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Minireview

Protective Effects of Estrogen and Selective Estrogen Receptor Modulators in the Brain[1]

Krishnan M. Dhandapani and Darrell W. Brann[2]

Institute of Molecular Medicine and Genetics, Program in Neurobiology, and Department of Neurology, Medical College of Georgia, Augusta, Georgia 30912

ABSTRACT

Within the last few years, there has been a growing interest in the neuroprotective effects of estrogen and the possible beneficial effects of estrogen in neurodegenerative diseases such as stroke, Alzheimer disease, and Parkinson disease. Here, we review the progress in this field, with a particular focus upon estrogen-induced protection from stroke-induced ischemic damage. The important issue of whether clinically relevant selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene and estrogen replacement therapy can exert neuroprotection is also addressed. Although the mechanism of estrogen and SERM neuroprotection is not clearly resolved, we summarize the leading possibilities, including 1) a genomic estrogen receptor-mediated pathway that involves gene transcription, 2) a nongenomic signaling pathway involving activation of cell signalers such as mitogen-activated protein kinases and/or phosphatidylinositol-3-kinase /protein kinase B, and 3) a nonreceptor antioxidant free-radical scavenging pathway that is primarily observed with pharmacological doses of estrogen. The role of other potential mediatory factors such as growth factors and the possibility of an astrocyte role in neuroprotection is also discussed.

estradiol, neuroendocrinology, steroid hormones

INTRODUCTION

Recently, there has been a growing interest in the actions and functions of 17β-estradiol (17β-E_2) and selective estrogen receptor modulators (SERMs) in the brain, particularly in whether they are neuroprotective for such neurodegenerative conditions as stroke, Alzheimer disease, and Parkinson disease. The concept that 17β-E_2 and SERMs (such as the clinically relevant tamoxifen and raloxifene) may be neuroprotective has only begun to gain momentum within the last decade, and the field is thus quite young. Nevertheless, 17β-E_2 has been shown in an impressive number of studies to be protective against a wide variety of death-inducing agents both in vitro and in vivo, and initial reports are beginning to appear of similar neuroprotective effects of SERMs.

Collateral support for a neuroprotective role of endogenous 17β-E_2 has also arisen from the observations of greater brain damage observed in males than in females and in ovariectomized (OVX) compared with intact female animals in ischemic stroke models [1–8]. As women age and enter menopause, they lose most of their ability to produce 17β-E_2 because of depletion of ovarian follicles, and 17β-E_2 blood levels in postmenopausal women thus are typically only 1% of those observed in normally cycling young women. This 17β-E_2-deplete state in postmenopausal women has been correlated with increased incidence of stroke, cognitive defects, hot flashes, mood changes, and early onset and severity of Alzheimer disease, although a causative relationship has not been firmly established. As the field of neurobiological and neuroprotective actions of estrogens has matured, it has approached a point where a review of the area would be beneficial. Hence, the goal of this article is to assess the state of the field of 17β-E_2 and SERM neuroprotection. To maintain focus, we concentrate primarily on ischemic stroke damage and 17β-E_2/SERM protection.

EVIDENCE FOR A NEUROPROTECTIVE ROLE FOR 17β-E_2 AND SERMs

17β-Estradiol

One of the first reports to hint at a possible sex steroid-based neuroprotection mechanism was published over a decade ago. In that study, female gerbils experienced a lower incidence and less severe brain damage following carotid artery occlusion than did male gerbils [9]. This gender difference in ischemic stroke damage was subsequently extended to the rat through studies that showed that intact adult female rats sustain lower mortality and less neuronal damage than do age-matched males following middle cerebral artery occlusion (MCAO) [2]. The possibility that the gender-based protection observed in females is due to an ovarian factor was suggested by studies in which ovariectomy was shown to eliminate the protective effect observed in females following cerebral ischemia [2]. Although there are many products secreted by the ovary, there is now abundant evidence that the major neuroprotective factor from the ovary responsible for the gender-based difference in ischemic stroke damage is 17β-E_2. Work by a large number of groups has shown that exogenous administration of 17β-E_2 dramatically reduces infarct volume fol-

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[2]Correspondence: Darrell W. Brann, Institute of Molecular Medicine and Genetics, Neurobiology Program, 1120 15th Street, Augusta, GA 30912. FAX: 706 721 8685; e-mail: dbrann@mail.mcg.edu

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lowing MCAO in OVX female rats [1, 3–6], in male rats [7], and in aged, reproductively senescent female rats [10], which represent a model of female menopause. The doses of 17β-E_2 used in these studies did not influence cerebral blood flow, implying that the neuroprotective effect of 17β-E_2 occurs directly at the level of the brain rather than involving the vasculature [6, 11]. Progesterone replacement in OVX rats did not give similar protection from MCAO-induced ischemic damage, as was observed with 17β-E_2 replacement [12].

Additional support for a neuroprotective role of 17β-E_2 has come from studies showing that serum 17β-E_2 levels are inversely correlated with ischemic stroke damage in female rats and from the observation that female animals treated with an antiestrogen compound, ICI182,780, have significantly enhanced stroke infarct size as compared with vehicle-treated female rats [13, 14]. There are also recent reports that the most frequently prescribed estrogen replacement hormones in the United States, conjugated equine estrogens, are neuroprotective against neuronal cell death induced by β-amyloid(25–35), hydrogen peroxide, and glutamate and induce neurite outgrowth in cortical, hippocampal, and basal forebrain neurons [15, 16]. This finding raises the exciting possibility that estrogen replacement therapy may also have beneficial neurotrophic and neuroprotective effects on the brain.

Selective Estrogen Receptor Modulators

Although estrogen replacement therapy is widely prescribed, it can have certain disadvantages because of its multiple effects in the body. For instance, 17β-E_2 can have undesired stimulatory effects on the breast and uterus, and thus estrogen replacement therapy has been associated with an increased risk of developing breast and uterine cancers. In an attempt to circumvent the limitations of estrogen replacement therapy, there has been intense interest in the development and therapeutic use of nonsteroidal SERMs. Although an ideal SERM has yet to be developed, theoretically it would exhibit antagonist activity in the breast and uterus and agonist activity in the cardiovascular system, bone, and brain. Of the SERMs available today, tamoxifen and raloxifene are approved by the Food and Drug Administration for the treatment/prevention of breast cancer and osteoporosis, respectively. Recently, several laboratories have explored whether these clinically relevant SERMs or analogues thereof could exert neuroprotective actions in animal models of cerebral ischemia. In one study, pretreatment with LY353381.HCl, a raloxifene analogue, protected the caudate-putamen region of the brain of OVX female rats in an ischemia-reperfusion model of ischemic stroke [17]. The effect of LY353381.HCl was independent of cerebral blood flow changes, indicating a potential direct neuroprotective effect of this SERM in the brain. Tamoxifen also appears to be neuroprotective. In recent work [18–20], it significantly reduced infarct size in permanent MCAO and transient occlusion/reperfusion models of cerebral ischemia. Like the raloxifene analogue, the protective effect of tamoxifen was independent of cerebral blood flow changes, indicating a potential direct neuroprotective effect of this SERM in the brain. Tamoxifen was also recently shown to protect the striatum against 1-methyl-4-phenylpyridine-induced toxicity, suggesting that its protective abilities may extend to regions of the brain that are known to be affected in Parkinson disease [21]. In addition to exerting neuroprotection, there is increasing evidence that SERMs may also be neurotrophic. For instance, tamoxifen increased synaptic density in the hippocampus of OVX rats, and raloxifene enhanced neurite outgrowth of PC12 cells in vitro [22, 23]. These studies suggest that SERMs can exert agonist effects in the brain and that clinically relevant SERMs such as tamoxifen and raloxifene may have heretofore unrecognized but potentially clinically important neuroprotective and neurotrophic effects on the brain.

Several mechanisms have been proposed to explain how 17β-E_2 and SERMs may protect the brain (Fig. 1). These proposals include 1) a genomic estrogen receptor (ER)-mediated mechanism, 2) a nongenomic mechanism involving mitogen-activated protein kinase (MAPK) and/or phosphotidylinositol-3-kinase (PI3K) signaling, and 3) a receptor-independent antioxidant free-radical scavenging mechanism. The first two mechanisms, genomic and nongenomic signaling, can be observed at low physiological doses of 17β-E_2. In contrast, the third mechanism (antioxidant free-radical scavenging) is only observed at high nonphysiological doses of 17β-E_2. Thus, the first two mechanisms are thought to underlie physiological neuroprotection by 17β-E_2, whereas the third mechanism may come into play if high pharmacological doses of estrogen are used. These three potential mechanisms and the evidence supporting each are discussed below.

EVIDENCE FOR AN ER-MEDIATED GENOMIC ACTIVATION MECHANISM

Genomic Activation

Evidence suggesting a genomic mechanism of action for 17β-E_2 in its neuroprotective effects has arisen from several areas. First, Wise and coworkers [3] showed that pretreatment is required to observe the neuroprotective effect of 17β-E_2 in the in vivo MCAO cerebral ischemia model. In these studies, acute pretreatment or administration of 17β-E_2 at the time of MCAO failed to protect against brain injury, whereas 24 h of pretreatment was protective. A similar requirement for 24-h pretreatment to observe the 17β-E_2 protective effect has also been reported in rat cortical neurons and in rat cortical explants in vitro [24, 25]. These studies suggest a genomic mechanism of 17β-E_2 action in protecting the brain; 17β-E_2 at physiological doses primarily exerts effects through activation of an ER and subsequently gene transcription. The precise genes regulated by 17β-E_2 to exert its neuroprotective effect have not been identified, but 17β-E_2 increases the expression of the antiapoptotic gene, *bcl-2*, in the ischemic penumbra following MCAO [26]. 17β-Estradiol also increases *bcl-2* levels in human NT2 neurons in vitro [27]. The role of *bcl-2* in protection of the brain against ischemic stroke damage was recently confirmed by studies demonstrating a decreased infarct volume following MCAO in OVX transgenic mice overexpressing the *bcl-2* gene as compared with wild-type OVX female mice [28].

ER Mediation

The requirement for 24-h pretreatment and the activation of gene expression by 17β-E_2 seem to suggest a role for the ER in mediation of the neuroprotective effects of 17β-E_2. In support of this contention, Sawada et al. [14] showed that administration of the potent ER antagonist, ICI182,780, dramatically increased infarct size in intact female rats following MCAO. Furthermore, ICI182,780 also blocked neuroprotection by low physiological doses of 17β-E_2 (1, 10,

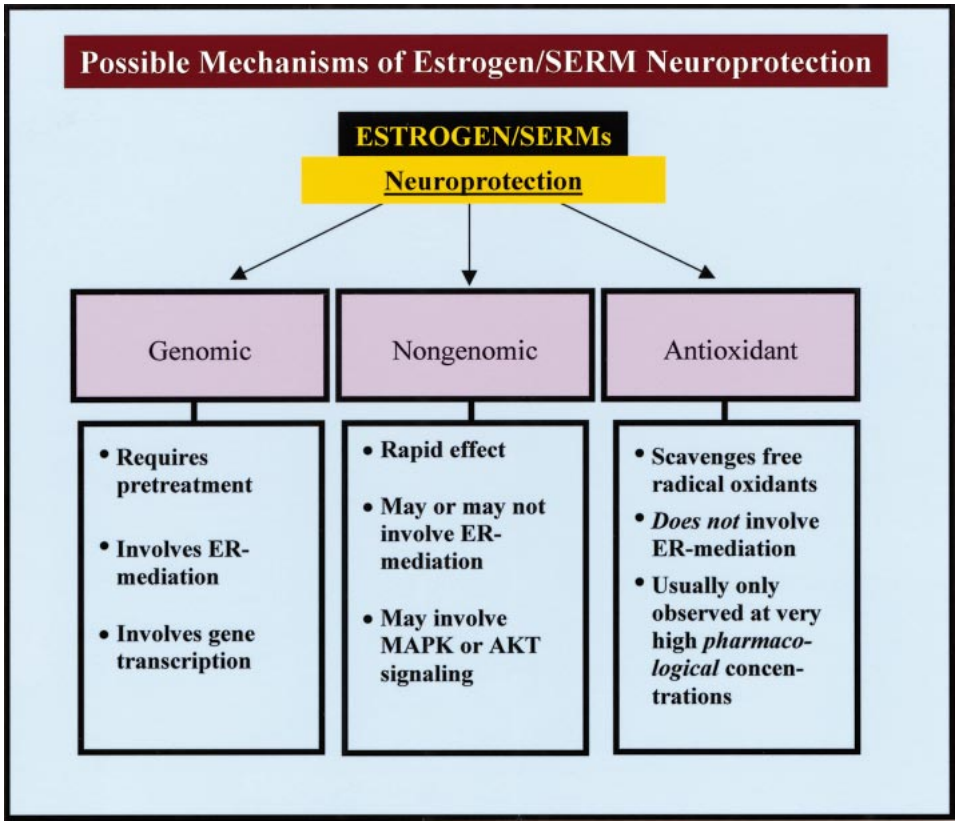

FIG. 1. Possible mechanisms of estrogen/SERM neuroprotection. See text for full description. SERM, Selective estrogen receptor modulator; ER, estrogen receptor; MAPK, mitogen activated protein kinase; AKT, protein kinase B.

and 30 nM) in cortical explant cultures in vitro [25]. Two ER isoforms have been identified to date, ERα and ERβ. Both ERs are expressed in the adult brain, and either one or both could mediate the neuroprotection by 17β-E_2 [29, 30]. To elucidate the individual ER subtype involved in 17β-E_2-mediated neuroprotection in vivo, specific ER knockout mice models have been used, which include ERα knockout mice (ERKO) and ERβ knockout mice (βERKO). The results so far with these transgenic animal models have been somewhat contradictory.

Evidence supporting a possible mediatory role for ERα in estrogen neuroprotection has come from the work of Dubal et al. [26], who demonstrated that following MCAO in rats, ERα mRNA was highly upregulated in the ischemic penumbra in the presence or absence of 17β-E_2. Furthermore, 17β-E_2 failed to protect the brain of OVX ERKO mice [31]. In contrast, 17β-E_2 protected the brain of OVX βERKO mice in a manner similar to that observed in OVX wild-type mice [31]. These findings, coupled with the upregulation of the ERα gene following MCAO, suggest a critical role for ERα in the protection of the brain by 17β-E_2. However, these findings are in contrast to those of Sampei et al. [32], who failed to demonstrate a critical role for ERα in neuroprotection following ischemic stroke. Sampei et al. found that intact wild-type and ERKO mice sustained a similar infarct volume following ischemic stroke, leading them to conclude that ERα is not necessary for protection by endogenous 17β-E_2. However, intact ERKO mice reportedly have dramatically higher serum levels of E_2 than do wild-type controls, which may complicate interpretations [33]. For instance, increased estrogen levels in the intact ERKO mice may allow a compensatory activation of the ERβ subtype and/or exert a pharmacological ER-independent antioxidant effect.

Recent work has also implicated a possible role for ERβ in neuroprotection. For instance, Wang et al. [34] showed that βERKO mice experienced a significant loss of neurons coupled with astroglia proliferation in the cerebral cortex. The loss of neurons was most pronounced between the somatosensory and parietal cortex. In addition, the size of the brain of 2-yr-old, but not 2-mo-old, βERKO mice was dramatically reduced compared with wild-type controls, suggesting that the loss of neurons occurs throughout the life of the animal rather than exclusively during the process of development. This finding contrasts with the observations in the ERKO mouse, which lacks gross brain morphological changes. As a whole, these data imply that ERβ is important in the basal maintenance of neuronal survival. However, it is unclear what role if any ERβ has in protecting neurons following brain injury. In a recent study by Dubal et al. [31], 17β-E_2 was still protective against MCAO-induced stroke damage in OVX βERKO mice, suggesting that ERβ may not mediate the protective effects of 17β-E_2. Likewise, infarct size in wild-type versus βERKO intact mice has also been reported not to differ, but the caveats about elevated 17β-E_2 levels in intact βERKO mice must be considered when interpreting these findings. As a whole, the current data suggests that both ERα and ERβ may exert neuroprotection in the brain. Although definitive roles cannot be assigned, the findings suggest a possible role for ERβ in basal neuroprotection and for ERα in injury-induced 17β-E_2-mediated protection. Further studies are needed to clarify the role(s) of the individual ER isoforms in 17β-E_2-mediated neuroprotection.

EVIDENCE FOR A NONGENOMIC MECHANISM FOR 17β-E_2 NEUROPROTECTION

Although a genomic mechanism of action for 17β-E_2 neuroprotection has been implicated in many studies, it may

not be the only mechanism utilized by 17β-E_2 to exert its neuroprotective effect. There is a growing body of evidence to suggest that a rapid induction of cell signaling pathways may play a crucial role in the actions of 17β-E_2 in the brain. Specifically, two signaling pathways, the MAPK pathway and the PI3-K/protein kinase B (Akt) pathway have been implicated in 17β-E_2 actions in the brain and nervous tissue, including its neuroprotective actions [35–39].

MAPK Activation

Recent studies have shown that 17β-E_2 rapidly activates the MAPK pathway in primary neuronal cortical cultures and in organotypic cerebrocortical explant cultures [40–43]. In rat cortical neurons incubated in vitro, 17β-E_2 induced phosphorylation of MAPK within 30 min of exposure, an effect blocked by the ER antagonist ICI182,780 [40]. The possibility that activation of the MAPK pathway by 17β-E_2 is important for the neuroprotective actions of 17β-E_2 was suggested by the finding that PD98059, a specific MAPK inhibitor, blocked the neuroprotective effect of 17β-E_2 against glutamate excitotoxicity. Singh et al. [41] extended these observations to organotypic cerebrocortical explant cultures by showing that 17β-E_2 elicited phosphorylation of MAPK within 15 min, an effect that was sustained for 2 h. However, ICI182,780 failed to inhibit the 17β-E_2 effect in this model, suggesting that a novel ER subtype or a novel mode of action may mediate the MAPK regulatory effect of 17β-E_2. In support of this hypothesis, 17β-E_2 maintained the ability to induce ER activation in organotypic explant cultures derived from ERKO mice, implying ERα was not involved in this effect. Further, neither 16α-17β-iodo-17β-E_2, an ERα-specific ligand, nor genistein, an ERβ-selective ligand, elicited MAPK phosphorylation [42]. In addition to the potential neuroprotective effect of 17β-E_2-induced MAPK phosphorylation in the cerebral cortex, 17β-E_2 also was recently demonstrated to protect hippocampal neurons from excitotoxicity via the MAPK activation pathway [35, 44]. The protection of hippocampal neurons is of interest because of the postulated protective actions of 17β-E_2 in Alzheimer disease. Cyclic changes in 17β-E_2 have also recently been shown to be correlated with activation of brain MAPK, further suggesting a regulatory link between 17β-E_2 and the MAPK signaling pathway in the brain [45].

PI3-K/Akt Activation

PI3-K is an enzyme responsible for phosphorylation/activation of Akt, a serine kinase that has been implicated in a variety of models as a survival factor [46, 47]. Akt phosphorylates death signals such as BAD (Bcl-2 antagonist of cell death) and glycogen synthase kinase 3β, leading to their inactivation [47, 48]. In recent work in cultured rat cortical neurons, PI3-K was rapidly activated following treatment with low doses of 17β-E_2 [49, 50]. Phosphorylation of Akt, a downstream target of PI3-K, was increased as early as 15 min following the addition of 17β-E_2 and stayed elevated over basal levels up to 24 h following treatment. Activation of PI3-K was crucial for the protective effect of 17β-E_2 against glutamate excitotoxicity, and the 17β-E_2 protection was abolished by coadministration of the selective PI3-K inhibitor LY294002. The protection was also abolished by the coadministration of ICI182,780, suggesting that 17β-E_2 activates PI3-K/Akt through an ICI182,780-sensitive ER. However, ICI182,780 only partially attenuated the induction of Akt phosphorylation induced by 17β-E_2, implying that an ICI182,780-insensitive receptor may mediate PI3-K activation by 17β-E_2. 17β-Estradiol also phosphorylated Akt in mouse organotypic cerebral cortical explants in vitro [43]. Additionally, recent work has extended 17β-E_2 regulation of Akt to hippocampal neurons and shown that estrogen protection against β-amyloid-induced cell death in hippocampal neurons can be blocked by a PI3-K inhibitor [51]. Correlative studies with SERMs and Akt in the brain have not been performed, but the raloxifene analog LY117018 rapidly activated Akt in vascular endothelial cells, suggesting that a similar regulation in the brain by SERMs is possible [52].

The mechanism whereby 17β-E_2 and SERMs can regulate PI3-K/Akt is poorly understood. However, work in Chinese hamster ovary cells has shown that raloxifene activation of Akt occurs in cells expressing ERα but not in those expressing ERβ [52]. Simoncini et al. [53] recently demonstrated that ERα binds in a ligand-dependent fashion to the p85α regulatory subunit of PI3-K, leading the authors to suggest that there exists a nonnuclear estrogen signaling pathway involving direct interaction of ERα with PI3-K. Such a pathway would be consistent with recent reports that ERα can be localized at the cell membrane in addition to its well-known nuclear localization [54, 55]

EVIDENCE FOR AN ANTIOXIDANT MECHANISM FOR 17β-E_2 NEUROPROTECTION

Recent work suggests that 17β-E_2 at pharmacological concentrations (the micromolar range) can act as an antioxidant [56]. The antioxidant ability of 17β-E_2 may be mediated by the phenolic A ring of the steroid, which is a potent electron donor and free-radical scavenger and prevents lipid peroxidation-induced membrane damage [9, 56–60]. In support of a possible antioxidant protective effect of pharmacological estrogen doses, Simpkins et al. [1] recently reported that administration of pharmacological doses of 17β-E_2 up to 90 min following ischemic stroke in rats significantly reduced infarct volume. However, administration of physiological doses of 17β-E_2 within the same time frame failed to similarly protect the brain, suggesting a nongenomic mechanism of action for pharmacological estrogen protection. The pharmacological estrogen antioxidant effect can also be observed in vitro. Bae et al. [61] demonstrated that 17β-E_2 at pharmacological levels protected mouse cortical neuronal cultures from cell death from various insults, including serum deprivation, $FeCl_2$, excitotoxicity, and Aβ25–35. The protective ability was also observed with 17α-E_2, which has weak estrogenic activity but possesses antioxidant activity similar to that of 17β-E_2. A number of reports have now appeared in the literature confirming that pharmacological concentrations of 17β-E_2 attenuate β-amyloid-induced cell death through an antioxidant mechanism [57, 58, 62–65].

Despite the above evidence in favor of a receptor-independent mechanism of protection by 17β-E_2, this mechanism does not appear to account for the protection observed with physiological levels of 17β-E_2 in vivo. The antioxidant capability of 17β-E_2 is observed following administration of high, supraphysiological doses, far exceeding the levels found under physiological conditions. Additionally, some of the protective effects observed with high doses of 17β-E_2 are associated with vasodilation and increased blood flow. However, the protection of the brain following physiological 17β-E_2 administration in vivo is independent of cerebral blood flow changes [3, 9]. Thus, pharmacological

doses of 17β-E_2 may be an effective clinical therapy following ischemia stroke injury, but this mechanism most likely cannot explain the well-documented protection of the brain by physiological levels of 17β-E_2 in vivo.

OTHER MECHANISMS?

Although the above mechanisms represent the major focal areas of research into estrogen neuroprotection, other possible mechanisms exist and are being actively investigated by several laboratories. Preeminent among these are the possible involvement of growth factors in 17β-E_2 neuroprotection and the potential role of other cell types such as astrocytes. With respect to growth factors, there is increasing evidence of interactions between insulin-like growth factor I (IGF-I) and estrogen in the brain. For instance, the IGF-I receptor has been reported to be necessary for 17β-E_2 regulation of bcl-2 in the brain, and a synergistic effect has also been reported between 17β-E_2 and IGF-I in the regulation of Akt. The interaction of estrogen and IGF-I in brain function and neuroprotection was elegantly reviewed by Cardona-Gomez et al. [66]. With regard to astrocytes, it has been known for some time that astrocytes are important in protecting neurons from cell death [67–71]. We and others [72–76] have reported that astrocytes in various regions of the brain, including hypothalamic, cortical, and hippocampal astrocytes, express ERs, suggesting that brain astrocytes may be targets for 17β-E_2 action. 17β-Estradiol and tamoxifen can enhance release from brain astrocytes of transforming growth factor β, which is neuroprotective against a wide variety of death-inducing agents [69, 74, 77–81]. Thus, it has been hypothesized that 17β-E_2 protects neurons through both indirect and direct mechanisms, and the protection involves multiple cell types and mobilization of growth factors. This intriguing possibility will certainly be an important area for future investigation.

CONCLUSIONS AND FUTURE DIRECTIONS

An increasing amount of data suggests a neuroprotective role for 17β-E_2; however, the molecular mechanisms whereby 17β-E_2 exerts these actions are unclear. Several different signaling mechanisms probably act in concert to achieve the protection observed following neuronal injury. Future investigations will determine the role of various 17β-E_2-regulated pathways on the protection of neurons from injury. With the genomic revolution, the employment of gene chip arrays undoubtedly will aid in the elucidation of target genes and pathways regulated by 17β-E_2 and SERMs to provide neuroprotection. The possibility that other cell types, such as astrocytes, represent an intermediary of 17β-E_2-mediated neuroprotection will also be a fertile area for future research. Although studies of 17β-E_2 protection in the human brain are still controversial, many studies support a role for estrogen replacement therapy in the reduction of injury associated with neurodegenerative diseases. However, estrogen replacement therapy is not a viable option for the treatment of male patients because of numerous undesirable side effects. The results from animal model studies suggest that SERMs, like 17β-E_2, possess potent neuroprotective capability. Thus, current SERMs or newly designed SERMs may be protective and may circumvent the adverse side effects associated with estrogen replacement therapy. Future clinical studies to investigate the role of current and new SERMs in the reduction of risk and severity of neurodegeneration in humans certainly seem warranted. The investigation of estrogen neuroprotection has mushroomed in the past few years, and continued growth is expected because of high interest in the potential for treatment of a variety of important neurodegenerative diseases. The beneficial effects of SERMs on the brain that have been observed in the limited studies performed to date is exciting and undoubtedly will serve as a springboard for launching many more studies in the future both at the clinical and basic science level.

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