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Review

Mechanisms of Estrogens' Dose-Dependent Neuroprotective and Neurodamaging Effects in Experimental Models of Cerebral Ischemia

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Abstract: Ever since the hypothesis was put forward that estrogens could protect against cerebral ischemia, numerous studies have investigated the mechanisms of their effects. Despite initial studies showing ameliorating effects, later trials in both humans and animals have yielded contrasting results regarding the fundamental issue of whether estrogens are neuroprotective or neurodamaging. Therefore, investigations of the possible mechanisms of estrogen actions in brain ischemia have been difficult to assess. A recently published systematic review from our laboratory indicates that the dichotomy in experimental rat studies may be caused by the use of insufficiently validated estrogen administration methods resulting in serum hormone concentrations far from those intended, and that physiological estrogen concentrations are neuroprotective while supraphysiological concentrations augment the damage from cerebral ischemia. This evidence offers a new perspective on the mechanisms of estrogens' actions in cerebral ischemia, and also has a direct bearing on the hormone replacement therapy debate. Estrogens affect their target organs by several different pathways and receptors, and the mechanisms proposed for their effects on stroke probably prevail in different concentration ranges. In the current article, previously suggested neuroprotective and neurodamaging mechanisms are reviewed in a hormone concentration perspective in an effort to provide a mechanistic framework for the dose-dependent paradoxical effects of estrogens in stroke. It is concluded that five

protective mechanisms, namely decreased apoptosis, growth factor regulation, vascular modulation, indirect antioxidant properties and decreased inflammation, and the proposed damaging mechanism of increased inflammation, are currently supported by experiments performed in optimal biological settings.

Keywords: estrogen; cerebral ischemia; stroke; animal experiments; administration methods

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List of Abbreviations

ACh	Acetylcholine
AMPA	α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
Apaf-1	Apoptotic Protein-Activating Factor-1
BDNF	Brain-Derived Neurotrophic Factor
BH3	Bcl Homology domain-3
CA1	Cornu Ammonis area-1
cAMP	Cyclic Adenosine Monophosphate
cGMP	Cyclic Guanosine Monophosphate
COX	Cyclooxygenase
CREB	cAMP Response Element Binding protein
eNOS	Extracellular Nitric Oxide Synthase
ER	Estrogen Receptor
ERE	Estrogen Response Elements
ERK	Extracellular signal-Regulated Kinases
GABA	Gamma-Aminobutyric Acid
GAP-43	Growth-Associated Protein-43
GPR-30	G-Protein coupled Receptor-30
GSK-3β	Glycogen synthase kinase 3β
IGF-I	Insulin-like Growth Factor-I
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthase
IRA	Innovative Research of America
LPS	Lipopolysaccharide
LTP	Long Term Potentiation
MAPK	Mitogen-Activated Protein Kinase
MCAo	Middle Cerebral Artery Occlusion
MPO	Myeloperoxidase
NADPH	Reduced form of Nicotinamide Adenine Dinucleotide Phosphate
NFKB	Nuclear Factor Kappa-light-chain-enhancer of activated B cells
NGF	Nerve Growth Factor
NMDA	*N*-Methyl-D-Aspartate
nNOS	Neuronal Nitric Oxide Synthase

NO	Nitric Oxide
NT-4	Neurotrophin-4
PGE2	Prostaglandin E2
PI3	Phosphatidylinositol-3
PUMA	p53-Upregulated Modulator of Apoptosis
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
Sp1	Specificity Protein-1
STAT3	Signal Transducer and Activator of Transcription 3
SVZ	Subventricular Zone
TGF-α	Transforming Growth Factor-α
TLR	Toll-Like Receptor
TNF-α	Tumor Necrosis Factor-α
VEGF	Vascular Endothelial Growth Factor

1. Introduction

In the 1990s, several studies demonstrated neuroprotective effects of estrogens in animal models of cerebral ischemia [1–3]. This supported a hypothesis of estrogen neuroprotection that earlier had been postulated from the clinical observation that women are less likely to suffer from stroke compared to men, and that this protection diminishes by the advent of menopause [4]. Several previous epidemiological studies had also corroborated this hypothesis by indicating decreased stroke incidence in women on hormone replacement therapy [4]. Encouraged by the potential of estrogens as a mean of preventing illnesses including stroke and other cardiovascular diseases, substantial research efforts have been invested in further studies of the matter. However, later studies have been contradictory regarding estrogens' effects on stroke, exemplified by the large randomized controlled trial Women's Health Initiative, which was interrupted prematurely because of increased incidences of breast cancer, stroke and cardiovascular disease, thus apparently antagonizing the hypothesis that estrogens are neuroprotective (it requires mention that in the case of this study conjugated equine estrogens were used, and not 17β-estradiol that has been used in most animal trials) [5].

A few animal studies also reported increased ischemic damage from estrogens [6–11], in contrast to a large number of experiments in which neuroprotection was found [12–14]. This dichotomy concerning estrogens' effects in animal models of cerebral ischemia was analyzed in a recent systematic review of rat studies, designating the dose and mode of estrogen administration as the culprits [15], which was later experimentally confirmed [16]. Slow-release pellets for subcutaneous implantation produced by the company IRA (all abbreviations are listed above) was identified as the only mode of estrogen administration which has led to increased ischemic lesions, plausibly due to the prolonged, supra-physiological plasma concentration peak (300–600 pg/mL, in comparison to the physiological 5–65 pg/mL) which characterizes these implants [17]. This is well in line with the concept of hormesis, stating that steroid hormones can have diametrically different actions in different concentration ranges [18]. The fact that estrogens seem to exert their effects via several different pathways, such as the classical nuclear receptors ER-α and ER-β, membrane-linked receptors and

through direct molecular mechanisms further adds to the complexity, and could account for the hormesis phenomenon. The highest dosed pellets were in the abovementioned systematic review found to be most neurodamaging, while pellets containing lower doses of estrogens were more likely to be protective. The two other main methods of administering estrogens to rats; subcutaneous silastic capsules (generated serum concentrations of 40 pg/mL diminishing to 5 pg/mL in 42 days [17]) and subcutaneous injections (generated baseline 17β-estradiol concentrations of 10–110 pg/mL [17]), showed consistent neuroprotection in the doses tested (Table 1) [15].

Table 1. Administration method dose ranges in relation to neuroprotection and neurodamage. 17β-estradiol doses and capsule concentration ranges reported to induce neuroprotection and neurodamage, respectively, in rat models of cerebral ischemia. For injection regimens, daily doses are presented. Silastic capsules containing crystallized estrogen are omitted from the table, but were consistently neuroprotective [15].

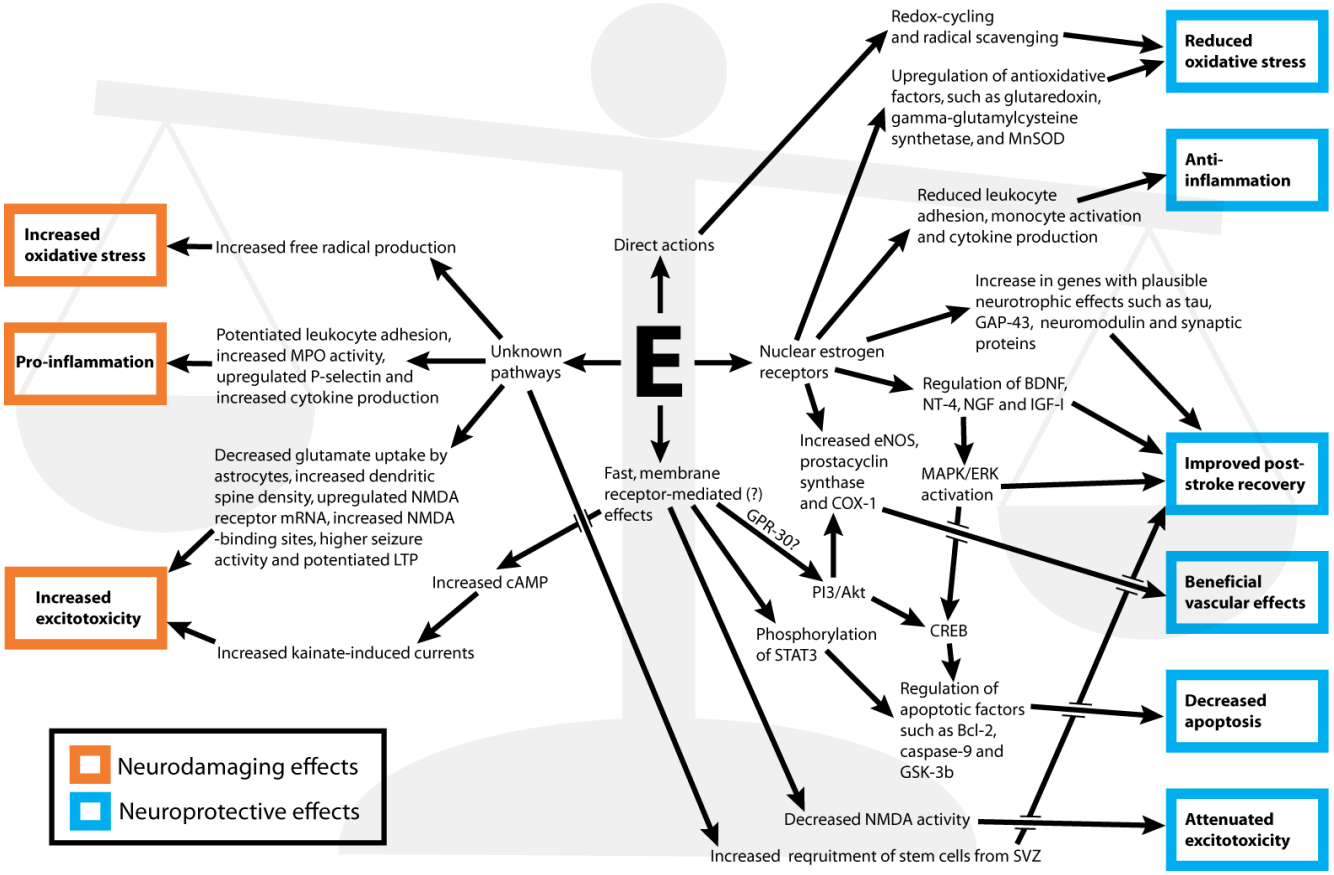
Administration method	**Pellet dose/silastic capsule concentration/injection dose ranges**	
	Neuroprotection	Neurodamage
Slow-release pellets, subcutaneous	0.025–0.25 mg [19,20]	0.025–1.5 mg [6,8]
Silastic capsules filled with 17β-estradiol dissolved in oil, subcutaneous	180–4000 μg/mL [21,22]	Not reported
Injections, subcutaneous	10–5000 μg/kg BW [23,24]	Not reported
Injection, intravenous	10–1000 μg/kg BW [2,25]	Not reported
Injection, intraperitoneal	100–20,000 μg/kg BW [26,27]	Not reported
Injection, intramuscular	100 μg/kg BW [28]	Not reported
Infusion, intraventricular	50–150 μg [29,30]	Not reported
Oral administration	10 μg/kg BW [31]	Not reported

Concerning mechanisms of estrogens' effects in cerebral ischemia, there have been numerous explanations, mainly focusing on estrogens' protective properties, but also with some suggestions of detrimental pathways. In the following sections, we will review the five most extensively investigated potential neuroprotective mechanisms, namely decreased oxidative stress (Section 2.1) decreased inflammation (2.2), decreased apoptosis (2.4), growth factor regulation (2.5) and vascular modulation (2.6), and the three suggested neurodamaging mechanisms increased oxidative stress (2.1), increased inflammation (2.2) and increased excitotoxicity (2.3). Each section consists of a brief summary of data supporting the mechanism hypothesis, when required also with a complementary description of pathways. Subsequently the estrogen administration methods used and in the cited studies are described, and it is discussed how well this matches with the notion that estrogens are protective in physiological doses and damaging in very high doses. Concerning the actual serum estrogen concentrations in the cited studies, it is unfortunately very uncommon that researchers do measurements of 17β-estradiol on more than one occasion during an experiment. Concentrations measured in a single blood sample provide very little information about the serum concentrations at the other time-points of the study. Therefore, presenting the (in the *vast* majority of cases) single measurements from the studies cited run the risk of misleading the reader and is therefore not done here. Further, analysis of minute amounts of 17β-estradiol, most often performed with

radioimmunoassay, should—because of the difficulties in calibrating the methods and the large inter-assay variations—always be performed including serum from native, cycling female rats to obtain reference intervals, which sadly is even rarer [32]. However, it is important to bear in mind that even if blood levels are monitored, these only represent a crude estimate of the concentrations in the brain, where the actual effects take place.

In vitro experiments are cited throughout the review, although it should be noted that the concentrations of estrogens used are generally several orders of magnitude higher than in whole-animal experiments and therefore hard to interpret to *in vivo* conditions. Interestingly, the dose-dependent dichotomy of studies reporting protective *versus* damaging results found in whole-animal experiments is not found in cell culture experiments.

Figure 1. A simplified map of suggested pathways and mechanisms for estrogens' effects in stroke. Orange and blue rectangles mark plausibly detrimental and protective effects, respectively. The balance in the background symbolizes that depending on the circumstances, such as the dose of estrogen, either the protective or detrimental mechanisms may dominate. The "E" in the middle of the figure is short for "Estrogens" (other abbreviations are detailed above the Introduction). Depicted pathways and mechanisms have previously been reviewed in numerous publications [12,13,34,37–43]. Each part of the figure is matched with exact citations in respective sections throughout the article.



Although not reviewed below, a number of additional suggested protective mechanisms also deserve mention, even though research efforts into their pathways are still in early stages. These include increased recruitment of stem cells from the subventricular zone [33], avoidance of apoptosis by balancing phosphatase activity [34] and decrease of excitotoxicity by reducing NMDA-signaling (please note that the opposite; that estrogens may increase excitotoxicity and thereby increase ischemic damage, is reviewed under 2.3) [35,36]. A simplified map of pathways and actions of estrogens that have been postulated to influence cerebral ischemia in a protective or detrimental direction is presented in Figure 1.

2. Mechanisms for Estrogens' Neuroprotective and Neurodamaging Effects

2.1. Decreased and Increased Oxidative Stress as Mechanisms of Estrogen Neuroprotection and Neurodamage

Oxidative stress is an important mechanism in cellular damage in general and cerebral ischemia in particular. Ischemia prompts mitochondria to produce ROS, which causes direct damaging oxidative reactions such as lipid peroxidations, as well as triggering apoptotic cascades. The cell has intricate defense systems against oxidative damage, including scavenging activity by SOD, glutathione peroxidase, and catalase, and further detoxification by small molecules such as glutathione, ascorbic acid, and α-tocopherol. However, during cerebral ischemia, especially reperfusion, these systems are generally overrun by the massive oxidative stress [44]. Estrogens have been stipulated to exert their neuroprotective effects both through direct chemical effects and indirectly via upregulation of the cell's anti-oxidative defense mechanisms (Figure 1) [34].

2.1.1. Direct Anti-Oxidative Effects

Direct anti-oxidative effects have been found in several studies. More specifically, estrogens have been reported to prevent intracellular peroxide accumulation in an ER-independent manner [45], decrease ROS production [46], limit lipid peroxidation [47–50], protect against oxidative stress $FeSO_4$ [51], and to decrease hydrogen peroxide concentrations [30]. In one of these studies, no extra protection was afforded by adding known potent free radical scavengers, indicating that estrogens exert all the protective effects available through anti-oxidative mechanisms [48]. Further, 17α-estradiol, a less feminizing enantiomer of 17β-estradiol, has been shown to protect against glutamate and hydrogen peroxide stress to a similar extent as 17β-estradiol, indicating the importance of receptor-independent pathways [52]. Anti-oxidative mechanisms have also been suggested merely on the basis that estrogens can protect against oxidative stress, although it should be emphasized that protection against an oxidative assault is not necessarily dependent on a primary anti-oxidative mechanism [53,54]. A further mechanism for estrogens' direct anti-oxidative effect was proposed by Prokai *et al.*, providing evidence that estrogens can engage in a redox cycle in which estrogens turn into a quinol when eliminating a radical, to subsequently be converted back to the parent estrogen using NADPH as a reducing agent [55,56]. Interestingly, it was demonstrated that the quinol-cycling actually made the compound function as a prodrug that was selectively activated in the brain, importantly *without* uterotropic actions [55]. 17β-estradiol's anti-oxidative effect has also been

attributed to the hydroxyl group in the C3 position of the A ring, even though one study found no protection by neither of 2-hydroxyestradiol nor 2-methoxyestradiol, despite the fact that these estrogen metabolites have intact hydroxyl groups [57]. However, the last-mentioned study is difficult to assess because of possible differences in brain-uptake by the three compared compounds, and further, in another article it is on the contrary reported that 2-methoxyestradiol protects against ischemia [58].

Taken together, the current evidence indicates that estrogens exert direct anti-oxidative effects under certain circumstances. However, it requires emphasis that the genomic and non-genomic actions of estrogens are impossible to firmly separate, especially *in vivo*. Also, an important concern is that the abovementioned studies mainly have been performed *in vitro* and using hormone concentrations that are extremely high (in the magnitude of 0.1–100 μM) compared to what is normally achieved in animal models of cerebral ischemia [15,45,46,49,50]. An exception, which seems to support the anti-oxidative evidence, is a study by Kii *et al.*, in which estrogens were shown to decrease levels of hydrogen peroxide measured by microdialysis, which were not decreased by tamoxifen in a rat model of cerebral ischemia [30]. However, the results of this study remain to be confirmed in studies less fraught with assessment difficulties. Since estrogens are protective in cerebral ischemia only within a relatively narrow and low range of concentrations, studies showing the part played by estrogens anti-oxidative effects in physiological concentrations *in vivo* remain to be done. Thus, although estrogens are likely neurodamaging in high concentrations, even much higher levels, only relevant in cell cultures, seem to be required to produce the direct anti-oxidative effects of estrogens.

2.1.2. Indirect Anti-Oxidative Effects

Indirect anti-oxidative effects of estrogens have been reported including attenuation of microglial superoxide release [59], increase of glutathione reductase, gamma-glutamylcystein synthetase, glutaredoxin and glutathione [60–64], increased MnSOD activity [65,66] and expression [67,68], upregulation of Cu/Zn SOD expression [67], reduction of free radical production via an increase mitochondrial efficiency [69,70], attenuation of NADPH oxidase activation [71,72] and decrease of the oxidative stress marker nitrotyrosine [67]. These effects have been found to at least in part result from nuclear ER-mediated upregulation of anti-oxidative proteins [34].

In contrast to the direct anti-oxidative mechanisms presented in the above section, these examples of upregulation of the oxidative defense system has been demonstrated in many studies in relevant biological contexts, such as mice and rats receiving subcutaneous and intraperitoneal injections [64,67,69–71]. Thus, at present the proposed indirect anti-oxidative mechanisms seem more likely to be relevant in actual whole-animal cerebral ischemia models than the direct anti-oxidative mechanisms do.

2.1.3. Pro-Oxidative Effects

As aforementioned, estrogens have also been shown to *increase* oxidative stress, and thereby possibly augment ischemic damage (Figure 1) [9,73]. The reported pro-oxidative effects include increased mitochondrial ROS production [74,75], oxidative DNA-damage in sperm and ovarian surface epithelium [76,77], reduced levels of anti-oxidant proteins in rat brain [78], promotion of oxidative damage in rat liver cells [79] and increased ROS-production from the estrogen metabolites

2-methoxyestradiol and 4-hydroxyestradiol [80–82]. However, these pro-oxidative effects of estrogens have mainly been reported from *in vitro* experiments and in other tissues than the brain, while studies on the nervous system almost uniformly have found estrogens to exert anti-oxidant properties [73]. This could possibly reflect tissue-specific estrogen response patterns, which has been proposed to result from differences in cellular balance of ER-β *versus* ER-α [73].

Apart from a study by Pajovic *et al.* [78], studies demonstrating pro-oxidative properties of estrogens in a biological context relevant to cerebral ischemia are lacking. In this study, levels of glutathione peroxidase, glutathione-S-transferase and glutathione reductase in male rat brains were decreased in response to moderately dosed exogenous estrogen. Theoretically, this decrease is likely to hamper the cells' anti-oxidative defense, increasing the risk of ischemia-induced cellular damage [78]. Even taking this study into account, the evidence for estrogenic pro-oxidative actions as a mechanism for increased damage in cerebral ischemia appears scarce, and it cannot be included as a plausible pathway for estrogens' damaging effects.

2.2. Anti- and Pro-Inflammatory Actions as Mechanisms of Estrogen Neuroprotection and Neurodamage

Cerebral ischemia triggers an acute and prolonged inflammatory process in the brain, characterized by activation of microglia, production of inflammatory cytokines and infiltration of various inflammatory cells, including neutrophils, T-cells and monocytes/macrophages, into the damaged tissue. The inflammatory process is considered an important component of the pathophysiology of stroke, and especially the early inflammatory cell infiltration and cytokine production seem to be predominantly deleterious [83]. Experiments in rats have shown that intraventricular administration of TNF-α, IL-1 and IL-6 exacerbates stroke damage, suggesting a detrimental role of inflammation in the ischemic process [84–86]. Further support for this hypothesis is found in the observation that blockage of pro-inflammatory cytokines ameliorates ischemic damage [86–91].

2.2.1. Anti-Inflammatory Effects

Anti-inflammatory properties of estrogens have been demonstrated in a large number of studies, and are commonly taken as important mechanisms for estrogens' neuroprotective effects in stroke [86]. Estrogens have been shown to induce a wide range of anti-inflammatory effects via, for example, reducing leukocyte adhesion [92–94], decreasing pro-inflammatory cytokine production [95–102], decreasing monocyte activation [103] and altering the microglial activation pattern [104]. Both leukocytes and microglia express ER, offering a direct pathway for estrogens' actions in inflammatory processes [86], and ER activation is e.g., thought to regulate iNOS transcription [105]. The classical pro-inflammatory cytokines IL-1, IL-6 and TNF-α lack ERE, but are thought to be affected by for example activated ER's down regulation of nuclear c-Jun and JunD, leading to decreased occupation of AP-1 which in turn could increase the expression of TNF-α (Figure 1) [106].

The studies designed to investigate estrogens' actions in inflammation have to a large extent been performed in cell cultures, where hormone concentrations are hard to extrapolate to concentrations in intact organisms. Of the studies performed in animals, most have focused on other organs than the brain, which potentially could lead to misinterpretation if the data are extrapolated to estrogens effects in cerebral ischemia. The effects of estrogens on inflammation are in many aspects organ specific,

vividly exemplified by the estrogen-induced prostatitis in rats [107] in contrast to the amelioration of soft tissue inflammatory conditions [108]. To elucidate at which estrogen concentrations anti-inflammatory effects in the brain occur, we here narrow our focus to studies performed to assess effect on cerebral inflammation in animals. These are comparatively few, but include experiments that have shown that estrogens limit the activity of the pro-inflammatory transcription factor NFKB in a rat MCAo model [109], decrease leukocyte adhesion both before and after transient forebrain ischemia in rats [92], reduce number of microglia and astrocytes in mice [110], decrease cytokine production in animal models of MCAo [96] and NMDA-induced toxicity [97], block COX-2 activity and PGE2 production after IL-1β administration in rats [102], reduce iNOS activity [105], and decrease monocyte activation and recruitment in response to LPS [103]. In two studies, the importance of anti-inflammation for estrogens' actions have been demonstrated by the lack of 17β-estradiol neuroprotection in iNOS knockout mice [111] and mice treated with the iNOS inhibitor aminoguanidine [112].

Of these studies, all but two have adopted presumptive low-dose or short-term 17β-estradiol regimes, such as various intraperitoneal or subcutaneous injection schedules and low-dose silastic capsules, which are in the dose range likely to induce protection against ischemic damage [15,92,95,96,102,103,105,109–112]. The remaining two of the abovementioned studies used pellets from IRA, which are high-dose regimes capable of inducing either protection or damage in cerebral ischemia [97,110]. In one of the studies using pellets [110], 17β-estradiol merely decreased the number of astrocytes and microglia without relation to stroke, which could be interpreted as a degenerative as well as an anti-inflammatory effect. Further, in the other high-dose pellet study, older rats given the same treatment showed *increased* cerebral inflammation [97]. Thus, of the studies reporting estrogen-induced decreases in animal brain inflammation, a majority have been performed with short-term or low-dose estrogens similar to regimens that previously have been reported to decrease cerebral ischemic damage, which is as expected if anti-inflammation is one of the actual protective mechanisms [15].

2.2.2. Pro-Inflammatory Effects

Paradoxically, one of the suggested mechanisms for estrogens' ability to *increase* damage in cerebral ischemia is the hormones' *pro*-inflammatory capacity [7,37]. In several rat experiments, estrogens have been reported to potentiate leukocyte adhesion, increase P-selectin and MPO enzyme activity in cerebral ischemia [7,113], increase TNF-α, TLR-2 and IL-12 in response to LPS stress [114,115], increase IL-1β in a NMDA-toxicity model [97] and to worsen functional outcome in a model of chronic cerebral inflammation (Figure 1) [116].

Most interestingly, in sharp contrast to the majority of studies reporting decreased inflammation, all but one [113] of these studies adopted administration regimens that are likely to produce highly supraphysiological 17β-estradiol concentrations in the range that have been shown to exacerbate ischemic damage in rats [15,17]. The high-dose regimens used were slow-release capsules from IRA [7,97,115] and silastic capsules containing dissolved 17β-estradiol in concentrations about 10 [114] to 250 [116] times higher than the highest dissolved silastic capsule 17β-estradiol concentration that, to the best of our knowledge, has been reported to be neuroprotective [117]. The

pro-inflammatory effects of estrogens have generally not been interpreted as resulting from the high hormone dose, but rather as synergistic effects of diabetes [7,113] and old age [97,115,118]. However, a possible contribution of factors such as age and disease do not explain the striking dominance of high-dose regimens in these experiments, thereby suggesting that estrogens in supraphysiological concentrations are likely to have a higher propensity for increasing inflammation, supporting the hypothesis that high-dose estrogens increase damage from cerebral ischemia. It should also be mentioned that estrogens indeed have been reported to protect both diabetic and old animals in several studies, contradicting a clear relation between age, diabetes and neurodamaging effects of estrogens [119–123].

2.3. Increased Excitotoxicity as a Mechanism of Estrogen Neurodamage

Excitotoxicity is a well-established feature in cerebral ischemia, and contributes to the pathophysiology by a series of events characterized by abnormal excitation by neurons due to pathological release of excitatory neurotransmitters from damaged cells. In the process, both NMDA and AMPA glutamate receptors are over stimulated, contributing to uptake of Na^+, Cl^- and Ca^{2+} ions, which depolarizes neurons and leads to subsequent transmitter release, further stimulating receptors in a vicious cycle. The ion uptake leads to cellular edema and to activation of various detrimental Ca^{2+}-dependent enzymes, which in turn damage the cell by degrading cytoskeletal proteins, damaging DNA and by increasing the generation of free radicals [124].

It has been stipulated that estrogens could augment the pathological process in cerebral ischemia by potentiating the excitotoxicity since estrogens have been reported to increase NMDA mRNA in the hippocampal CA1-region [125], increase NMDA-binding sites in CA1 [126,127], increase dendritic spine density or decreased ovariectomy-induced dendritic spine loss in CA1 [126,128–130], increased sensitivity of CA1 pyramidal cells to NMDA receptor-mediated synaptic input [126], facilitate seizure activity [131], augment LTP [132,133], increase the excitability of different neurons [134,135], decrease glutamate-uptake by astrocytes [136] and to facilitate kainite induced currents via cAMP-dependant phosphorylation (Figure 1) [137]. It is likely that a substance that facilitates NMDA activity and increases excitability could potentiate excitotoxicity and augment ischemic damage. In line with this hypothesis, it has been reported in several articles that decreased excitotoxicity, either by reducing the number of collaterals [138–140] or potentiating GABA-ergic transmission [141,142], is associated with amelioration of ischemic damage.

However, of the many aforementioned studies performed on animals, the vast majority have seen the potentially excitatory effects from low-dose or short-time estrogen administration regimens that are likely to protect from rather than increase ischemic damage [15,17,126,127,129,132,135]. None of the studies adopted the high-dose pellets from IRA that have been shown to be detrimental in cerebral ischemia. Thus it is as yet not established whether estrogens are able to increase excitotoxicity in doses that are relevant to the animal models that have reported *increased* ischemic damage from estrogens. Also, the abovementioned studies have merely presented indirect evidence of increased excitotoxicity by estrogens. In contrast, several studies have shown *decreased* excitotoxicity from estrogens in the same dose range (Figure 1) [36,143–149]. The studies indicating estrogen-induced increased

excitotoxicity have notably largely been restricted to hippocampus, while the ischemic damage in the most common animal stroke model (MCAo) primarily involve the striatum and cerebral cortex.

In conclusion, the hypothesis that estrogens exacerbate ischemic damage by potentiating excitotoxicity has limited support since (1) the potentially excitotoxicity-increasing effects have mainly been demonstrated in experimental paradigms involving presumably neuroprotective hormone regimens; (2) no direct evidence of increased excitotoxicity is as yet available; (3) several studies have reported direct signs of *decreased* excitotoxicity from estrogen treatment; and (4) the studies reporting excitatory effects have largely been restricted to hippocampus, possibly reflecting site-specific effects.

2.4. Decreased Apoptosis as a Mechanism of Estrogen Neuroprotection

Apoptosis is a major pathophysiological mode of cell death in ischemic brain injury [150,151]. Ischemia triggers mitochondria to produce reactive oxygen species, which do not only directly damage lipids, proteins and nucleic acids in the cell, but also activate various intracellular pathways that return to the mitochondria to induce apoptotic cell death, in part through regulation of pro- and antiapoptotic proteins such as the Bcl-2 family [151]. The Bcl-2 family is an essential group of proteins that regulate the integrity of the mitochondrial membrane, and is subdivided into three subgroups based on structural homology: antiapoptotic proteins including Bcl-2, Bcl-X_L and Bcl-w; proapoptotic proteins such as Bax and Bak and the BH3-only proteins including Bad, Bim, Noxa and PUMA [151]. An overweight of pro-apoptotic proteins at the membrane triggers the release of cytochrome c into the cytosol, which in turn combines with Apaf-1 and procaspase-9 to activate various caspases, such as caspase-3. The caspases are the proteins that perform the cellular degradation in apoptosis, exemplified by caspase-3's cleavage of DNA repair enzymes leading to DNA damage [152]. Another feature of apoptotic cell death is the seemingly mandatory increase in expression of the so-called immediate early genes, such as c-Jun and c-Fos [153,154], which can be used as markers of apoptosis [155]. The importance of apoptosis in stroke is suggested by the neuroprotection afforded by increased expression of the antiapoptotic Bcl-2 [156,157] and by the ischemia-induced upregulation of proapoptotic proteins in animal models of cerebral ischemia [158].

Estrogens have been reported to reduce apoptosis in a number of studies. The antiapoptotic effects of estrogens include blocking the ischemia-induced reduction of Bcl-2 following MCAo [157,159], reducing caspase-3 after global ischemia [160], increasing expression of Bcl-2, Bcl-w and Bcl-X_L while decreasing Bax, Bad and Bim [161–167], attenuating injury-mediated DNA fragmentation [21], reducing the level of the 120 kDa caspase-mediated spectrin breakdown product [21], decreasing c-Fos induction [155], limiting apoptosis induced by staurosporine in cell cultures [168], inducing cGMP-dependent expression of thioredoxin—a redox protein with potent antioxidative and antiapoptotic properties [169]—and preventing glutamate-induced translocation of cytochrome c from mitochondria to cytosol (Figure 1) [170]. ER activation is also thought to limit apoptosis through increased expression of components in oxidative phosphorylation, making energy production more stable and thus maintaining mitochondrial membrane integrity [34]. Further, Bcl-2 over-expressing male mice sustained smaller infarct sizes compared to their male wild type counterparts, while this difference was not observed in females, which is likely to mean that apoptosis is one of the mechanisms of estrogen neuroprotection [157].

Considering the potential pathways for estrogens' antiapoptotic actions, it is of interest that the Bcl-2 gene promotor has no ideal consensus sequence for an ERE, but that estrogens can interact with Sp1 for which there are several binding sites in the Bcl-2 gene promoter [171]. Also, estrogens have been shown to induce Bcl-2 expression through STAT3 and phosphoinositide-3-kinase/Akt-dependent CREB, which in turn possibly is activated by GPR-30 [164,168,172]. Akt also targets procaspase-9, members of the Forkhead family of transcription factors, which promote pro-death gene transcription [41]. These increases in antiapoptotic factors compared to proapoptotic factors afforded by estrogens are generally considered to convey neuroprotection by preventing activation of the permeability transition pore, thereby protecting against a cytosolic Ca^{2+}-overload and release of cytochrome c into the cytosol [42,173].

Many of the aforementioned studies have been performed in animal models catering for *in vivo* relevant dose intervals in which the mechanism occurs. The reported antiapoptotic effects of estrogens presented above have been demonstrated using several different hormone administration protocols, though with an overwhelming dominance of low-dose and/or short-term regimens [15,17,21,155,159,165,166]. This corroborates the combined hypotheses that estrogens are neuroprotective through antiapoptotic mechanisms and that neuroprotection due to estrogens are mainly seen in low dose and/or short-term hormone regimens. However, to our knowledge no study has been performed where estrogens' influence on apoptosis has been inhibited, and where the effect of such an inhibition has been assessed.

2.5. Growth Factor Regulation as a Mechanism of Estrogen Neuroprotection

Estrogens are known to regulate growth factors, an attribute that has been suggested as another mechanism for the hormones' beneficial effects in cerebral ischemia [12,41]. Growth factors contribute to improved outcome after cerebral ischemia both by facilitating recovery and by decreasing apoptosis, thereby reducing infarct size [174]. This mechanism overlaps considerably with apoptosis, even if the extensive research focused on estrogens interaction with growth factors merits special attention. Also, the positive, possibly neuroprotective, effects of estrogens on neural cell proliferation, synaptogenesis, modulation of synaptic connectivity and regeneration [175,176] are probably mediated through regulation of growth factors and neurotrophins, including TGF-β, IGF-I, NGF, BDNF and NT-4 (Figure 1) [177–182].

17β-estradiol regulates the transcription of numerous growth factor genes through ERs' binding to ERE in gene promoters. The factors influenced in this manner include, *i.e.*, VEGF [183], TGF-α [184], tau [185], BDNF, NT-4 and NGF [41,179]. ER not only co-localizes with and regulates the expression of neurotrophins and their cognate receptors, but estrogens and neurotrophins also share converging signaling pathways in the MAPK cascade, which includes activation of B-Raf and ERK, in turn regulating a broad array of cytoskeletal and growth-associated genes [186]. Additional evidence implying that estrogens exert their positive effects via growth factor interaction includes the cooperation with IGF-I to exert neuroprotection, possibly by sharing the MAPK and PI3/Akt signaling pathways [177,187]. Interestingly, IGF-I receptor blockade prevents estrogen neuroprotection while the ER antagonist ICI 182,780 can block IGF-I neuroprotection [188,189]. Similar results have been seen in models of cerebral ischemia [41,190], and in another study, a combination of IGF-I and 17β-estradiol did not add any extra protection against ischemia compared to the two substances

administered separately [191], emphasizing to the relation between estrogens and growth factors as a protective mechanism in stroke. Moreover, estrogens have been postulated to promote recovery after stroke by directly regulating genes required for growth, such as tau microtubule-associated protein [185], GAP-43, [192], structural lipoproteins such as apolipoprotein E [193], and neurofilament proteins [194].

Thus, ample evidence exists for the notion that estrogens increase the activity of growth factors as a major mechanism for neuroprotection. As expected, the interactions of estrogens with growth factors have been demonstrated *in vivo* resulting from predominantly low-dose or short term estrogen administration regimens [15,17,178,180,181,187–189,192,194]. Thus there is good coherence between the biological environments in which the growth factor interactions have been shown and the notion that estrogens mainly are neuroprotective in physiological concentrations.

2.6. Vascular Modulation as a Mechanism of Estrogen Neuroprotection

The importance of vascular properties, such as vessel wall reactivity and contraction propensity, for the development of stroke is self-evident. Even though this category of factors may seem less important in animal models of cerebral ischemia where the vessel occlusion is artificial, it still influences the crucial aspects of collateral circulation and reperfusion. It is thus likely that increased vasodilatation in the cerebral vascular bed is beneficial in cerebral ischemia by facilitating blood flow to compromised brain regions [39]. The reactivity and contraction propensity of a blood vessel is strongly influenced by locally produced vasodilators including prostacyclin and NO, and vasoconstrictors such as endothelin-1, which in turn are regulated by other factors.

Estrogens have been shown to affect cerebral blood vessels in a number of studies; by relaxing cerebral arteries through inhibition of extracellular Ca^{2+} influx in vascular smooth muscle [195], moderating thrombotic mechanisms [196], influencing the biosynthesis of prostacyclin [197,198], potentiating ACh-induced endothelium-dependent relaxation [199], enhancing nNOS and eNOS levels [24,200–204] and thus increasing NO production [205–208], increasing COX-1 levels [200], and by less well characterized pathways which increase cerebral blood flow (Figure 1) [3,22,209–212]. It deserves mention that although eNOS could be neuroprotective through vasodilatation, it has also been shown to induce peroxynitrite formation under certain disease states [213], which in turn potentially could compromise cellular viability [214]. Most of these effects, such as influence on eNOS, COX-1 and prostacyclin synthase leading to vasodilatation and improved collateral flow, seem to be exerted via the classical genomic pathway or via the PI3/Akt pathway [39].

Several of the effects of estrogens on the cerebral vasculature have been demonstrated *in vivo* using estrogen regimens that are in the dose-range likely to mediate neuroprotection [15], such as low-dose subcutaneous injections [24] or physiologically cycling hormones [209]. There are therefore strong indications that estrogens affect important vessel properties in biological contexts relevant for the question of its effect on cerebral ischemia, even though evidence of this mechanism's indispensability is lacking. Thus, to the best of our knowledge, no study has been performed where estrogens' impact on blood vessels have been inhibited, and where the effect of such an inhibition has been assessed. Further, numerous studies have been unable to corroborate vascular effects of estrogens in stroke models, notably by absence of blood flow differences before or during MCAo between estrogen

treated and estrogen deficient animals, even though differences in stroke outcome were observed [122,215–218].

3. Conclusions

3.1. Quality of Mechanism Experiments

Before an overall summary of the mechanisms dealt with here, brief considerations regarding study design, quality and causality may be in place.

In the process of elucidating which mechanisms are important for a certain biological target effect exerted by an investigated substance, different studies obviously contribute evidence of different weight, primarily depending on the experimental design. It is particularly difficult to draw conclusions about causality, exemplified by the fact that decreased/increased inflammatory response and oxidative stress resulting from estrogen supplementation may be a consequence of other mechanisms rather than a primary cause of the decreased/increased damage. Studies investigating mechanisms may be allocated three alternative ranks according to increasing degree of evidence:

1. The lowest degree of "evidence" for a certain mechanism comes from the discovery of a biological alteration, which *potentially* could bring about the biological target effect, in response to the investigated substance. An example is the finding that estrogens increase the concentration of the synaptic protein syntaxin, which *hypothetically* (without direct experimental evidence) could facilitate recovery after cerebral ischemia [219].
2. If the investigated substance has an effect on a presumed mechanism that in itself has been *proven* to exert the biological target effect the evidence is evidently stronger, even if the relative contribution of the mechanism cannot be quantified. An example is the fact that estrogens upregulate Bcl-2 [164], a protein in itself proven to decrease the damage from cerebral ischemia [156,157].
3. A yet higher degree of evidence for a mechanism's importance is afforded when the presence of a specific blockage inactivates the biological target effect. An example is estrogens' lack of protective effects in iNOS knocked out mice [111].

Two of the mechanisms of estrogens' neuroprotection are supported by studies of the highest evidence rank, providing the best evidence for causality between the mechanism and the outcome. These are the abovementioned importance of iNOS [111,112] and the indispensable interactions between IGF-1 and estrogens [41,188–191]. Numerous studies in the current field do not reach the highest level of evidence, e.g., due to the lack of a suitable blocker, and thus the weight of the evidence in these studies needs to be adjusted accordingly.

3.2. Summary of Mechanism Evaluations

Decreased apoptosis, growth factor regulation, vascular modulation, *indirect* decrease of oxidative stress by altering the anti-oxidative defense and decreased inflammation have all been demonstrated in experimental settings fitting the dose-concentration range pattern established for neuroprotection and are thus likely candidates for being true protective mechanisms. Anti-inflammation (iNOS) [111,112],

and interaction with growth factors (IGF-I) [41,188–191] are due to the abovementioned studies contributing with the highest evidence rank particularly well established. The *direct* anti-oxidative effect still needs to be demonstrated in relevant biological settings in estrogen concentration intervals known to be protective in whole-animal models, and this mechanism is, as aforementioned, especially difficult to assess because of the difficulties in separating genomic from non-genomic actions.

Of the suggested neurodamaging counterparts, only increased inflammation has been reported to occur under biological settings imitating conditions under which estrogens have been shown to be detrimental in whole-animal stroke experiments, and is thus to date the only real candidate of being a true damaging mechanism. The pro-oxidative effects, with their inherent problem of proving non-genomic actions, have as yet mainly been demonstrated in non-neuronal *in vitro* trials, and increased excitotoxicity has even less experimental support.

Thus while several mechanisms seem to contribute to the neuroprotective effects of estrogens in lower concentration ranges, it seems that possibly the anti-inflammatory effect of estrogens turning pro-inflammatory in supraphysiological concentrations could explain the observation that estrogens have opposing effects in different concentrations. It should however be emphasized that which mechanisms are true and false as assessed above is a highly complex issue, and that a review of this kind is better viewed as hypothesis-generating than hypothesis-testing.

3.3. Difficulties in Studying the Complex Estrogenic Mechanisms

When reviewing the abundance of studies investigating possible mechanisms of estrogens' neuroprotective actions, risk of bias is evident in cases when different estrogen effects depending on its concentrations are not taken into account (relating to the concept of hormesis). Effects of estrogens assessed as potentially protective may sometimes just as well be interpreted as risks of increased damage. For example, in a study by Weiland, estrogens were found to increase NMDA binding sites in the hippocampus, which may be taken as a neurotropic effect (protective by facilitating recovery) or as a risk of increased excitotoxicity (harmful in cerebral ischemia) [127]. In another study estrogen replacement decreased the number of astrocytes and microglia in the hippocampus, which was taken as evidence for decreased inflammation instead of the possible alternative assessment that the estrogens induced neurodegeneration [110]. To minimize the risk of similar pitfalls, several controls are needed including e.g., the verification that the biological serum concentrations of estrogens are in the range proven to afford neuroprotection. Unfortunately, careful investigation of the administration methods used are scarce, and in the majority of instances when blood samples are drawn for analysis of serum estrogen levels the sampling is only performed at only one single time point (most often at animal sacrifice), which thus conveys little information of the serum concentrations before and after this specific moment.

3.4. Final Remarks

Investigations of different mechanisms for estrogens' actions in stroke have been performed in a very wide range of concentrations, profoundly affecting the plausibility of the suggested mechanism given that the hormones' neuroprotective and neurodamaging properties are in fact restricted to certain dose intervals. In future studies it is crucial that mechanisms are verified in relevant biological contexts

where special care has been taken to control estrogen concentrations. This in combination with experimental designs catering for the highest level of evidence will provide the solid ground needed for characterizing estrogens' actions and pathways in cerebral ischemia.

References

1. Hall, E.D.; Pazara, K.E.; Linseman, K.L. Sex differences in postischemic neuronal necrosis in gerbils. *J. Cereb. Blood Flow Metab.* **1991**, *11*, 292–298.
2. Simpkins, J.W.; Rajakumar, G.; Zhang, Y.Q.; Simpkins, C.E.; Greenwald, D.; Yu, C.J.; Bodor, N.; Day, A.L. Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. *J. Neurosurg.* **1997**, *87*, 724–730.
3. Hurn, P.D.; Littleton-Kearney, M.T.; Kirsch, J.R.; Dharmarajan, A.M.; Traystman, R.J. Postischemic cerebral blood flow recovery in the female: Effect of 17beta-estradiol. *J. Cereb. Blood Flow Metab.* **1995**, *15*, 666–672.
4. Lobo, R.A. The risk of stroke in postmenopausal women receiving hormonal therapy. *Climacteric* **2009**, *12* (Suppl 1), 81–85.
5. Rossouw, J.E.; Anderson, G.L.; Prentice, R.L.; LaCroix, A.Z.; Kooperberg, C.; Stefanick, M.L.; Jackson, R.D.; Beresford, S.A.; Howard, B.V.; Johnson, K.C.; Kotchen, J.M.; Ockene, J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the Women's Health Initiative randomized controlled trial. *Jama* **2002**, *288*, 321–333.
6. Harukuni, I.; Hurn, P.D.; Crain, B.J. Deleterious effect of beta-estradiol in a rat model of transient forebrain ischemia. *Brain Res.* **2001**, *900*, 137–142.
7. Yong, Y.; Xie, H.J.; Zhang, Y.F.; Yang, Q.D.; Liao, D.F.; Yang, H.L.; Yan, P.K.; Liu, Z.J. 17beta-estradiol potentiates ischemia-reperfusion injury in diabetic ovariectomized female rats. *Brain Res.* **2005**, *1054*, 192–199.
8. Theodorsson, A.; Theodorsson, E. Estradiol increases brain lesions in the cortex and lateral striatum after transient occlusion of the middle cerebral artery in rats: No effect of ischemia on galanin in the stroke area but decreased levels in the hippocampus. *Peptides* **2005**, *26*, 2257–2264.
9. Gordon, K.B.; Macrae, I.M.; Carswell, H.V. Effects of 17beta-oestradiol on cerebral ischaemic damage and lipid peroxidation. *Brain Res.* **2005**, *1036*, 155–162.
10. Carswell, H.V.; Bingham, D.; Wallace, K.; Nilsen, M.; Graham, D.I.; Dominiczak, A.F.; Macrae, I.M. Differential effects of 17beta-estradiol upon stroke damage in stroke prone and normotensive rats. *J. Cereb. Blood Flow Metab.* **2004**, *24*, 298–304.
11. Bingham, D.; Macrae, I.M.; Carswell, H.V. Detrimental effects of 17beta-oestradiol after permanent middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* **2005**, *25*, 414–420.
12. Liu, M.; Dziennis, S.; Hurn, P.D.; Alkayed, N.J. Mechanisms of gender-linked ischemic brain injury. *Restor. Neurol. Neurosci.* **2009**, *27*, 163–179.
13. Brown, C.M.; Suzuki, S.; Jelks, K.A.; Wise, P.M. Estradiol is a potent protective, restorative, and trophic factor after brain injury. *Semin. Reprod. Med.* **2009**, *27*, 240–249.
14. Suzuki, S.; Brown, C.M.; Wise, P.M. Neuroprotective effects of estrogens following ischemic stroke. *Front. Neuroendocrinol.* **2009**, *30*, 201–211.

15. Strom, J.O.; Theodorsson, A.; Theodorsson, E. Dose-related neuroprotective *versus* neurodamaging effects of estrogens in rat cerebral ischemia: A systematic analysis. *J. Cereb. Blood Flow Metab.* **2009**, *29*, 1359–1372.
16. Strom, J.O.; Theodorsson, E.; Holm, L.; Theodorsson, A. Different methods for administering 17beta-estradiol to ovariectomized rats result in opposite effects on ischemic brain damage. *BMC Neurosci.* **2010**, *11*, 39.
17. Strom, J.O.; Theodorsson, E.; Theodorsson, A. Order of magnitude differences between methods for maintaining physiological 17beta-oestradiol concentrations in ovariectomized rats. *Scand. J. Clin. Lab. Invest.* **2008**, *68*, 814–822.
18. Calabrese, E.J.; Baldwin, L.A. Hormesis: The dose-response revolution. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 175–197.
19. Dziennis, S.; Jia, T.; Ronnekleiv, O.K.; Hurn, P.D.; Alkayed, N.J. Role of signal transducer and activator of transcription-3 in estradiol-mediated neuroprotection. *J. Neurosci.* **2007**, *27*, 7268–7274.
20. Schreihofer, D.A.; Do, K.D.; Schreihofer, A.M. High-soy diet decreases infarct size after permanent middle cerebral artery occlusion in female rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2005**, *289*, R103–108.
21. Rau, S.W.; Dubal, D.B.; Bottner, M.; Gerhold, L.M.; Wise, P.M. Estradiol attenuates programmed cell death after stroke-like injury. *J. Neurosci.* **2003**, *23*, 11420–11426.
22. He, Z.; He, Y.J.; Day, A.L.; Simpkins, J.W. Proestrus levels of estradiol during transient global cerebral ischemia improves the histological outcome of the hippocampal CA1 region: Perfusion-dependent and-independent mechanisms. *J. Neurol. Sci.* **2002**, *193*, 79–87.
23. Sandstrom, N.J.; Rowan, M.H. Acute pretreatment with estradiol protects against CA1 cell loss and spatial learning impairments resulting from transient global ischemia. *Horm. Behav.* **2007**, *51*, 335–345.
24. Pelligrino, D.A.; Santizo, R.; Baughman, V.L.; Wang, Q. Cerebral vasodilating capacity during forebrain ischemia: Effects of chronic estrogen depletion and repletion and the role of neuronal nitric oxide synthase. *Neuroreport* **1998**, *9*, 3285–3291.
25. Saleh, T.M.; Connell, B.J.; Legge, C.; Cribb, A.E. Estrogen attenuates neuronal excitability in the insular cortex following middle cerebral artery occlusion. *Brain Res.* **2004**, *1018*, 119–129.
26. Plamondon, H.; Morin, A.; Charron, C. Chronic 17beta-estradiol pretreatment and ischemia-induced hippocampal degeneration and memory impairments: A 6-month survival study. *Horm. Behav.* **2006**, *50*, 361–369.
27. Choi, Y.C.; Lee, J.H.; Hong, K.W.; Lee, K.S. 17Beta-estradiol prevents focal cerebral ischemic damages via activation of Akt and CREB in association with reduced PTEN phosphorylation in rats. *Fundam. Clin. Pharmacol.* **2004**, *18*, 547–557.
28. Yu, S.J.; Kim, J.R.; Lee, C.K.; Kim, J.H.; Kam, K.Y.; Hong, J.H.; Kang, S.G. Involvement of purα gene in neuroprotection effects of estrogen in rat ischemic brain model. *Korean J. Genet.* **2006**, *28*, 403–412.
29. Gulinello, M.; Lebesgue, D.; Jover-Mengual, T.; Zukin, R.S.; Etgen, A.M. Acute and chronic estradiol treatments reduce memory deficits induced by transient global ischemia in female rats. *Horm. Behav.* **2006**, *49*, 246–260.

30. Kii, N.; Adachi, N.; Liu, K.; Arai, T. Acute effects of 17beta-estradiol on oxidative stress in ischemic rat striatum. *J. Neurosurg. Anesthesiol.* **2005**, *17*, 27–32.
31. Littleton-Kearney, M.T.; Klaus, J.A.; Hurn, P.D. Effects of combined oral conjugated estrogens and medroxyprogesterone acetate on brain infarction size after experimental stroke in rat. *J. Cereb. Blood Flow Metab.* **2005**, *25*, 421–426.
32. Strom, J.O.; Theodorsson, A.; Theodorsson, E. Substantial discrepancies in 17beta-oestradiol concentrations obtained with three different commercial direct radioimmunoassay kits in rat sera. *Scand. J. Clin. Lab. Invest.* **2008**, *68*, 806–813.
33. Suzuki, S.; Gerhold, L.M.; Bottner, M.; Rau, S.W.; Dela Cruz, C.; Yang, E.; Zhu, H.; Yu, J.; Cashion, A.B.; Kindy, M.S.; Merchenthaler, I.; Gage, F.H.; Wise, P.M. Estradiol enhances neurogenesis following ischemic stroke through estrogen receptors alpha and beta. *J. Comp. Neurol.* **2007**, *500*, 1064–1075.
34. Simpkins, J.W.; Yi, K.D.; Yang, S.H. Role of protein phosphatases and mitochondria in the neuroprotective effects of estrogens. *Front. Neuroendocrinol.* **2009**, *30*, 93–105.
35. Singer, C.A.; Rogers, K.L.; Strickland, T.M.; Dorsa, D.M. Estrogen protects primary cortical neurons from glutamate toxicity. *Neurosci. Lett.* **1996**, *212*, 13–16.
36. Weaver, C.E., Jr; Park-Chung, M.; Gibbs, T.T.; Farb, D.H. 17Beta-Estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. *Brain Res.* **1997**, *761*, 338–341.
37. Carswell, H.V.; Macrae, I.M.; Farr, T.D. Complexities of oestrogen in stroke. *Clin. Sci. (London)* **2010**, *118*, 375–389.
38. Macrae, I.M.; Carswell, H.V. Oestrogen and stroke: The potential for harm as well as benefit. *Biochem. Soc. Trans.* **2006**, *34*, 1362–1365.
39. Duckles, S.P.; Krause, D.N. Cerebrovascular effects of oestrogen: Multiplicity of action. *Clin. Exp. Pharmacol. Physiol.* **2007**, *34*, 801–808.
40. Simpkins, J.W.; Singh, M. More than a decade of estrogen neuroprotection. *Alzheimers Dement.* **2008**, *4*, S131–136.
41. Lebesgue, D.; Chevaleyre, V.; Zukin, R.S.; Etgen, A.M. Estradiol rescues neurons from global ischemia-induced cell death: Multiple cellular pathways of neuroprotection. *Steroids* **2009**, *74*, 555–561.
42. Arnold, S.; Beyer, C. Neuroprotection by estrogen in the brain: The mitochondrial compartment as presumed therapeutic target. *J. Neurochem.* **2009**, *110*, 1–11.
43. Sherwin, B.B. Estrogen therapy: Is time of initiation critical for neuroprotection? *Nat. Rev. Endocrinol.* **2009**, *5*, 620–627.
44. Fujimura, M.; Tominaga, T.; Chan, P.H. Neuroprotective effect of an antioxidant in ischemic brain injury: Involvement of neuronal apoptosis. *Neurocrit. Care* **2005**, *2*, 59–66.
45. Behl, C.; Skutella, T.; Lezoualc'h, F.; Post, A.; Widmann, M.; Newton, C.J.; Holsboer, F. Neuroprotection against oxidative stress by estrogens: Structure-activity relationship. *Mol. Pharmacol.* **1997**, *51*, 535–541.
46. Culmsee, C.; Vedder, H.; Ravati, A.; Junker, V.; Otto, D.; Ahlemeyer, B.; Krieg, J.C.; Krieglstein, J. Neuroprotection by estrogens in a mouse model of focal cerebral ischemia and in

cultured neurons: Evidence for a receptor-independent antioxidative mechanism. *J. Cereb. Blood Flow Metab.* **1999**, *19*, 1263–1269.

47. Vedder, H.; Anthes, N.; Stumm, G.; Wurz, C.; Behl, C.; Krieg, J.C. Estrogen hormones reduce lipid peroxidation in cells and tissues of the central nervous system. *J. Neurochem.* **1999**, *72*, 2531–2538.
48. Ayres, S.; Abplanalp, W.; Liu, J.H.; Subbiah, M.T. Mechanisms involved in the protective effect of estradiol-17beta on lipid peroxidation and DNA damage. *Am. J. Physiol.* **1998**, *274*, E1002–1008.
49. Rattanajarasroj, S.; Unchern, S. Comparable attenuation of Abeta(25–35)-induced neurotoxicity by quercitrin and 17beta-estradiol in cultured rat hippocampal neurons. *Neurochem. Res.* **2010**, *35*, 1196–1205.
50. Wang, X.; Dykens, J.A.; Perez, E.; Liu, R.; Yang, S.; Covey, D.F.; Simpkins, J.W. Neuroprotective effects of 17beta-estradiol and nonfeminizing estrogens against H_2O_2 toxicity in human neuroblastoma SK-N-SH cells. *Mol. Pharmacol.* **2006**, *70*, 395–404.
51. Keller, J.N.; Germeyer, A.; Begley, J.G.; Mattson, M.P. 17Beta-estradiol attenuates oxidative impairment of synaptic Na+/K+-ATPase activity, glucose transport, and glutamate transport induced by amyloid beta-peptide and iron. *J. Neurosci. Res.* **1997**, *50*, 522–530.
52. Prokai, L.; Simpkins, J.W. Structure-nongenomic neuroprotection relationship of estrogens and estrogen-derived compounds. *Pharmacol. Ther.* **2007**, *114*, 1–12.
53. Behl, C.; Widmann, M.; Trapp, T.; Holsboer, F. 17-beta estradiol protects neurons from oxidative stress-induced cell death *in vitro*. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 473–482.
54. Bonnefont, A.B.; Munoz, F.J.; Inestrosa, N.C. Estrogen protects neuronal cells from the cytotoxicity induced by acetylcholinesterase-amyloid complexes. *FEBS Lett.* **1998**, *441*, 220–224.
55. Prokai, L.; Prokai-Tatrai, K.; Perjesi, P.; Zharikova, A.D.; Perez, E.J.; Liu, R.; Simpkins, J.W. Quinol-based cyclic antioxidant mechanism in estrogen neuroprotection. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 11741–11746.
56. Prokai-Tatrai, K.; Perjesi, P.; Rivera-Portalatin, N.M.; Simpkins, J.W.; Prokai, L. Mechanistic investigations on the antioxidant action of a neuroprotective estrogen derivative. *Steroids* **2008**, *73*, 280–288.
57. Picazo, O.; Azcoitia, I.; Garcia-Segura, L.M. Neuroprotective and neurotoxic effects of estrogens. *Brain Res.* **2003**, *990*, 20–27.
58. Chen, C.; Hu, Q.; Yan, J.; Lei, J.; Qin, L.; Shi, X.; Luan, L.; Yang, L.; Wang, K.; Han, J.; Nanda, A.; Zhou, C. Multiple effects of 2ME2 and D609 on the cortical expression of HIF-1alpha and apoptotic genes in a middle cerebral artery occlusion-induced focal ischemia rat model. *J. Neurochem.* **2007**, *102*, 1831–1841.
59. Bruce-Keller, A.J.; Keeling, J.L.; Keller, J.N.; Huang, F.F.; Camondola, S.; Mattson, M.P. Antiinflammatory effects of estrogen on microglial activation. *Endocrinology* **2000**, *141*, 3646–3656.

60. Diwakar, L.; Kenchappa, R.S.; Annepu, J.; Ravindranath, V. Downregulation of glutaredoxin but not glutathione loss leads to mitochondrial dysfunction in female mice CNS: Implications in excitotoxicity. *Neurochem. Int.* **2007**, *51*, 37–46.
61. Schmidt, A.J.; Krieg, J.C.; Vedder, H. Differential effects of glucocorticoids and gonadal steroids on glutathione levels in neuronal and glial cell systems. *J. Neurosci. Res.* **2002**, *67*, 544–550.
62. Diwakar, L.; Kenchappa, R.S.; Annepu, J.; Saeed, U.; Sujanitha, R.; Ravindranath, V. Down-regulation of glutaredoxin by estrogen receptor antagonist renders female mice susceptible to excitatory amino acid mediated complex I inhibition in CNS. *Brain Res.* **2006**, *1125*, 176–184.
63. Urata, Y.; Ihara, Y.; Murata, H.; Goto, S.; Koji, T.; Yodoi, J.; Inoue, S.; Kondo, T. 17Beta-estradiol protects against oxidative stress-induced cell death through the glutathione/glutaredoxin-dependent redox regulation of Akt in myocardiac H9c2 cells. *J. Biol. Chem.* **2006**, *281*, 13092–13102.
64. Ozacmak, V.H.; Sayan, H. The effects of 17beta estradiol, 17alpha estradiol and progesterone on oxidative stress biomarkers in ovariectomized female rat brain subjected to global cerebral ischemia. *Physiol. Res.* **2009**, *58*, 909–912.
65. Gottipati, S.; Cammarata, P.R. Mitochondrial superoxide dismutase activation with 17 beta-estradiol-treated human lens epithelial cells. *Mol. Vis.* **2008**, *14*, 898–905.
66. Pedram, A.; Razandi, M.; Wallace, D.C.; Levin, E.R. Functional estrogen receptors in the mitochondria of breast cancer cells. *Mol. Biol. Cell* **2006**, *17*, 2125–2137.
67. Tripanichkul, W.; Sripanichkulchai, K.; Duce, J.A.; Finkelstein, D.I. 17Beta-estradiol reduces nitrotyrosine immunoreactivity and increases SOD1 and SOD2 immunoreactivity in nigral neurons in male mice following MPTP insult. *Brain Res.* **2007**, *1164*, 24–31.
68. Strehlow, K.; Rotter, S.; Wassmann, S.; Adam, O.; Grohe, C.; Laufs, K.; Bohm, M.; Nickenig, G. Modulation of antioxidant enzyme expression and function by estrogen. *Circ. Res.* **2003**, *93*, 170–177.
69. Nilsen, J.; Irwin, R.W.; Gallaher, T.K.; Brinton, R.D. Estradiol *in vivo* regulation of brain mitochondrial proteome. *J. Neurosci.* **2007**, *27*, 14069–14077.
70. Irwin, R.W.; Yao, J.; Hamilton, R.T.; Cadenas, E.; Brinton, R.D.; Nilsen, J. Progesterone and estrogen regulate oxidative metabolism in brain mitochondria. *Endocrinology* **2008**, *149*, 3167–3175.
71. Miller, A.A.; Drummond, G.R.; Mast, A.E.; Schmidt, H.H.; Sobey, C.G. Effect of gender on NADPH-oxidase activity, expression, and function in the cerebral circulation: Role of estrogen. *Stroke* **2007**, *38*, 2142–2149.
72. Zhang, Q.G.; Raz, L.; Wang, R.; Han, D.; De Sevilla, L.; Yang, F.; Vadlamudi, R.K.; Brann, D.W. Estrogen attenuates ischemic oxidative damage via an estrogen receptor alpha-mediated inhibition of NADPH oxidase activation. *J. Neurosci.* **2009**, *29*, 13823–13836.
73. Kumar, S.; Lata, K.; Mukhopadhyay, S.; Mukherjee, T.K. Role of estrogen receptors in pro-oxidative and anti-oxidative actions of estrogens: A perspective. *Biochim. Biophys. Acta* **2010**, *1800*, 1127–1135.
74. Sastre-Serra, J.; Valle, A.; Company, M.M.; Garau, I.; Oliver, J.; Roca, P. Estrogen down-regulates uncoupling proteins and increases oxidative stress in breast cancer. *Free Radic. Biol. Med.* **2010**, *48*, 506–512.

75. Felty, Q.; Xiong, W.C.; Sun, D.; Sarkar, S.; Singh, K.P.; Parkash, J.; Roy, D. Estrogen-induced mitochondrial reactive oxygen species as signal-transducing messengers. *Biochemistry* **2005**, *44*, 6900–6909.
76. Rempel, M.A.; Hester, B.; Deharo, H.; Hong, H.; Wang, Y.; Schlenk, D. Effects of 17beta-estradiol, and its metabolite, 4-hydroxyestradiol on fertilization, embryo development and oxidative DNA damage in sand dollar (Dendraster excentricus) sperm. *Sci. Total Environ.* **2009**, *407*, 2209–2215.
77. Symonds, D.A.; Merchenthaler, I.; Flaws, J.A. Methoxychlor and estradiol induce oxidative stress DNA damage in the mouse ovarian surface epithelium. *Toxicol. Sci.* **2008**, *105*, 182–187.
78. Pajovic, S.B.; Saicic, Z.S.; Spasic, M.B.; Petrovic, V.M. The effect of ovarian hormones on antioxidant enzyme activities in the brain of male rats. *Physiol. Res.* **2003**, *52*, 189–194.
79. Genc, S.; Gurdol, F.; Oner-Iyidogan, Y.; Suzme, R. Acute effects of estradiol and of diethylstilbestrol: Pro- or antioxidant potential? *Res. Commun. Mol. Pathol. Pharmacol.* **1999**, *105*, 253–261.
80. Ting, C.M.; Lee, Y.M.; Wong, C.K.; Wong, A.S.; Lung, H.L.; Lung, M.L.; Lo, K.W.; Wong, R.N.; Mak, N.K. 2-Methoxyestradiol induces endoreduplication through the induction of mitochondrial oxidative stress and the activation of MAPK signaling pathways. *Biochem. Pharmacol.* **2010**, *79*, 825–841.
81. Park, S.A.; Na, H.K.; Kim, E.H.; Cha, Y.N.; Surh, Y.J. 4-hydroxyestradiol induces anchorage-independent growth of human mammary epithelial cells via activation of IkappaB kinase: potential role of reactive oxygen species. *Cancer Res.* **2009**, *69*, 2416–2424.
82. She, M.R.; Li, J.G.; Guo, K.Y.; Lin, W.; Du, X.; Niu, X.Q. Requirement of reactive oxygen species generation in apoptosis of leukemia cells induced by 2-methoxyestradiol. *Acta Pharmacol. Sin.* **2007**, *28*, 1037–1044.
83. Jin, R.; Yang, G.; Li, G. Inflammatory mechanisms in ischemic stroke: Role of inflammatory cells. *J. Leukoc. Biol.* **2010**, *87*, 779–789.
84. Barone, F.C.; Arvin, B.; White, R.F.; Miller, A.; Webb, C.L.; Willette, R.N.; Lysko, P.G.; Feuerstein, G.Z. Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury. *Stroke* **1997**, *28*, 1233–1244.
85. Yamasaki, Y.; Matsuura, N.; Shozuhara, H.; Onodera, H.; Itoyama, Y.; Kogure, K. Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke* **1995**, *26*, 676–680; discussion 681.
86. Czlonkowska, A.; Ciesielska, A.; Gromadzka, G.; Kurkowska-Jastrzebska, I. Gender differences in neurological disease: Role of estrogens and cytokines. *Endocrine* **2006**, *29*, 243–256.
87. Mulcahy, N.J.; Ross, J.; Rothwell, N.J.; Loddick, S.A. Delayed administration of interleukin-1 receptor antagonist protects against transient cerebral ischaemia in the rat. *Br. J. Pharmacol.* **2003**, *140*, 471–476.
88. Relton, J.K.; Martin, D.; Thompson, R.C.; Russell, D.A. Peripheral administration of interleukin-1 receptor antagonist inhibits brain damage after focal cerebral ischemia in the rat. *Exp. Neurol.* **1996**, *138*, 206–213.
89. Nawashiro, H.; Martin, D.; Hallenbeck, J.M. Inhibition of tumor necrosis factor and amelioration of brain infarction in mice. *J. Cereb. Blood Flow Metab.* **1997**, *17*, 229–232.

90. Martin-Villalba, A.; Hahne, M.; Kleber, S.; Vogel, J.; Falk, W.; Schenkel, J.; Krammer, P.H. Therapeutic neutralization of CD95-ligand and TNF attenuates brain damage in stroke. *Cell Death Differ.* **2001**, *8*, 679–686.
91. Loddick, S.A.; Rothwell, N.J. Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischaemia in the rat. *J. Cereb. Blood Flow Metab.* **1996**, *16*, 932–940.
92. Santizo, R.A.; Anderson, S.; Ye, S.; Koenig, H.M.; Pelligrino, D.A. Effects of estrogen on leukocyte adhesion after transient forebrain ischemia. *Stroke* **2000**, *31*, 2231–2235.
93. Mori, M.; Tsukahara, F.; Yoshioka, T.; Irie, K.; Ohta, H. Suppression by 17beta-estradiol of monocyte adhesion to vascular endothelial cells is mediated by estrogen receptors. *Life Sci.* **2004**, *75*, 599–609.
94. Nathan, L.; Pervin, S.; Singh, R.; Rosenfeld, M.; Chaudhuri, G. Estradiol inhibits leukocyte adhesion and transendothelial migration in rabbits *in vivo*: Possible mechanisms for gender differences in atherosclerosis. *Circ. Res.* **1999**, *85*, 377–385.
95. Suzuki, S.; Brown, C.M.; Dela Cruz, C.D.; Yang, E.; Bridwell, D.A.; Wise, P.M. Timing of estrogen therapy after ovariectomy dictates the efficacy of its neuroprotective and antiinflammatory actions. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 6013–6018.
96. Chiappetta, O.; Gliozzi, M.; Siviglia, E.; Amantea, D.; Morrone, L.A.; Berliocchi, L.; Bagetta, G.; Corasaniti, M.T. Evidence to implicate early modulation of interleukin-1beta expression in the neuroprotection afforded by 17beta-estradiol in male rats undergone transient middle cerebral artery occlusion. *Int. Rev. Neurobiol.* **2007**, *82*, 357–372.
97. Nordell, V.L.; Scarborough, M.M.; Buchanan, A.K.; Sohrabji, F. Differential effects of estrogen in the injured forebrain of young adult and reproductive senescent animals. *Neurobiol. Aging* **2003**, *24*, 733–743.
98. Tenenbaum, M.; Azab, A.N.; Kaplanski, J. Effects of estrogen against LPS-induced inflammation and toxicity in primary rat glial and neuronal cultures. *J. Endotoxin Res.* **2007**, *13*, 158–166.
99. Vegeto, E.; Bonincontro, C.; Pollio, G.; Sala, A.; Viappiani, S.; Nardi, F.; Brusadelli, A.; Viviani, B.; Ciana, P.; Maggi, A. Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. *J. Neurosci.* **2001**, *21*, 1809–1818.
100. Lewis, D.K.; Johnson, A.B.; Stohlgren, S.; Harms, A.; Sohrabji, F. Effects of estrogen receptor agonists on regulation of the inflammatory response in astrocytes from young adult and middle-aged female rats. *J. Neuroimmunol.* **2008**, *195*, 47–59.
101. Ray, P.; Ghosh, S.K.; Zhang, D.H.; Ray, A. Repression of interleukin-6 gene expression by 17beta-estradiol: Inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF-kappa B by the estrogen receptor. *FEBS Lett.* **1997**, *409*, 79–85.
102. Ospina, J.A.; Brevig, H.N.; Krause, D.N.; Duckles, S.P. Estrogen suppresses IL-1beta-mediated induction of COX-2 pathway in rat cerebral blood vessels. *Am. J. Physiol. Heart Circ. Physiol.* **2004**, *286*, H2010–2019.
103. Vegeto, E.; Belcredito, S.; Etteri, S.; Ghisletti, S.; Brusadelli, A.; Meda, C.; Krust, A.; Dupont, S.; Ciana, P.; Chambon, P.; Maggi, A. Estrogen receptor-alpha mediates the brain antiinflammatory activity of estradiol. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9614–9619.

104. Vegeto, E.; Benedusi, V.; Maggi, A. Estrogen anti-inflammatory activity in brain: A therapeutic opportunity for menopause and neurodegenerative diseases. *Front. Neuroendocrinol.* **2008**, *29*, 507–519.
105. Sunday, L.; Osuna, C.; Krause, D.N.; Duckles, S.P. Age alters cerebrovascular inflammation and effects of estrogen. *Am. J. Physiol. Heart Circ. Physiol.* **2007**, *292*, H2333–2340.
106. Srivastava, S.; Weitzmann, M.N.; Cenci, S.; Ross, F.P.; Adler, S.; Pacifici, R. Estrogen decreases TNF gene expression by blocking JNK activity and the resulting production of c-Jun and JunD. *J. Clin. Invest.* **1999**, *104*, 503–513.
107. Yatkin, E.; Bernoulli, J.; Talvitie, E.M.; Santti, R. Inflammation and epithelial alterations in rat prostate: Impact of the androgen to oestrogen ratio. *Int. J. Androl.* **2009**, *32*, 399–410.
108. Salem, M.L.; Hossain, M.S.; Nomoto, K. Mediation of the immunomodulatory effect of beta-estradiol on inflammatory responses by inhibition of recruitment and activation of inflammatory cells and their gene expression of TNF-alpha and IFN-gamma. *Int. Arch. Allergy Immunol.* **2000**, *121*, 235–245.
109. Wen, Y.; Yang, S.; Liu, R.; Perez, E.; Yi, K.D.; Koulen, P.; Simpkins, J.W. Estrogen attenuates nuclear factor-kappa B activation induced by transient cerebral ischemia. *Brain Res.* **2004**, *1008*, 147–154.
110. Lei, D.L.; Long, J.M.; Hengemihle, J.; O'Neill, J.; Manaye, K.F.; Ingram, D.K.; Mouton, P.R. Effects of estrogen and raloxifene on neuroglia number and morphology in the hippocampus of aged female mice. *Neuroscience* **2003**, *121*, 659–666.
111. Brown, C.M.; Dela Cruz, C.D.; Yang, E.; Wise, P.M. Inducible nitric oxide synthase and estradiol exhibit complementary neuroprotective roles after ischemic brain injury. *Exp. Neurol.* **2008**, *210*, 782–787.
112. Park, E.M.; Cho, S.; Frys, K.A.; Glickstein, S.B.; Zhou, P.; Anrather, J.; Ross, M.E.; Iadecola, C. Inducible nitric oxide synthase contributes to gender differences in ischemic brain injury. *J. Cereb. Blood Flow Metab.* **2006**, *26*, 392–401.
113. Xu, H.L.; Baughman, V.L.; Pelligrino, D.A. Estrogen replacement treatment in diabetic ovariectomized female rats potentiates postischemic leukocyte adhesion in cerebral venules. *Stroke* **2004**, *35*, 1974–1978.
114. Soucy, G.; Boivin, G.; Labrie, F.; Rivest, S. Estradiol is required for a proper immune response to bacterial and viral pathogens in the female brain. *J. Immunol.* **2005**, *174*, 6391–6398.
115. Johnson, A.B.; Sohrabji, F. Estrogen's effects on central and circulating immune cells vary with reproductive age. *Neurobiol. Aging* **2005**, *26*, 1365–1374.
116. Marriott, L.K.; Hauss-Wegrzyniak, B.; Benton, R.S.; Vraniak, P.D.; Wenk, G.L. Long-term estrogen therapy worsens the behavioral and neuropathological consequences of chronic brain inflammation. *Behav. Neurosci.* **2002**, *116*, 902–911.
117. O'Donnell, M.E.; Lam, T.I.; Tran, L.Q.; Foroutan, S.; Anderson, S.E. Estradiol reduces activity of the blood-brain barrier Na-K-Cl cotransporter and decreases edema formation in permanent middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* **2006**, *26*, 1234–1249.
118. Krause, D.N.; Duckles, S.P.; Pelligrino, D.A. Influence of sex steroid hormones on cerebrovascular function. *J. Appl. Physiol.* **2006**, *101*, 1252–1261.

119. Wise, P.M.; Dubal, D.B. Estradiol protects against ischemic brain injury in middle-aged rats. *Biol. Reprod.* **2000**, *63*, 982–985.
120. Dubal, D.B.; Wise, P.M. Neuroprotective effects of estradiol in middle-aged female rats. *Endocrinology* **2001**, *142*, 43–48.
121. Toung, T.J.; Chen, T.Y.; Littleton-Kearney, M.T.; Hurn, P.D.; Murphy, S.J. Effects of combined estrogen and progesterone on brain infarction in reproductively senescent female rats. *J. Cereb. Blood Flow Metab.* **2004**, *24*, 1160–1166.
122. Alkayed, N.J.; Murphy, S.J.; Traystman, R.J.; Hurn, P.D.; Miller, V.M. Neuroprotective effects of female gonadal steroids in reproductively senescent female rats. *Stroke* **2000**, *31*, 161–168.
123. Toung, T.K.; Hurn, P.D.; Traystman, R.J.; Sieber, F.E. Estrogen decreases infarct size after temporary focal ischemia in a genetic model of type 1 diabetes mellitus. *Stroke* **2000**, *31*, 2701–2706.
124. Brouns, R.; De Deyn, P.P. The complexity of neurobiological processes in acute ischemic stroke. *Clin. Neurol. Neurosurg.* **2009**, *111*, 483–495.
125. Brann, D.W.; Zamorano, P.L.; Chorich, L.P.; Mahesh, V.B. Steroid hormone effects on NMDA receptor binding and NMDA receptor mRNA levels in the hypothalamus and cerebral cortex of the adult rat. *Neuroendocrinology* **1993**, *58*, 666–672.
126. Woolley, C.S.; Weiland, N.G.; McEwen, B.S.; Schwartzkroin, P.A. Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: Correlation with dendritic spine density. *J. Neurosci.* **1997**, *17*, 1848–1859.
127. Weiland, N.G. Estradiol selectively regulates agonist binding sites on the N-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. *Endocrinology* **1992**, *131*, 662–668.
128. Woolley, C.S.; McEwen, B.S. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J. Comp. Neurol.* **1993**, *336*, 293–306.
129. Woolley, C.S.; McEwen, B.S. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *J. Neurosci.* **1992**, *12*, 2549–2554.
130. Pozzo-Miller, L.D.; Inoue, T.; Murphy, D.D. Estradiol increases spine density and NMDA-dependent Ca2+ transients in spines of CA1 pyramidal neurons from hippocampal slices. *J. Neurophysiol.* **1999**, *81*, 1404–1411.
131. Buterbaugh, G.G.; Hudson, G.M. Estradiol replacement to female rats facilitates dorsal hippocampal but not ventral hippocampal kindled seizure acquisition. *Exp. Neurol.* **1991**, *111*, 55–64.
132. Warren, S.G.; Humphreys, A.G.; Juraska, J.M.; Greenough, W.T. LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrus rats. *Brain Res.* **1995**, *703*, 26–30.
133. Foy, M.R.; Xu, J.; Xie, X.; Brinton, R.D.; Thompson, R.F.; Berger, T.W. 17Beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J. Neurophysiol.* **1999**, *81*, 925–929.
134. Teyler, T.J.; Vardaris, R.M.; Lewis, D.; Rawitch, A.B. Gonadal steroids: Effects on excitability of hippocampal pyramidal cells. *Science* **1980**, *209*, 1017–1018.
135. Smith, S.S.; Waterhouse, B.D.; Woodward, D.J. Sex steroid effects on extrahypothalamic CNS. I. Estrogen augments neuronal responsiveness to iontophoretically applied glutamate in the cerebellum. *Brain Res.* **1987**, *422*, 40–51.

136. Sato, K.; Matsuki, N.; Ohno, Y.; Nakazawa, K. Estrogens inhibit l-glutamate uptake activity of astrocytes via membrane estrogen receptor alpha. *J. Neurochem.* **2003**, *86*, 1498–1505.
137. Gu, Q.; Moss, R.L. 17Beta-Estradiol potentiates kainate-induced currents via activation of the cAMP cascade. *J. Neurosci.* **1996**, *16*, 3620–3629.
138. Johansen, F.F.; Jorgensen, M.B.; Diemer, N.H. Ischemic CA-1 pyramidal cell loss is prevented by preischemic colchicine destruction of dentate gyrus granule cells. *Brain Res.* **1986**, *377*, 344–347.
139. Onodera, H.; Sato, G.; Kogure, K. Lesions to Schaffer collaterals prevent ischemic death of CA1 pyramidal cells. *Neurosci. Lett.* **1986**, *68*, 169–174.
140. Wieloch, T.; Lindvall, O.; Blomqvist, P.; Gage, F.H. Evidence for amelioration of ischaemic neuronal damage in the hippocampal formation by lesions of the perforant path. *Neurol. Res.* **1985**, *7*, 24–26.
141. Ito, H.; Watanabe, Y.; Isshiki, A.; Uchino, H. Neuroprotective properties of propofol and midazolam, but not pentobarbital, on neuronal damage induced by forebrain ischemia, based on the GABAA receptors. *Acta Anaesthesiol. Scand.* **1999**, *43*, 153–162.
142. Schwartz-Bloom, R.D.; McDonough, K.J.; Chase, P.J.; Chadwick, L.E.; Inglefield, J.R.; Levin, E.D. Long-term neuroprotection by benzodiazepine full *versus* partial agonists after transient cerebral ischemia in the gerbil [corrected]. *J. Cereb. Blood Flow Metab.* **1998**, *18*, 548–558.
143. Goodman, Y.; Bruce, A.J.; Cheng, B.; Mattson, M.P. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J. Neurochem.* **1996**, *66*, 1836–1844.
144. Azcoitia, I.; Fernandez-Galaz, C.; Sierra, A.; Garcia-Segura, L.M. Gonadal hormones affect neuronal vulnerability to excitotoxin-induced degeneration. *J. Neurocytol.* **1999**, *28*, 699–710.
145. Mendelowitsch, A.; Ritz, M.F.; Ros, J.; Langemann, H.; Gratzl, O. 17beta-Estradiol reduces cortical lesion size in the glutamate excitotoxicity model by enhancing extracellular lactate: A new neuroprotective pathway. *Brain Res.* **2001**, *901*, 230–236.
146. DeGiorgio, L.A.; Attardi, B.; Shimizu, Y.; Ogata, M.; Volpe, B.T. 17Beta-estradiol treatment retards excitotoxic delayed degeneration in substantia nigra reticulata neurons. *Brain Res.* **2002**, *936*, 15–20.
147. Ritz, M.F.; Schmidt, P.; Mendelowitsch, A. 17Beta-estradiol effect on the extracellular concentration of amino acids in the glutamate excitotoxicity model in the rat. *Neurochem. Res.* **2002**, *27*, 1677–1683.
148. Ritz, M.F.; Schmidt, P.; Mendelowitsch, A. Acute effects of 17beta-estradiol on the extracellular concentration of excitatory amino acids and energy metabolites during transient cerebral ischemia in male rats. *Brain Res.* **2004**, *1022*, 157–163.
149. Zhang, H.; Xie, M.; Schools, G.P.; Feustel, P.F.; Wang, W.; Lei, T.; Kimelberg, H.K.; Zhou, M. Tamoxifen mediated estrogen receptor activation protects against early impairment of hippocampal neuron excitability in an oxygen/glucose deprivation brain slice ischemia model. *Brain Res.* **2009**, *1247*, 196–211.
150. Zhang, F.; Yin, W.; Chen, J. Apoptosis in cerebral ischemia: Executional and regulatory signaling mechanisms. *Neurol. Res.* **2004**, *26*, 835–845.

151. Niizuma, K.; Yoshioka, H.; Chen, H.; Kim, G.S.; Jung, J.E.; Katsu, M.; Okami, N.; Chan, P.H. Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. *Biochim. Biophys. Acta* **2010**, *1802*, 92–99.
152. Broughton, B.R.; Reutens, D.C.; Sobey, C.G. Apoptotic mechanisms after cerebral ischemia. *Stroke* **2009**, *40*, e331–339.
153. Estus, S.; Tucker, H.M.; van Rooyen, C.; Wright, S.; Brigham, E.F.; Wogulis, M.; Rydel, R.E. Aggregated amyloid-beta protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction. *J. Neurosci.* **1997**, *17*, 7736–7745.
154. Estus, S.; Zaks, W.J.; Freeman, R.S.; Gruda, M.; Bravo, R.; Johnson, E.M., Jr. Altered gene expression in neurons during programmed cell death: Identification of c-jun as necessary for neuronal apoptosis. *J. Cell Biol.* **1994**, *127*, 1717–1727.
155. Rau, S.W.; Dubal, D.B.; Bottner, M.; Wise, P.M. Estradiol differentially regulates c-Fos after focal cerebral ischemia. *J. Neurosci.* **2003**, *23*, 10487–10494.
156. Kitagawa, K.; Matsumoto, M.; Tsujimoto, Y.; Ohtsuki, T.; Kuwabara, K.; Matsushita, K.; Yang, G.; Tanabe, H.; Martinou, J.C.; Hori, M.; Yanagihara, T. Amelioration of hippocampal neuronal damage after global ischemia by neuronal overexpression of BCL-2 in transgenic mice. *Stroke* **1998**, *29*, 2616–2621.
157. Alkayed, N.J.; Goto, S.; Sugo, N.; Joh, H.D.; Klaus, J.; Crain, B.J.; Bernard, O.; Traystman, R.J.; Hurn, P.D. Estrogen and Bcl-2: Gene induction and effect of transgene in experimental stroke. *J. Neurosci.* **2001**, *21*, 7543–7550.
158. Krajewski, S.; Mai, J.K.; Krajewska, M.; Sikorska, M.; Mossakowski, M.J.; Reed, J.C. Upregulation of bax protein levels in neurons following cerebral ischemia. *J. Neurosci.* **1995**, *15*, 6364–6376.
159. Dubal, D.B.; Shughrue, P.J.; Wilson, M.E.; Merchenthaler, I.; Wise, P.M. Estradiol modulates bcl-2 in cerebral ischemia: A potential role for estrogen receptors. *J. Neurosci.* **1999**, *19*, 6385–6393.
160. Jover, T.; Tanaka, H.; Calderone, A.; Oguro, K.; Bennett, M.V.; Etgen, A.M.; Zukin, R.S. Estrogen protects against global ischemia-induced neuronal death and prevents activation of apoptotic signaling cascades in the hippocampal CA1. *J. Neurosci.* **2002**, *22*, 2115–2124.
161. Pike, C.J. Estrogen modulates neuronal Bcl-xL expression and beta-amyloid-induced apoptosis: Relevance to Alzheimer's disease. *J. Neurochem.* **1999**, *72*, 1552–1563.
162. Singer, C.A.; Rogers, K.L.; Dorsa, D.M. Modulation of Bcl-2 expression: A potential component of estrogen protection in NT2 neurons. *Neuroreport* **1998**, *9*, 2565–2568.
163. Kandouz, M.; Siromachkova, M.; Jacob, D.; Chretien Marquet, B.; Therwath, A.; Gompel, A. Antagonism between estradiol and progestin on Bcl-2 expression in breast-cancer cells. *Int. J. Cancer* **1996**, *68*, 120–125.
164. Yune, T.Y.; Park, H.G.; Lee, J.Y.; Oh, T.H. Estrogen-induced Bcl-2 expression after spinal cord injury is mediated through phosphoinositide-3-kinase/Akt-dependent CREB activation. *J. Neurotrauma* **2008**, *25*, 1121–1131.
165. Fan, L.; Pandey, S.C.; Cohen, R.S. Estrogen affects levels of Bcl-2 protein and mRNA in medial amygdala of ovariectomized rats. *J. Neurosci. Res.* **2008**, *86*, 3655–3664.

166. Sharma, K.; Mehra, R.D. Long-term administration of estrogen or tamoxifen to ovariectomized rats affords neuroprotection to hippocampal neurons by modulating the expression of Bcl-2 and Bax. *Brain Res.* **2008**, *1204*, 1–15.
167. Gollapudi, L.; Oblinger, M.M. Estrogen and NGF synergistically protect terminally differentiated, ERalpha-transfected PC12 cells from apoptosis. *J. Neurosci. Res.* **1999**, *56*, 471–481.
168. Honda, K.; Shimohama, S.; Sawada, H.; Kihara, T.; Nakamizo, T.; Shibasaki, H.; Akaike, A. Nongenomic antiapoptotic signal transduction by estrogen in cultured cortical neurons. *J. Neurosci. Res.* **2001**, *64*, 466–475.
169. Lee, S.Y.; Andoh, T.; Murphy, D.L.; Chiueh, C.C. 17Beta-estradiol activates ICI 182,780-sensitive estrogen receptors and cyclic GMP-dependent thioredoxin expression for neuroprotection. *FASEB J.* **2003**, *17*, 947–948.
170. Zhang, Y.; Bhavnani, B.R. Glutamate-induced apoptosis in primary cortical neurons is inhibited by equine estrogens via down-regulation of caspase-3 and prevention of mitochondrial cytochrome c release. *BMC Neurosci.* **2005**, *6*, 13.
171. Teixeira, C.; Reed, J.C.; Pratt, M.A. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. *Cancer Res.* **1995**, *55*, 3902–3907.
172. Funakoshi, T.; Yanai, A.; Shinoda, K.; Kawano, M.M.; Mizukami, Y. G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. *Biochem. Biophys. Res. Commun.* **2006**, *346*, 904–910.
173. Simpkins, J.W.; Wang, J.; Wang, X.; Perez, E.; Prokai, L.; Dykens, J.A. Mitochondria play a central role in estrogen-induced neuroprotection. *Curr. Drug. Targets CNS Neurol. Disord.* **2005**, *4*, 69–83.
174. Hansen, T.M.; Moss, A.J.; Brindle, N.P. Vascular endothelial growth factor and angiopoietins in neurovascular regeneration and protection following stroke. *Curr. Neurovasc. Res.* **2008**, *5*, 236–245.
175. Garcia-Segura, L.M.; Azcoitia, I.; DonCarlos, L.L. Neuroprotection by estradiol. *Prog. Neurobiol.* **2001**, *63*, 29–60.
176. Jones, K.J. Gonadal steroids as promoting factors in axonal regeneration. *Brain Res. Bull.* **1993**, *30*, 491–498.
177. Garcia-Segura, L.M.; Sanz, A.; Mendez, P. Cross-talk between IGF-I and estradiol in the brain: Focus on neuroprotection. *Neuroendocrinology* **2006**, *84*, 275–279.
178. Duenas, M.; Luquin, S.; Chowen, J.A.; Torres-Aleman, I.; Naftolin, F.; Garcia-Segura, L.M. Gonadal hormone regulation of insulin-like growth factor-I-like immunoreactivity in hypothalamic astroglia of developing and adult rats. *Neuroendocrinology* **1994**, *59*, 528–538.
179. Jezierski, M.K.; Sohrabji, F. Region- and peptide-specific regulation of the neurotrophins by estrogen. *Brain Res. Mol. Brain Res.* **2000**, *85*, 77–84.
180. Gibbs, R.B. Treatment with estrogen and progesterone affects relative levels of brain-derived neurotrophic factor mRNA and protein in different regions of the adult rat brain. *Brain Res.* **1999**, *844*, 20–27.

181. Sohrabji, F.; Miranda, R.C.; Toran-Allerand, C.D. Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11110–11114.
182. Dhandapani, K.M.; Wade, F.M.; Mahesh, V.B.; Brann, D.W. Astrocyte-derived transforming growth factor-{beta} mediates the neuroprotective effects of 17{beta}-estradiol: Involvement of nonclassical genomic signaling pathways. *Endocrinology* **2005**, *146*, 2749–2759.
183. Mueller, M.D.; Vigne, J.L.; Minchenko, A.; Lebovic, D.I.; Leitman, D.C.; Taylor, R.N. Regulation of vascular endothelial growth factor (VEGF) gene transcription by estrogen receptors alpha and beta. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 10972–10977.
184. El-Ashry, D.; Chrysogelos, S.A.; Lippman, M.E.; Kern, F.G. Estrogen induction of TGF-alpha is mediated by an estrogen response element composed of two imperfect palindromes. *J. Steroid Biochem. Mol. Biol.* **1996**, *59*, 261–269.
185. Ferreira, A.; Caceres, A. Estrogen-enhanced neurite growth: Evidence for a selective induction of Tau and stable microtubules. *J. Neurosci.* **1991**, *11*, 392–400.
186. Singh, M.; Setalo, G., Jr.; Guan, X.; Frail, D.E.; Toran-Allerand, C.D. Estrogen-induced activation of the mitogen-activated protein kinase cascade in the cerebral cortex of estrogen receptor-alpha knock-out mice. *J. Neurosci.* **2000**, *20*, 1694–1700.
187. Mendez, P.; Azcoitia, I.; Garcia-Segura, L.M. Estrogen receptor alpha forms estrogen-dependent multimolecular complexes with insulin-like growth factor receptor and phosphatidylinositol 3-kinase in the adult rat brain. *Brain Res. Mol. Brain Res.* **2003**, *112*, 170–176.
188. Quesada, A.; Micevych, P.E. Estrogen interacts with the IGF-1 system to protect nigrostriatal dopamine and maintain motoric behavior after 6-hydroxdopamine lesions. *J. Neurosci. Res.* **2004**, *75*, 107–116.
189. Azcoitia, I.; Sierra, A.; Garcia-Segura, L.M. Neuroprotective effects of estradiol in the adult rat hippocampus: Interaction with insulin-like growth factor-I signalling. *J. Neurosci. Res.* **1999**, *58*, 815–822.
190. Jover-Mengual, T.; Zukin, R.S.; Etgen, A.M. MAPK signaling is critical to estradiol protection of CA1 neurons in global ischemia. *Endocrinology* **2007**, *148*, 1131–1143.
191. Traub, M.L.; De Butte-Smith, M.; Zukin, R.S.; Etgen, A.M. Oestradiol and insulin-like growth factor-1 reduce cell loss after global ischaemia in middle-aged female rats. *J. Neuroendocrinol.* **2009**, *21*, 1038–1044.
192. Shughrue, P.J.; Dorsa, D.M. Estrogen modulates the growth-associated protein GAP-43 (Neuromodulin) mRNA in the rat preoptic area and basal hypothalamus. *Neuroendocrinology* **1993**, *57*, 439–447.
193. Teter, B.; Harris-White, M.E.; Frautschy, S.A.; Cole, G.M. Role of apolipoprotein E and estrogen in mossy fiber sprouting in hippocampal slice cultures. *Neuroscience* **1999**, *91*, 1009–1016.
194. Scoville, S.A.; Bufton, S.M.; Liuzzi, F.J. Estrogen regulates neurofilament gene expression in adult female rat dorsal root ganglion neurons. *Exp. Neurol.* **1997**, *146*, 596–599.

195. Salom, J.B.; Burguete, M.C.; Perez-Asensio, F.J.; Torregrosa, G.; Alborch, E. Relaxant effects of 17-beta-estradiol in cerebral arteries through Ca(2+) entry inhibition. *J. Cereb. Blood Flow Metab.* **2001**, *21*, 422–429.
196. Ono, H.; Sasaki, Y.; Bamba, E.; Seki, J.; Giddings, J.C.; Yamamoto, J. Cerebral thrombosis and microcirculation of the rat during the oestrous cycle and after ovariectomy. *Clin. Exp. Pharmacol. Physiol.* **2002**, *29*, 73–78.
197. Ospina, J.A.; Krause, D.N.; Duckles, S.P. 17Beta-estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase. *Stroke* **2002**, *33*, 600–605.
198. Ospina, J.A.; Duckles, S.P.; Krause, D.N. 17Beta-estradiol decreases vascular tone in cerebral arteries by shifting COX-dependent vasoconstriction to vasodilation. *Am. J. Physiol. Heart Circ. Physiol.* **2003**, *285*, H241–250.
199. Momoi, H.; Ikomi, F.; Ohhashi, T. Estrogen-induced augmentation of endothelium-dependent nitric oxide-mediated vasodilation in isolated rat cerebral small arteries. *Jpn. J. Physiol.* **2003**, *53*, 193–203.
200. Geary, G.G.; McNeill, A.M.; Ospina, J.A.; Krause, D.N.; Korach, K.S.; Duckles, S.P. Selected contribution: Cerebrovascular nos and cyclooxygenase are unaffected by estrogen in mice lacking estrogen receptor-alpha. *J. Appl. Physiol.* **2001**, *91*, 2391–2399; discussion 2389–2390.
201. McNeill, A.M.; Kim, N.; Duckles, S.P.; Krause, D.N.; Kontos, H.A. Chronic estrogen treatment increases levels of endothelial nitric oxide synthase protein in rat cerebral microvessels. *Stroke* **1999**, *30*, 2186–2190.
202. Geary, G.G.; Krause, D.N.; Duckles, S.P. Gonadal hormones affect diameter of male rat cerebral arteries through endothelium-dependent mechanisms. *Am. J. Physiol. Heart Circ. Physiol.* **2000**, *279*, H610–618.
203. Pelligrino, D.A.; Ye, S.; Tan, F.; Santizo, R.A.; Feinstein, D.L.; Wang, Q. Nitric-oxide-dependent pial arteriolar dilation in the female rat: Effects of chronic estrogen depletion and repletion. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 165–171.
204. Stirone, C.; Chu, Y.; Sunday, L.; Duckles, S.P.; Krause, D.N. 17Beta-estradiol increases endothelial nitric oxide synthase mRNA copy number in cerebral blood vessels: Quantification by real-time polymerase chain reaction. *Eur. J. Pharmacol.* **2003**, *478*, 35–38.
205. Geary, G.G.; Krause, D.N.; Duckles, S.P. Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries. *Am. J. Physiol.* **1998**, *275*, H292–300.
206. Geary, G.G.; Krause, D.N.; Duckles, S.P. Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. *Am. J. Physiol. Heart Circ. Physiol.* **2000**, *279*, H511–519.
207. Skarsgard, P.; van Breemen, C.; Laher, I. Estrogen regulates myogenic tone in pressurized cerebral arteries by enhanced basal release of nitric oxide. *Am. J. Physiol.* **1997**, *273*, H2248–2256.
208. Sobey, C.G.; Weiler, J.M.; Boujaoude, M.; Woodman, O.L. Effect of short-term phytoestrogen treatment in male rats on nitric oxide-mediated responses of carotid and cerebral arteries: Comparison with 17beta-estradiol. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 135–140.

209. Alkayed, N.J.; Harukuni, I.; Kimes, A.S.; London, E.D.; Traystman, R.J.; Hurn, P.D. Gender-linked brain injury in experimental stroke. *Stroke* **1998**, *29*, 159–165; discussion 166.
210. Krejza, J.; Siemkowicz, J.; Sawicka, M.; Szylak, A.; Kochanowicz, J.; Mariak, Z.; Lewko, J.; Spektor, V.; Babikian, V.; Bert, R. Oscillations of cerebrovascular resistance throughout the menstrual cycle in healthy women. *Ultrasound Obstet. Gynecol.* **2003**, *22*, 627–632.
211. McCullough, L.D.; Alkayed, N.J.; Traystman, R.J.; Williams, M.J.; Hurn, P.D. Postischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. *Stroke* **2001**, *32*, 796–802.
212. Watanabe, Y.; Littleton-Kearney, M.T.; Traystman, R.J.; Hurn, P.D. Estrogen restores postischemic pial microvascular dilation. *Am. J. Physiol. Heart Circ. Physiol.* **2001**, *281*, H155–160.
213. Coma, M.; Guix, F.X.; Uribesalgo, I.; Espuna, G.; Sole, M.; Andreu, D.; Munoz, F.J. Lack of oestrogen protection in amyloid-mediated endothelial damage due to protein nitrotyrosination. *Brain* **2005**, *128*, 1613–1621.
214. Radi, R. Nitric oxide, oxidants, and protein tyrosine nitration. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 4003–4008.
215. Wang, Q.; Santizo, R.; Baughman, V.L.; Pelligrino, D.A.; Iadecola, C. Estrogen provides neuroprotection in transient forebrain ischemia through perfusion-independent mechanisms in rats. *Stroke* **1999**, *30*, 630–637.
216. Carswell, H.V.; Anderson, N.H.; Morton, J.J.; McCulloch, J.; Dominiczak, A.F.; Macrae, I.M. Investigation of estrogen status and increased stroke sensitivity on cerebral blood flow after a focal ischemic insult. *J. Cereb. Blood Flow Metab.* **2000**, *20*, 931–936.
217. Shi, J.; Bui, J.D.; Yang, S.H.; He, Z.; Lucas, T.H.; Buckley, D.L.; Blackband, S.J.; King, M.A.; Day, A.L.; Simpkins, J.W. Estrogens decrease reperfusion-associated cortical ischemic damage: An MRI analysis in a transient focal ischemia model. *Stroke* **2001**, *32*, 987–992.
218. Rusa, R.; Alkayed, N.J.; Crain, B.J.; Traystman, R.J.; Kimes, A.S.; London, E.D.; Klaus, J.A.; Hurn, P.D. 17Beta-estradiol reduces stroke injury in estrogen-deficient female animals. *Stroke* **1999**, *30*, 1665–1670.
219. Choi, J.M.; Romeo, R.D.; Brake, W.G.; Bethea, C.L.; Rosenwaks, Z.; McEwen, B.S. Estradiol increases pre- and post-synaptic proteins in the CA1 region of the hippocampus in female rhesus macaques (Macaca mulatta). *Endocrinology* **2003**, *144*, 4734–4738.

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