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Estradiol rescues neurons from global ischemia-induced cell death: Multiple cellular pathways of neuroprotection

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ABSTRACT

The potential neuroprotective role of sex hormones in chronic neurodegenerative disorders and acute brain ischemia following cardiac arrest and stroke is of a great therapeutic interest. Long-term pretreatment with estradiol and other estrogens affords robust neuroprotection in male and female rodents subjected to focal and global ischemia. However, the receptors (e.g., cell surface or nuclear), intracellular signaling pathways and networks of estrogen-regulated genes that intervene in neuronal apoptosis are as yet unclear. We have shown that estradiol administered at physiological levels for two weeks before ischemia rescues neurons destined to die in the hippocampal CA1 and ameliorates ischemia-induced cognitive deficits in ovariectomized female rats. This regimen of estradiol treatment involves classical intracellular estrogen receptors, transactivation of IGF-1 receptors and stimulation of the ERK/MAPK signaling pathway, which in turn maintains CREB activity in the ischemic CA1. We also find that a single, acute injection of estradiol administrated into the brain ventricle immediately after an ischemic event reduces both neuronal death and cognitive deficits. Because these findings suggest that hormones could be used to treat patients when given after brain ischemia, it is critical to determine whether the same or different pathways mediate this form of neuroprotection. We find that an agonist of the membrane estrogen receptor GPR30 mimics short latency estradiol facilitation of synaptic transmission in the hippocampus. Therefore, we are testing the hypothesis that GPR30 may act together with intracellular estrogen receptors to activate cell signaling pathways to promote neuron survival after global ischemia.

1. Introduction

It is now well established that estrogens exert profound protective effects in animal models of focal and global ischemia (for review, see [1–4]). Global ischemia in humans or induced experimentally in animals causes selective and delayed neuronal death in pyramidal neurons of the hippocampal CA1 [5]. Histological evidence of degeneration is not observed until 2–3 days after ischemia in rats or 3–4 days in gerbils [6,7]. Although the mechanisms underlying ischemia-induced death are as yet unclear, the substantial delay between insult and onset of cell death suggests a critical role for transcriptional changes activating specific apoptotic pathways. Thus, classical estrogen receptors (ERs), which are ligand-activated transcription factors, might mediate neuroprotection by directly regulating the transcription of pro- and anti-apoptotic molecules. Genomic screens for estrogen responses element (ERE) motifs reveal genes such as IGF-1 (insulin like growth factor-1), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and anti-apoptotic members of the Bcl-2 family known to be involved in neural survival [8,9]. However, the likely existence of classical ER-mediated mechanisms does not rule out the possibility that cell signaling initiated by estrogen-binding molecules located at the plasma membrane could also block apoptotic cascades via ER-independent regulation of gene transcription.

Indeed, estrogens produce rapid actions in neurons, on the order of seconds to minutes, consistent with a role for membrane receptors [10,11]. Estradiol rapidly activates cellular pathways associated with growth factor signaling, such as the ERK/MAPK and PI3K/Akt cascades, and promotes accumulation of second messengers in a G protein-dependent manner. One scenario is that classical ER-α and ER-β are expressed at the plasma membrane [12]; however, the mechanisms by which they might act to regulate second messenger production is unclear. Moreover, findings that estradiol can elicit rapid actions on neurons even in the presence of ER-α and ER-β blockade or in neurons lacking ER-α and ER-β (for review see [13]) suggest that estradiol signaling depends on receptors in addition to ER-α and ER-β. Whether classical ERs (ER-α and ER-β) located in the nucleus or on the plasma membrane, or other membrane

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estrogen receptors are critical mediators of the neuroprotective actions of estradiol in an animal model of global ischemia is the topic of this review.

2. Estradiol and global ischemia

2.1. Long-term exposure to estradiol: histological and behavioral neuroprotection

We and others have shown that estradiol, chronically administrated at physiological levels, affords robust protection against ischemia-induced cell death in hippocampal CA1 of intact male gerbils [14] and ovariectomized female rats [15,16] and gerbils [16]. The histological outcomes after ischemic insult were quite similar in estradiol-treated animals irrespective of sex or species. Dramatic cell loss (close to 90%) compared to sham-operated animals is observed in the pyramidal layer of the hippocampal CA1 at 7 days after a 5–10 min episode of global ischemia. Cell loss is significantly reduced by about 60% when animals subjected to ischemia are implanted with pellets providing physiological levels of 17β-estradiol (E2) beginning 2 weeks before and continuing for 1 week after ischemic insult. We verified that this chronic treatment with physiological E2 also confers behavioral neuroprotection [17]. Performance on memory tasks that depend on the hippocampus and that show deficits after ischemia was improved by chronic exposure to E2 in ovariectomized rats. Long-term E2 prevented the ischemia-induced deficit in visual working memory, maintaining normal performance in tests with retention intervals of up to 1 h. Long-term E2 treatment also prevented ischemia-induced deficits in spatial memory tests with short (1 and 7 min), but not longer (15 min) retention intervals.

2.2. Long-term exposure to estradiol: cellular pathways of neuroprotection

2.2.1. Both ER-α and ER-β are involved

Determining whether ER-α, ER-β, and/or other membrane estrogen receptors mediate estradiol neuroprotection following global ischemia is of great interest both for the development of therapeutic strategies and for elucidating underlying molecular mechanisms of delayed neuronal death. Our published work using chronic administration of selective agonists for either ER-α or ER-β in female rats indicated that the activation of either receptor was able to rescue hippocampal CA1 pyramidal neurons following transient global ischemia [15]. However, only ERα was upregulated in the CA1 by E2 and ischemia. We also showed that ICI 182,780, a competitive antagonist for both ER-α or ER-β, completely blocks long-term E2 neuroprotection when administered in the early post-ischemic period (Fig. 1). These findings confirm that neuroprotection afforded by chronic treatment with E2 likely involves activation of the classical receptors ER-α and ER-β. However, this does not rule out the possibility that other estrogen receptors, which could also be sensitive to ICI 182,780, may play a role in long-term E2 neuroprotection following ischemic insult.

2.2.2. ERK/MAPK signaling is critical for estradiol neuroprotection

Neuronal apoptosis can proceed via two parallel pathways, the intrinsic pathway (mitochondrial) and the extrinsic pathway (involving death receptors), to activate caspase-3 and lead to DNA fragmentation and other hallmarks of cell suicide [18]. Our early study on E2 neuroprotection in male gerbils suggested that chronic hormone treatment rescued neurons destined to die by interfering with apoptotic death cascades that activate caspase-3 [14]. Under conditions of transient brain ischemia, the mitochondrial pathway predominates, with activation of pro-apoptotic members of the Bcl-2 family triggering the release of killer proteins (smac/DIABLO,

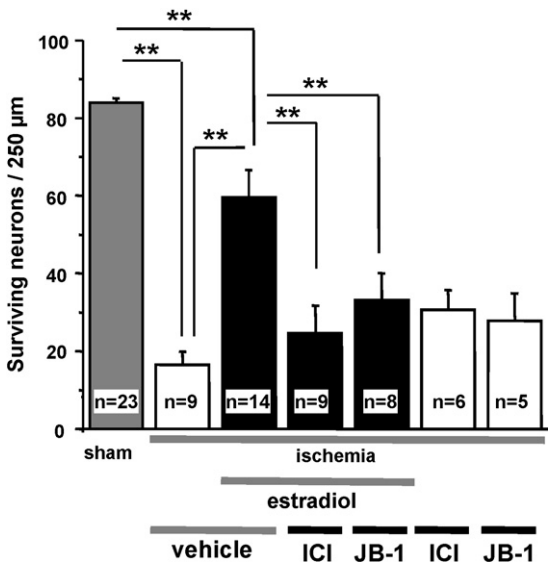

Fig. 1. Long-term estradiol protection requires both estrogen receptors and IGF-1 receptors.
Ovariectomized female gerbils were implanted with placebo or estradiol pellets 2 weeks before global ischemia or sham operation. They received icv infusions of the broad spectrum estrogen receptor antagonist ICI 182,780 (100 μg), the IGF-1 receptor antagonist JB-1 (10 μg), or vehicle at 0 and 12 h after ischemia. Plasma estradiol levels at the time of death were 15.4 ± 1.4 pg/ml in the placebo group and 136.35 ± 12.9 pg/ml in the estradiol group. Neither ICI 182,780 nor JB-1 affected neuronal survival in ischemic or sham animals implanted with placebo pellets, but they abolished the neuroprotective actions of estradiol. Data are from Jover-Mengual et al. (2007).

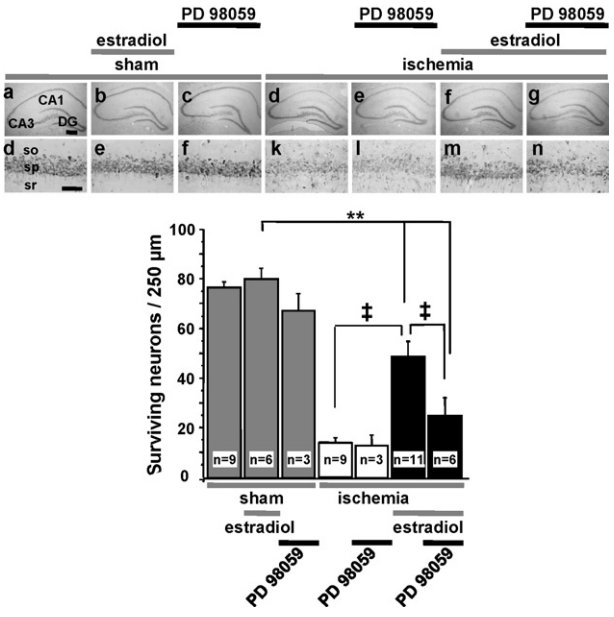

Fig. 2. Neuroprotection conferred by long-term estradiol pretreatment is blocked by the MEK inhibitor PD98059.
Ovariectomized female rats were treated as described in Fig. 1, except that the duration of global ischemia was 10 min. When animals were killed for histological evaluation, they exhibited physiological serum estradiol levels (33 ± 2.9 pg/ml). The *upper panel* shows representative low (4×) and high (20×) photomigrographs of CA1 pyramidal cells 7 days after ischemia or sham operation. The *lower panel* illustrates quantitation of surviving pyramidal neurons in CA1 at 7 days after surgery. Estradiol significantly increased the number of surviving neurons. Icv administration of the MEK inhibitor PD98059 (3 μg) at 0 and 12 h after reperfusion prevented the ability of estradiol to increase neuronal survival. Data are from Jover-Mengual et al. (2007). so, Stratum oriens; sp, stratum pyramidale; sr, stratum radiatum.
Scale bars, lower magnification, 400 μm; higher magnification, 60 μm.
$^{**}P < 0.01$ *vs.* all sham groups; $^{‡}P < 0.01$ ischemia + estradiol *vs.* ischemia or ischemia + estradiol + PD98059.