levels of estradiol protect against stroke injury and that this protection may be mediated through receptor-dependent alterations of gene expression. In this report, we tested the hypothesis that estrogen receptors play a pivotal role in mediating neuroprotective actions of estradiol and dissected the potential biological roles of each estrogen receptor (ER) subtype, ER α and ER β , in the injured brain. To investigate and delineate these mechanisms, we used ER α -knockout (ER α KO) and ER β -knockout (ER β KO) mice in an animal model of stroke. We performed our studies by using a controlled endocrine paradigm, because endogenous levels of estradiol differ dramatically among ER α KO, ER β KO, and wild-type mice. We ovariectomized ER α KO, ER β KO, and the respective wild-type mice and implanted them with capsules filled with oil (vehicle) or a dose of 17 β -estradiol that produces physiological hormone levels in serum. One week later, mice underwent ischemia. Our results demonstrate that deletion of ER α 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 ER β . Thus, our results clearly establish that the ER α subtype is a critical mechanistic link in mediating the protective effects of physiological levels of estradiol in brain injury. Our discovery that ER α mediates protection of the brain carries far-reaching implications for the selective targeting of ERs in the treatment and prevention of neural dysfunction associated with normal aging or brain injury.

Menopause marks the end of female reproduction and is accompanied by a dramatic and permanent decrease in estrogen levels. Although the life span of women has increased significantly in the past century, the average age of menopause has remained constant. Consequently, women may now spend more than one-third of their lives in a chronic hypoestrogenic postmenopausal state. Because estradiol is an important trophic and protective factor in the adult brain (1, 2), hypoestrogenic postmenopausal women may be more vulnerable to brain injury and dysfunction caused by neurodegenerative conditions and cognitive decline. It is, therefore, crucial that we gain a complete understanding of the mechanisms underlying the neuroprotective actions of estradiol.

A growing body of evidence has begun to reveal that estrogen replacement therapy may ameliorate neural dysfunctions resulting from Alzheimer's disease (3–5) and stroke (6, 7) through multiple and complex cellular and molecular mechanisms of action. The protective role of estrogen in brain function has been examined by using a variety of *in vivo* and *in vitro* models of brain injury that mimic neurotoxic environments found in Alzheimer's disease, stroke, and other neurodegenerative conditions (8–13). These studies demonstrate that physiological and pharmacological concentrations of 17 β -estradiol, the predominant and most biologically active estrogen secreted by the ovary, profoundly attenuate the extent of injury and decrease cell death in the brain.

Using *in vivo* and *in vitro* methods, our laboratory has focused on the mechanisms by which physiological levels of estradiol

protect the brain against stroke injury and, specifically, whether estrogen receptors (ERs) are essential to neuroprotection. After the discovery that two ER subtypes, ER α and ER β , exist throughout the body (14), we began to probe the potential roles of these receptors in the brain. We found that the neuroprotective effects of estradiol are not rapid and require a period of pretreatment, suggesting that ER-mediated alteration of gene expression is required to afford neuroprotection (8, 12). Indeed, our initial studies revealed that in response to injury estradiol modulates critical factors including bcl-2 (15), galanin (16), and immediate early genes (17). Furthermore, we found that ERs are differentially modulated in neurodegenerative injury. After injury, ER α mRNA is highly up-regulated in the presence and absence of estradiol. In contrast, injury down-regulates ER β mRNA and estradiol prevents this injury-induced down-regulation (15). Finally, our *in vitro* studies established that 17 α -estradiol, an estrogen isomer that has 100-fold less affinity for ERs, fails to protect against injury (12). In addition, these *in vitro* studies demonstrated that the protective actions of estradiol are blocked by ICI 162,780, an ER antagonist (12). Thus, these findings strongly implicate that estradiol acts through its receptor(s) in unique and novel ways in the injured brain.

The goal of this study was to test directly and dissect out the specific biological roles of ER subtypes, ER α and ER β , in estradiol-mediated protection against brain injury. We used ER α -knockout (ER α KO) and ER β -knockout (ER β KO) mice in an animal model of stroke. Our results show that ER α , but not ER β , is the critical mechanistic link that mediates the ability of physiological levels of estradiol to protect the brain against injury.

Materials and Methods

All surgical procedures were performed in compliance with the National Institutes of Health Guide and have been approved by the University of Kentucky, Chandler Medical Center, Institutional Animal Care and Use Committee.

Animals. The two strains of transgenic mice that were used in this study were obtained from Wyeth Ayerst Laboratories. Each transgenic strain was compared with its respective genetically matched wild-type strain. Homozygous ER α KO (C57BL/6J \times

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Abbreviations: ER, estrogen receptor; ER α , ER subtype α ; ER β , ER subtype β ; ER α KO, ER α knockout; ER β KO, ER β knockout; rCBF, regional cerebral blood flow.

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129) (18) and homozygous 129Sv ER β KO mice were bred and genotyped by PCR analysis of tail samples to assess the presence of the neomycin resistance, ER α , and/or ER β mRNAs. Briefly, ER β KO mice were generated as follows: A targeting vector was used in which a 2-kb fragment of exons 1 and 2 was replaced by a cassette including translational stop codons in all three reading frames, followed by a LoxP-flanked neomycin-resistance gene. This construct forces a translational block after the 19th amino acid. The construct was electroporated into R1 embryonic stem cells and neomycin-resistant clones were screened for homologous recombinants by Southern blot analysis. Two gene-targeted embryonic stem cell clones were identified, and each clone was expanded and used to generate germ-line chimeras by blast injection into C57BL/6 host blasts. The mice have been fully characterized to confirm the disruption of ER β by a variety of molecular and histological techniques (P.J.S., G. R. Askew, and I.M., unpublished results).

Hormone Replacement. Young mice (19–22 g) were ovariectomized to eliminate endogenous ovarian steroids and then implanted s.c. with a Silastic capsule [0.062 in/0.125 in, inner/outer diameter (1 in = 2.54 cm); volume, 0.035 ml] containing sesame oil (vehicle) or 17 β -estradiol (180 μ g/ml; n = 8–13 per group). The Silastic capsules consistently release hormone over time, producing stable levels of 17 β -estradiol in serum (19).

In Vivo Cerebral Ischemia. One week after ovariectomy and estradiol or vehicle treatment, mice were anesthetized with a mixture of chloral hydrate (350.0 mg/kg i.p.) and xylazine (4.0 mg/kg i.p.). Permanent cerebral ischemia was induced by a method modified from Huang *et al.* (20). Briefly, a 5/0 size blue nylon suture was fired at the tip and inserted into the external carotid artery. From the external carotid artery, it was advanced 11 mm into internal carotid artery, where it effectively occluded the anterior cerebral artery in the circle of Willis. In all mice, body temperature was maintained at normothermia until recovery from anesthesia.

Histological Preparation. Brains were collected 24 h after the onset of ischemia, frozen, and sectioned into 16- μ m sections. Coronal sections were stained with hematoxylin and eosin to clearly delineate the extent of ischemic injury. The volume of infarct was calculated by integrating the area of injury on 16- μ m stained coronal sections collected from the bregma points +2.46, +1.66, +0.86, +0.06, -0.74, -1.54, -2.34, and -3.14 mm of each brain (21). Total, cortical, striatal, and hippocampal infarct volumes were quantified with a computer-assisted imaging system (NIH IMAGE, Version 1.6).

Blood Flow Measurements. A laser Doppler probe was positioned through a craniotomy at 2.5–3.0 mm posterior to bregma and 2.0 mm lateral to the sagittal suture over the right parietal cortex (21), an area that is affected by cerebral ischemia and is protected by estradiol treatment. Baseline measurements were obtained every minute for 10 min. Ischemic laser Doppler measurements began 10 min after the onset of vascular occlusion and measurements were taken every minute for 30 min.

17 β -Estradiol Assay. In a pilot study, we determined the serum concentrations produced in the mice by our physiological paradigm of estradiol replacement. Mice were ovariectomized and implanted s.c. with a Silastic capsule containing sesame oil (vehicle) or 17 β -estradiol (180 μ g/ml) (n = 4 per group). One week after ovariectomy and treatment, we collected arterial blood from mice and froze the sera until the time of assay. Samples (250 μ l) were extracted with ether and then analyzed for levels of 17 β -estradiol by radioimmunoassay.

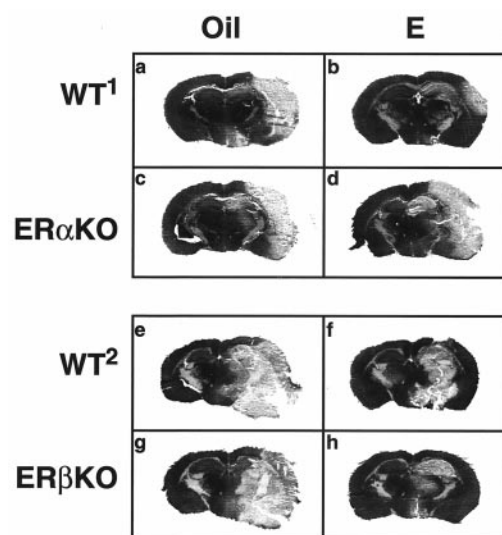


Fig. 1. Representative brain sections from oil- and estradiol-treated wild-type, ER α KO, and ER β KO mice that underwent permanent cerebral ischemia of the right hemisphere. In the absence of estradiol, injury is extensive in all mice (*a*, *c*, *e*, and *g*). Estradiol reduces the extent of infarct in wild-type mice of both genetic backgrounds (*b* and *f*) and in ER β KO mice (*h*), but not in ER α KO mice (*d*). The volume of infarct includes significant portions of the cerebral cortex, striatum, and hippocampus. Each brain section is a 16- μ m coronal slice obtained from bregma -1.54 mm.

Data Analyses. All data are expressed as mean \pm SEM. Infarct volumes and baseline laser Doppler measurements were analyzed by using a two-way analysis of variance (ANOVA). Post hoc analyses were carried out with Newman–Keuls test. Ischemic laser Doppler measurements were analyzed by using a mixed-factorial ANOVA. All differences were considered significant at P < 0.05.

Results

Effects of Estradiol in Stroke Injury. The extent of brain injury produced by permanent cerebral ischemia, an animal model of stroke, is clearly delineated by staining and is distributed throughout the right hemisphere of ER α KO, ER β KO, and the respective wild-type mice (Fig. 1 *a–h*). These representative brain sections clearly demonstrate that without estradiol, ischemic injury, or infarct, is extensive in all mice (Fig. 1 *a*, *c*, *e*, and *g*). Physiological levels of estradiol greatly reduce the extent of infarct in both wild-type mice (Fig. 1 *b* and *f*) and in ER β KO mice (Fig. 1*h*) but not in ER α KO mice (Fig. 1*d*). The volume of infarct, which includes significant portions of the cerebral cortex, striatum, and hippocampus, was analyzed by computer-assisted imaging.

Estradiol-Mediated Protection Against Total Brain Injury Is Abolished in the Absence of ER α . We analyzed total ischemic injury and found that the ability of physiological levels of estradiol to exert neuroprotection is completely different in wild-type and ER β KO mice than in ER α KO mice. In both wild-type backgrounds, estradiol decreases total ischemic injury by more than 50% compared with respective oil-treated controls (Fig. 2). In ER α KO mice, estradiol fails to exert any protective effect, and infarct volume is equally extensive in ovariectomized mice with oil- or estradiol-replacement (Fig. 2*a*). In marked contrast, in ER β KO mice, estradiol continues to exert profound protective effects against brain injury. Estradiol-induced protection in ER β KO mice is identical to its effects in wild-type mice: total ischemic injury in estradiol-treated ER β KO mice is decreased by more than 50% compared with oil-treated controls (Fig. 2*b*). Thus, the deletion of ER α totally abolishes the profound protection afforded by estradiol in the brain, whereas

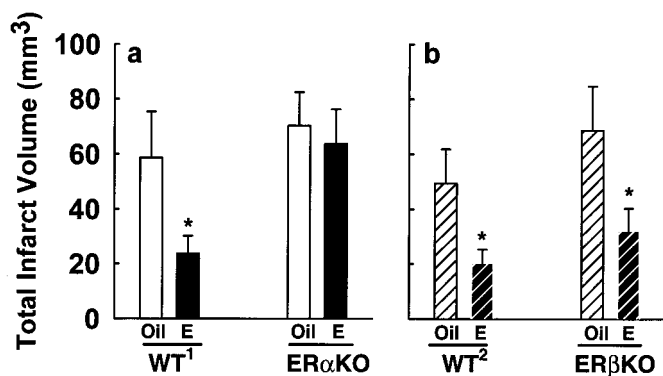


Fig. 2. Estradiol protects against total ischemic brain injury in wild-type mice of both genetic backgrounds and ER β KO mice, but not in ER α KO mice. (a) Estradiol ($n = 9$) significantly decreases total infarct volume in C57BL/6J \times 129 wild-type mice (WT¹), compared with oil-treated controls ($n = 7$) (*, $P < 0.04$). In contrast, in ER α KO mice, estradiol ($n = 13$) does not exert any protective effect on total infarct volume, compared with oil-treated controls ($n = 13$) ($P = 0.48$). (b) Estradiol ($n = 6$) significantly decreases total infarct volume in 129Sv wild-type mice (WT²), compared with oil-treated controls ($n = 10$) (*, $P < 0.02$). In ER β KO mice, estradiol ($n = 8$) significantly decreases total infarct, compared with oil-treated controls ($n = 9$) (*, $P < 0.02$). Values represent the mean \pm SEM.

deletion of ER β does not diminish the ability of estradiol to protect the brain against injury.

ER α Is Critical in All Brain Regions Protected by Estradiol. We further analyzed total infarct volume and compared treatment effects in cortex, striatum, and hippocampus to determine whether the biological role of ER α in estradiol-mediated neuroprotection is region-specific. Estradiol treatment significantly reduces cortical and striatal infarct volumes in both wild-type backgrounds and in ER β KO mice (Fig. 3*a–d*). Protection in these regions is truly profound because estradiol induces decreases of 59%–95% in wild-type mice and in ER β KO mice. In contrast, estradiol fails to protect cortex or striatum in ER α KO mice (Fig. 3*a–d*). Hippocampal injury is equivalent in all ER α KO, ER β KO, and wild-type brains, because we did not detect any protective effect of estradiol in this region (Fig. 3*e* and *f*). Thus, in this model of cerebral ischemia, estradiol exerts profound neuroprotective effects in the cerebral cortex and striatum of wild-type and ER β KO mice, but not in ER α KO mice. ER α plays a critical role throughout the brain in all regions where we observe estradiol-mediated neuroprotection.

Estradiol-Mediated Protection via ER α Is Independent of Blood Flow. We monitored baseline and ischemic regional cerebral blood flow (rCBF) in ER α KO, ER β KO, and the respective wild-type mice by laser Doppler flowmetry over the right parietal cortex. Estradiol does not alter the baseline, or preischemic, levels of cortical perfusion in ER α KO, ER β KO, or wild-type mice, compared with the respective oil-treated controls (Table 1). Furthermore, rCBF after the onset of ischemia does not differ between oil- and estradiol-treated mice (Fig. 4) in any of the experimental groups. Thus, estradiol's protective effects in wild-type and ER β KO mice cannot be explained by differences in basal regional cerebral blood flow or by differences in the extent of circulatory compromise after permanent cerebral ischemia. ER α plays a crucial role in neuroprotection via mechanisms that are likely independent of blood flow.

Estradiol Replacement Therapy Achieves Physiological Levels of Estradiol in Serum. We measured concentrations of 17 β -estradiol in serum to assure that our controlled endocrine paradigm of hormone replacement achieves equivalent and physiological

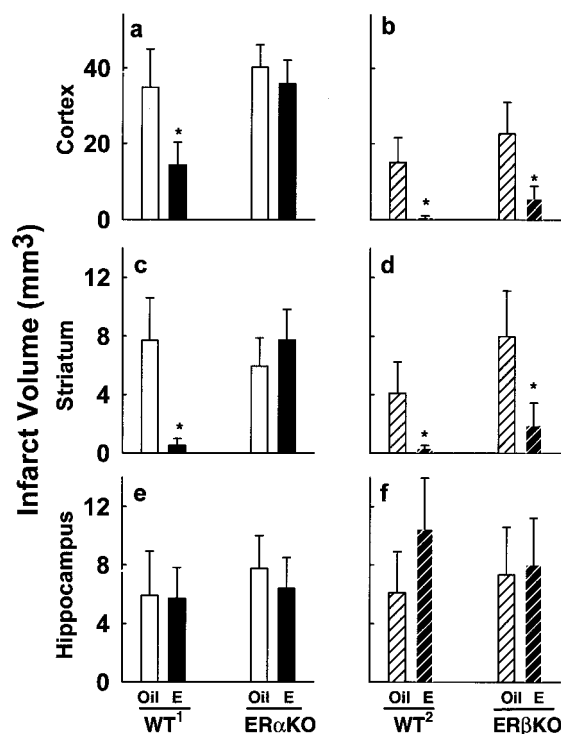


Fig. 3. ER α is critical in brain regions protected by estradiol. Effects of estradiol in cerebral ischemia are region-specific in wild-type mice of both genetic backgrounds, ER α KO, and ER β KO mice. (a) Estradiol ($n = 9$) significantly reduces cortical infarct volume in wild-type mice (WT¹), compared with oil-treated controls ($n = 7$) (*, $P < 0.02$). In contrast, estradiol ($n = 13$) fails to protect the cerebral cortex in ER α KO mice, compared with oil-treated controls ($n = 13$) ($P = 0.56$). (b) Estradiol ($n = 6$) significantly reduces cortical infarct volume in wild-type mice (WT²), compared with oil-treated controls ($n = 10$) (*, $P < 0.02$). In parallel to its effects in wild-type mice, estradiol ($n = 8$) significantly reduces cortical infarct volume in ER β KO mice, compared with oil-treated controls ($n = 9$) (*, $P < 0.02$). (c) Estradiol significantly reduces striatal infarct volume in WT¹, compared with oil-treated controls (*, $P < 0.05$), but fails to protect the striatum in ER α KO mice ($P = 0.81$). (d) Estradiol significantly reduces striatal infarct volume in both WT² mice and in ER β KO mice, compared with respective oil-treated controls (*, $P < 0.04$). (e) Estradiol does not protect against hippocampal injury in WT¹ or in ER α KO mice, compared with respective oil-treated controls ($P = 0.68$). (f) Likewise, estradiol does not protect against hippocampal injury in WT² or in ER β KO mice, compared with respective oil-treated controls ($P = 0.441$). Values represent mean \pm SEM.

levels of 17 β -estradiol in mice. This is critical because endogenous levels of this hormone are dramatically different in ER α KO (18) versus wild-type (18, 22) and ER β -knockout (23) mice. Our paradigm of physiological estradiol replacement in ovariectomized mice produces serum levels of approximately 25 pg/ml

Table 1. Baseline rCBF does not differ between oil- and estradiol-treated mice

Mice	Oil	E
WT ¹	12.03 \pm 0.47	12.08 \pm 0.55
ER α KO	13.18 \pm 0.41*	13.96 \pm 0.62*
WT ²	12.52 \pm 1.03	12.34 \pm 1.24
ER β KO	11.91 \pm 0.62	11.89 \pm 0.16

Baseline rCBF is slightly elevated in ER α KO compared with wild-type¹ (WT¹) mice (*, $P < 0.02$). However, estradiol (E) does not alter baseline rCBF compared with oil-treated mice in any of the experimental groups ($n = 3$ –4 per group). Values represent the mean \pm SEM.

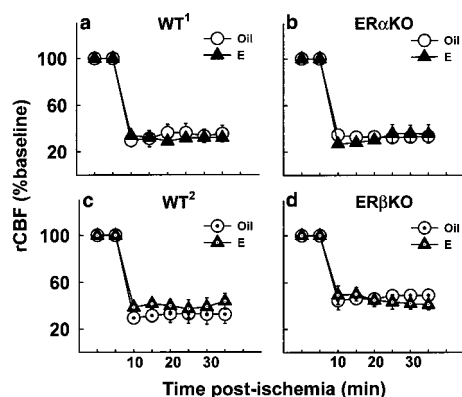


Fig. 4. rCBF during permanent cerebral ischemia does not differ between oil- and estradiol-treated mice. Ischemia significantly reduces cortical perfusion in oil- and estradiol-treated, WT¹ (a) and ER α KO mice (b) ($n = 3$ per group) ($P < 0.001$). Likewise, ischemia significantly reduces cortical perfusion in oil- and estradiol-treated, WT² (c) and ER β KO (d) mice ($n = 3$ or 4 per group) ($P < 0.001$). Estradiol does not affect the extent of the decrease in regional cortical flow in any of the mice (a–d). Ischemic laser Doppler measurements began 10 min after the onset of vascular occlusion and were taken every minute. Each data point represents a 5-min average and is represented as a percentage of baseline. Values represent mean \pm SEM.

(Fig. 5), a level that is equivalent to basal circulating levels in the mouse estrous cycle (22).

Discussion

The results of this study clearly establish the pivotal mechanistic role of ERs in estradiol-mediated protection against brain injury. We demonstrate that ER α , and not ER β , is a critical link that determines the ability of physiological levels of estradiol to exert neuroprotection. These findings uncover fundamental molecular mechanisms by which low levels of estradiol protect the brain against injury, neurodegeneration, and possibly cognitive decline. Therefore, these results carry far-reaching implications for the selective targeting of ERs in the treatment and prevention of neural dysfunction associated with normal aging or brain injury.

We have uncovered a critical role for ER α in estradiol-mediated protection of the brain. Our data demonstrate that estradiol exerts profound protective actions in the brains of

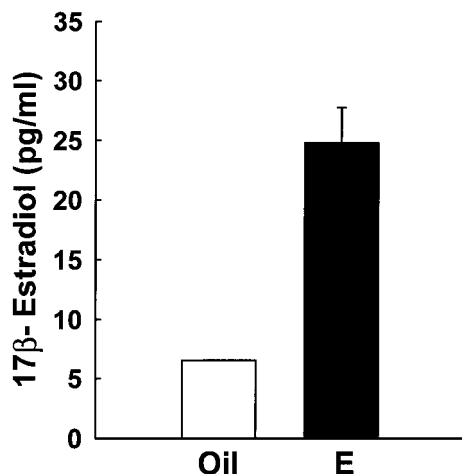


Fig. 5. Estradiol replacement in ovariectomized mice achieves physiological levels of 17 β -estradiol in serum. Physiological estradiol replacement in mice ($n = 4$ per group) produces serum levels that are equivalent to basal circulating levels in the mouse estrous cycle. The low range of sensitivity of the radioimmunoassay was determined to be approximately 5.0 pg/ml. Values represent mean \pm SEM.

wild-type and ER β KO mice but fails to protect in ER α KO mice. Physiological levels of estradiol dramatically decrease stroke infarct volumes in the cerebral cortex and striatum in wild-type mice of two genetic backgrounds and in ER β KO mice. In marked contrast, the deletion of ER α totally abolishes the ability of estradiol to protect in any region of the brain. Interestingly, no hippocampal protection by estradiol was observed. We speculate that the lack of estradiol-mediated neuroprotection in this brain region may be due to severely compromised and irreversibly injured tissue in this area.

Our findings elucidate a new functional role for ER α in brain regions of the adult that were not previously thought to express the receptor (24). Importantly, this biological role for ER α extends and complements our recent finding that ER α expression is dramatically up-regulated in regions of the adult brain that are protected by estradiol (15). The induction of ERs after brain injury is specific to only the ER α subtype and not the ER β subtype (15). Interestingly, the dramatic increase in ER α expression during injury is reminiscent of its expression during early postnatal development of the brain (25, 26), a time of extensive neurogenesis and differentiation. Therefore, perhaps, the ischemia-induced reexpression of ER α may allow recapitulation of the developmental actions of estradiol.

We performed these studies with a controlled endocrine paradigm of hormone replacement because endogenous levels of estradiol are dramatically different in ER α KO versus wild-type or ER β -KO mice. Because of the lack of negative feedback at the level of the hypothalamus and pituitary in ER α KO mice, estradiol synthesis and secretion increases exponentially. This increase leads to serum levels that are 10- to 15-fold higher in ER α KO mice (18) than normally circulate in wild-type (18, 22) or ER β -knockout mice (23). These chronically elevated concentrations of estradiol are considered pharmacological. Because physiological and pharmacological levels of estradiol may act through different mechanisms (1), it was critical that animals were exposed to equivalent concentrations of estradiol. To achieve equivalent levels of estradiol replacement, ER α KO, ER β KO, and their respective wild-type littermates were ovariectomized and implanted with Silastic capsules filled with oil (vehicle) or a low dose of 17 β -estradiol. This dose in capsules produces serum estradiol concentrations of 25 pg/ml in mice, a level of hormone that is equivalent to basal circulating levels in the mouse estrous cycle (18, 22). We used two groups of wild-type mice because the genetic backgrounds of the ER α KO and ER β KO mice were different, and could influence the extent of injury and the effects of steroids.

Our results differ from a previous study that compared stroke injury in gonadally intact wild-type and ER α -knockout mice and concluded that protection does not depend on ER α (27). The comparison between wild-type and ER α KO mice that are gonadally intact is difficult to interpret because the estradiol concentrations in ER α KO mice are dramatically higher than in wild-type controls. This is important to consider because the mechanisms by which estradiol exerts protective actions are diverse and depend, in part, on the dose of the steroid. Thus, previous studies suggest that physiological levels of estradiol may protect through receptor-dependent mechanisms (8, 12, 13, 28), whereas higher concentrations of estradiol may act through mechanisms that do not require the presence of ERs (29–36). Hence, the former study further confirms that pharmacological levels of estradiol protect through ER-independent mechanisms, but it does not address the potential role of ERs in mediating the physiological levels of estradiol to protect. In our current study, use of a controlled endocrine paradigm of physiological hormone replacement clearly establishes that estradiol acts through ER α to exert neuroprotection.

Our results demonstrate that estradiol protects against brain injury through blood flow-independent mechanisms. Estradiol

does not alter baseline, or preischemic, cortical perfusion in wild-type, ER α KO, or ER β KO mice. Likewise, the extent of decrease in regional ischemic flow is equivalent between oil- and estradiol-treated mice in all experimental groups, regardless of genetic background. It is interesting to note that preischemic perfusion is slightly increased in ER α KO mice, in both the presence and the absence of estradiol treatment. These data corroborate previous reports that show that low levels of estradiol do not modulate cerebral blood flow (8, 9, 16, 37). Thus, our current findings indicate that physiological levels of estradiol act via ER α to protect the brain through mechanisms that cannot be explained by differential levels of circulatory compromise.

It is interesting to speculate that the ER knockout mice may differ in target tissue sensitivities to estradiol; however, our data do not hint that this might be the case in our paradigm. We examined our data to determine whether the absence of ER α or ER β might alter sensitivity to the neuroprotective effects of estradiol. Our data do not reveal an exaggerated protection in brains of ER β KO mice compared with the respective wild-type mice. This result indicates that, in the context of cerebral ischemia, the absence of ER β does not induce hypersensitivity to estradiol's neuroprotective effects through ER α . Furthermore, in the absence of ER α , no hormone-mediated protection is afforded, indicating that estradiol requires ER α , not ER β , to protect. Whether ER β contributes to injury in ER α KO mice is unknown and our data do not allow us to ask this question. Whether altered sensitivity to estradiol exists in other paradigms or brain regions of ER knockout mice remains to be determined.

Our discovery that ER α is a crucial mechanistic component of estradiol-mediated protection highlights emerging roles for this receptor in traditional and novel actions that reach far beyond the reproductive axis and its classical targets. The long-held view that estradiol simply binds to its receptor, causing receptor transactivation, dimerization, DNA binding, and transcription of genes (38) may be too simple. Accumulating evidence shows that ERs may also activate second messenger signaling pathways, such as adenyl cyclase, phosphoinositol 3-kinase, and/or mitogen-activated protein kinase, or involve cross-talk with growth factor receptors, such as trkA and the IGF-I receptor (13, 39–42). These novel ER-mediated mechanisms may lead to altered gene expression downstream and/or increased phosphorylation of proteins that promote estradiol's actions.

The predominant mechanism of estradiol action may depend on the brain region, the type of neural injury or stimulus, and/or

the dose of hormone administered. It should be noted that physiological levels of estradiol generally require receptor-mediated genomic or nongenomic function for neuroprotection. Neuroprotection induced by physiological levels of estradiol often, but not always (11), requires pretreatment, is specific to the 17 β -estradiol stereoisomer, and/or can be blocked by ER antagonists (8, 12, 13, 28). In contrast, neuroprotection by pharmacological levels of estradiol appears to protect predominantly through non-ER-mediated antioxidant and/or membrane/channel effects (29–36).

The diverse and multifactorial ER-mediated interactions may induce a variety of cellular responses that promote trophic and protective effects in the brain. Physiological levels of estradiol can enhance the plasticity of synaptic connections (43–47), regulate the expression of neurotrophins and cognate receptors (48–51), and elevate the expression of cell survival factors (15, 52) in the brain. Any or all ER-mediated actions of estradiol that enhance the integrity and plasticity of the brain may ultimately promote neuroprotection through enhanced cellular function, resistance to injury, and/or facilitated regeneration from injury.

In summary, our discovery that the ER subtype ER α is a critical link in the protective actions of physiological concentrations of estradiol in neurodegenerative injury carries far-reaching implications for the selective targeting of ERs in the treatment of disease states, particularly for aging postmenopausal women. Since the recent discovery that two ERs exist in the body (14), biomedical researchers have aggressively investigated the specific biological roles of ER α and ER β in normal and pathological processes throughout the body (23, 53–57). We have now identified a critical biological role for ER α in the brain. As we continue to gain greater insights into the actions of ER subtypes in different organ systems, we will be better able to design estrogens that selectively elicit protective effects. Our findings suggest that estrogens that specifically target ER α in the brain may promote protective actions in neurodegenerative conditions such as Alzheimer's disease and stroke.

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