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17β-Estradiol as a Neuroprotective Agent

Katalin Prokai-Tatrai and Laszlo Prokai

Abstract

The pathophysiology of neurodegeneration in the central nervous system is complex and multifactorial in nature and yet to be fully understood. Broad-spectrum neuroprotective agents with multiple mechanisms of action rather than a single druggable target are, therefore, highly desirable. The main human estrogen, 17β-estradiol, can also be considered a neurosteroid as it forms de novo in the central nervous system, and it possesses beneficial effects against practically all critical contributors to neurodegeneration to collectively thwart both the initiation and the progression of neuronal cell death. This chapter details the main aspects of the hormone’s genomic and non-genomic actions important to protect the highly vulnerably neurons of the central nervous system, as well as translational efforts to successfully realize its powerful neuroprotective potential in clinical setting while ensuring both therapeutic safety and efficacy.

Keywords: antioxidant, brain, bioprecursor prodrug, broad-spectrum neuroprotectant, brain-selective estrogen therapy, cell death, 10β,17β-dihydroxyestra-1,4-dien-3-one (DHED), estrogens, genomic and non-genomic estrogenic actions, neuroprotection, para-quinol, Prokai antioxidant cycle, stroke, translational research

1. Introduction

The steroid hormone 17β-estradiol (E2, Figure 1) is the main human estrogen that is not only involved in sexual maturation and reproduction but also has a myriad of important roles throughout the body affecting, for example, the cardiovascular system, lipid metabolism and brain health [1–4]. Therefore, E2 cannot only be considered as just a “female hormone.” In humans, the other two endogenously formed estrogens are estrone (E1, Figure 1) and the lesser-known estriol (E3, Figure 1) that is the 16-hydroxy derivate of E2 and formed mostly during gestation by the placenta. E1 becomes the predominant estrogen in women after...
menopause, when it is synthesized largely in subcutaneous fat from androstenedione. The unique structure of estrogens among steroids arises from the presence of the aromatic A-ring (Figure 1).

E2 is now also considered as one of the neurosteroids, as its regioselective local formation in the brain has been established [5–8]. Indeed, with today’s modern analytical instrumentations and using validated bioassays that are devoid of the limitations of immunoassays [9], brain E2 level even in ovariectomized animals (i.e., in animals without gonadal E2 source) can be measured [10, 11]. It has been hypothesized that this de novo central formation of E2 due to the lack of gonadal E2 sources, for example, as in case of ovariectomy, is essentially a compensatory mechanism to protect the estrogen-deprived brain that cannot receive the hormone from the circulation any more, although plasma estrogen levels do not directly correlate with that of brain [12, 13]. Additionally, de novo synthesis of E2 with a presumed role of neuroprotection in the developing mammalian brain has also been shown [14].

Independent of the loci of estrogen’s gonadal or extra-gonadal biosynthesis from cholesterol via a number of enzyme-catalyzed steps, the final process is the oxidation of the 10-methyl group of testosterone, followed by elimination and subsequent aromatization into E2 by aromatase. This step is the one that has been controlled by aromatase inhibitors in clinical practice to prevent the reoccurrence of estrogen-dependent malignancies [15].

Among estrogens and estrogenic compounds, E2 also is the best-known estrogen to be used as a powerful neuroprotective agent in various in vitro and preclinical animal models of neurodegeneration impacting the central nervous system (CNS) [16–20]. It is important to emphasize, though, that E2 has a large array of other beneficial effects in the CNS, including regulating body temperature, enhancing cognition and memory and ameliorating neuropsychiatric conditions in both females and males [7, 11, 21–23]. While the brain is undoubtedly the most frequently studied part of the CNS in the context of neuroprotection [24–26], the utility of E2 in protecting the eye (retina and optic nerve) [27–29] and spinal cord [30–32] have also been explored with promising outcomes.

Altogether, extensive basic science investigations brought about convincing data on the plethora of mechanisms by which E2 promotes neuronal survival and protects neurons against a wide variety of stressors addressing, thereby, practically all proposed critical contributors to neurodegeneration such as inflammation, oxidative stress, excitotoxicity and collapse of mitochondrial membrane potential. Clinical and epidemiological observations also suggest that in humans the better outcome after neurotrauma (e.g., traumatic brain and spinal cord injuries) in premenopausal females compared with age-matched men, at least in part, is due to the protective role of endogenous estrogens against neuronal injuries [33, 34].
2. Mechanistic overview of neuroprotection by estrogens

The broad-spectrum protective mechanism of E2 on injured neurons is the end result of well-orchestrated and synergistic combination of genomic and non-genomic actions of the hormone that allows for prevention of both the initiation and progression of neuronal cell death. This implicates a significant translational value for the hormone upon restricting its action to the CNS, as detailed in the following section.

2.1. Genomic pathways in E2 neuroprotection

Estrogen receptors (ERs) are expressed throughout the brain [35, 36] indicating their role in various CNS functions including neuroprotection. ER density is higher in the hypothalamus than in extrahypothalamic areas with overlapping expression of the two isoforms ERα and ERβ [35, 36]. In some brain regions, ERα or ERβ may also be co-localized in cells [37]. However, ERβ is highly expressed in the cortex [38] and hippocampus [39, 40]. Consequently, estrogen impacts the function of extrahypothalamic areas that are not involved in sex maturational and reproduction [41].

The two ERs have similar affinities to endogenous estrogens [42]. Just like many other members of the nuclear receptor superfamily of proteins, they elicit their genomic effect through gene transcription [43]. The sequence of the classical ligand-dependent genomic mechanism of estrogens’ neuroprotective action is summarized in Figure 2. After E2 (or in general, an estrogen) distributes into the neuron and reaches the nucleus, it is ligated to its cognate receptor. The ligated ERs form homo- (ERα/ERα or ERβ/ERβ) or heterodimers (ERα/ERβ) that bind to the estrogen-response element (ERE) of the nuclear DNA. Transcriptional activation is enabled by

![Figure 2. A simplified model for the ligand-dependent genomic mechanism of E2 neuroprotection. After E2 distributes into the neuron (A) and enters the nucleus, it binds to the cognate receptor followed by dimerization of the ligated ER that binds, together with co-regulators, to the nuclear DNA’s ERE, which results in the transcription of the corresponding gene (B). E2, ER, co-regulator (Co-r) and the nuclear DNA are symbolized by the filled steroid shape, rounded rectangles and elongated rectangle showing an ERE (shaded area), respectively.](http://dx.doi.org/10.5772/intechopen.72682)
a constitutively active and a ligand-dependent function located at the amino-terminus and in the carboxy-terminal ligand-binding domain of ERs, respectively [44]. The DNA-bound dimers recruit co-regulator proteins [45], which can participate in and also recruit many enzymatic and structural proteins permitting the modulation of chromatin structure to facilitate or block gene expression [46]. Additionally, ERE-independent mechanisms have also been shown [47].

Neuroprotective target genes for E2 that directly support vital neuronal functions include neurotrophic factors such as the brain-derived neurotrophic factor [48]. Additional target genes are involved in apoptosis to remove unneeded, damaged or potentially deleterious cells [49] playing thereby a central role in development and homeostasis. Through the apoptosis-associated genomic mechanism, E2 has been shown to rescue neurons through the induction of anti-apoptotic proteins such as Bcl-2 [50] or suppression of apoptotic proteins such as the Bcl-2-associated X protein [50, 51]. Induction of several gene products that maintain cellular architecture such as neurofilament, tau and microtubulin-associated proteins and many additional genomic pathways potentially associated with E2’s neuroprotection have also been described [52].

2.2. Modulation of intracellular signaling by E2

E2 also rapidly induces numerous cellular responses, which cannot be explained by a delayed genomic effect. ERs have been shown to be present in membrane compartments and in the cytoplasm [53, 54]. Specifically, ERα and ERβ are also found as homo or heterodimers at the cell membrane; they are membrane-associated but not actually embedded in the membrane. In addition, a G protein-coupled estrogen receptor (GPER) is localized mainly to intracellular membranes, including the endoplasmic reticulum and Golgi apparatus, under steady-state conditions [55]. G protein-coupled receptors such as GPER can actually signal from intracellular locations [56] and activation results in intracellular Ca²⁺-mobilization and synthesis of phosphatidylinositol 3,4,5-triphosphate in the nucleus, which could impact gene transcription indirectly. The mitogen-activated protein kinase (MAPK) cascade [57] and the cyclic-AMP-responsive element-binding protein signaling pathway [58, 59] also respond rapidly to E2 and have been implicated in its neuroprotective effects.

An E2-ER complex can also function through cytoplasmic signaling to provide neuroprotection [60]. For example, ERs have been shown to bind in a ligand-dependent manner to the p85 alpha regulatory subunit of phosphatidylinositol 3-kinase (PI3K) [61, 62]. Therefore, stimulation with E2 increases ER-associated PI3K activity, leading to the activation of protein kinase B/Akt and endothelial nitric oxide synthase. However, modulation of intracellular pathways may occur though the binding of E2 to ERs, or independently of ligand binding [63, 64]. A representative of the non-genomic mechanism involving the modulation of intracellular signaling through ERs is summarized schematically in Figure 3.

E2 has been proposed to influence neurotransmission directly by binding to various transmembrane ion channels [65, 66]. Localization of ERβ to the mitochondria has also been shown [67], implicating E2 in the regulation of mitochondrial structure and function in the brain [68]. In addition to estrogen potentially influencing bioenergetics through long-lasting nuclear-associated processes, rapid mitochondria-intrinsic signaling mechanisms that promote the
maintenance of this organelle’s integrity could contribute therefore to the neuroprotective action of E2. Essentially, the hormone could minimize mitochondrial dysfunctions, which accompany neurotrauma, aging and neurodegenerative diseases [69]. However, continued research is needed to fully understand molecular details about the apparently complex interactions between ERs and cellular signaling pathways in the context of neuroprotective mechanisms.

2.3. Anti-inflammatory action

The influence of E2 on neuroinflammation, a process commonly accompanying neurotrauma and neurodegenerative diseases [70–72], has been well established. Direct action on microglia and astrocytes (the cellular component of the neuroimmune system) and response to peripheral blood cells’ infiltration to the brain have been implicated as major contributors to the observed anti-inflammatory action of the hormone often impacting the cerebral vasculature [73, 74]. For example, E2 can suppress chemokine-mediated induction of the cyclooxygenase-2 (COX-2) pathway in cerebral blood vessels thereby preventing migration of microglia into the brain after an inflammatory challenge [75]. Although inflammatory processes in the brain are usually associated with microglia and astrocytes, expression of the COX-2 gene in neurons and possible mechanisms by which E2 down-regulates this inflammation-associated gene have been shown recently [76]. Specifically, ERβ contributes to neuronal expression of COX-2, and E2 leads to increased recruitment of histone deacetylase 1 (HDAC1), switch-independent 3A (Sin3A) and a concomitant reduction of nuclear factor-κB (NF-κB) p65 occupancy and histone 4 acetylation levels. The hormone also prevents the activation of microglia and the recruitment of peripheral monocytes induced by a toxic stimulus. This effect involves ERα activation and reduces the expression of pro-inflammatory mediators and E2 have also shown to prevent morphological changes occurring in microglia during inflammatory response [77]. Decrease of microglial superoxide production and phagocytic activity by both an ER- and

Figure 3. An example of non-genomic action of E2 involving interaction with intracellular signaling pathways through ERs. As in Figure 2, E2, ER and the nuclear DNA are represented by the filled steroid shapes, rounded rectangles and elongated rectangle indicating a regulated gene promoter (shaded area), respectively, while the ER-interacting protein (↔) such as PI3K is shown by a shaded oval.
MAPK-dependent pathway have also been reported among the anti-inflammatory effects of E2 [78]. In addition, the hormone inhibits pro-inflammatory gene expression by controlling intracellular localization of NF-κB [79].

2.4. Antioxidant effects

Oxidative stress-induced damage has been linked to brain aging [80], neurodegenerative diseases [81] and neurotrauma [25, 26]. From a long time, therapeutic antioxidant interventions have been proposed to reduce the detrimental impact of oxidative stress [82]. E2’s ER-independent antioxidant effects are mainly due to its ability to attenuate free-radical reactions [83], although indirect mechanisms such as up regulation of antioxidant enzymes [84, 85] and chelation of redox-active metal ions [86] have been reported. The neuroprotective effect of the hormone through direct oxidative stress reduction has been recognized in part by structure-activity relationship studies [87–89]. Acute E2 neuroprotection in ischemic brain [90] or against damage by ionizing radiation [91] may be largely conferred through antioxidant mechanisms.

The quintessential feature of estrogens as neuroprotective antioxidants is their phenolic A-ring [83, 92, 93]. Because of its lipophilicity, E2 concentrates in lipid-rich regions of the cell such as cellular membranes [94]. Therefore, it is likely that estrogens act in vivo as a highly localized antioxidant [83]. The mechanism of direct oxyradical-scavenging by E2 functioning as a phenolic antioxidant is shown schematically in Figure 4.

The process involves H-atom transfer that causes an interruption of free-radical chain reactions, such as lipid peroxidation (R = LOO, where L represents a lipid). Estrogens, indeed, reduce lipid peroxidation in cells and tissues of the CNS [95]. However, the chain-breaking reaction leaves behind a radical product (phenoxyl radical) whose fate has to be explained in consideration of an efficient antioxidant action observed both in vitro and in vivo. Indeed, phenolic antioxidants can be regenerated from the corresponding phenoxyl radicals by a reaction with ascorbic acid (vitamin C) [96] or through glutathione-dependent free-radical reductase [97]; therefore, a continuous antioxidant cycle is established by E2.

Figure 4. E2’s effect through the classical phenolic antioxidant mechanism. The solid arrows represent the chain-breaking H-atom transfer, such as lipid peroxidation, while the dashed arrows indicate the conversion of the E2-derived phenoxyl radical back to the phenolic compound by an endogenous reductant (AH) such as ascorbic acid or glutathione.
Our laboratory pioneered in recognizing a complementary novel neuroprotective antioxidant cycle that involves a para-quinol as a molecular intermediate of oxyradical scavenging and, then, NADPH-mediated enzyme-catalyzed reductive aromatization \[98–100\] to regenerate E2, as shown in Figure 5. We wish to name this previously unrecognized antioxidant cycle for simple phenolic antioxidants as the “Prokai antioxidant cycle.” The enzyme activity driving the reductive phase of the cycle is observed predominantly in neuronal tissue \[101\]. Beyond its mechanistic significance regarding oxidative stress-reducing effect, this discovery has prompted a strategy for brain-selective estrogen therapy using a prodrug approach detailed in the following section.

3. CNS-selective estrogen neurotherapy

The pathophysiology of neurodegeneration in the central nervous system is complex and multifactorial in nature \[102\]. Therefore, it is not surprising that an agent like E2 can provide robust protection against a myriad of neuronal insults owing to its broad-spectrum activity resulting from well-orchestrated genomic and non-genomic actions, as detailed in Section 2. The need for clinical therapeutic interventions that can be used to target multiple parallel mechanisms of neuronal injury has been repeatedly expressed \[101–103\]. We argue that, despite profound dichotomy between basic science and clinical studies, E2 is ideally suited to be developed as a broad-spectrum neuroprotectant if its action can be restricted to the CNS, that is, to the site of action to avoid undesirable peripheral hormonal burdens. Since neurotrauma triggers a cascade of biochemical events leading to further damages decreasing thereby the chance of appreciable functional recovery \[17, 20, 102, 103\], chronic pharmacotherapeutic interventions should be considered in the context of translational research. This, on the other hand, brings about critical considerations for safety and efficacy, which highlights the need for brain-selective (or in general CNS-selective) neurotherapy, considering both a preventative and a curative modality.

When estrogen neurotherapy is considered, however, one cannot ignore the (in)famous Women’s Health Initiative (WHI) study \[104\]. This was a placebo-controlled, randomized trial of hormone “replacement” therapy in postmenopausal women that indicated detrimental consequences of estrogen and progesterone supplantations, among others, for brain...
health, propagating thereby a dogma that all estrogens (and progestins) are “created equal.” The fact is that WHI did not use human hormones and, thus, did not study the effect of hormone replacement per se in aging women. On the contrary, conjugated equine estrogens (CEE) and a synthetic progestin were used for women with intact uterus. CEE is a complex mixture of over 60 different estrogens from pregnant mares’ urine, and it only contains a small amount of E2: the main constituents are the sulfate esters of B-ring saturated and unsaturated estrogens [105]. The pharmacokinetic and toxicology profiles of these non-human estrogens are different from those of E2; therefore, direct comparison between E2 and CEE is fundamentally unjustified [106, 107]. Accordingly, the beneficial central effects of E2, including robust neuroprotection based on clinical, epidemiological and basic science observations should not be undermined in view of the confusion brought about by the WHI studies.

Nevertheless, an E2-based neurotherapy cannot be realized in clinical settings until E2’s actions are restricted to the site of action assuring therapeutic safety and efficacy. Currently approved E2 dosage forms expose the entire body to the hormone through the circulation, potentially leading to detrimental side-effects including cardiovascular problems and the development of certain type of cancers upon chronic administration that is required for long-term neuroprotection and functional recovery after neurotrauma. Feminization (e.g., gynecomastia) is also a critical negative aspect of estrogen therapy, especially in case of children and males.

Early attempts to restrict E2’s actions to the brain upon systemic administration included the so-called chemical delivery system, which was conceptually a complex prodrug approach carrying a 1,4-dihydrotrigonellyl promoiety and is capable to usher the hormone through the blood brain barrier (BBB). Once in the brain, the prodrug is oxidized analogously to that of NADP(H) = NADP+, locking thereby the oxidized prodrug into the brain before it releases E2 [108]. This approach does result in significantly increased brain-enhanced delivery of the hormone compared to that of simple prodrugs of E2; however, it still results in sufficient increase in circulating E2 that can produce unwanted peripheral hormonal burdens [10, 109]. Prodrugs are inert precursors of their corresponding biologically active parent drugs and they traditionally carry auxiliary bioreversible “promoiety(ies)” that are removed enzymatically (rarely via chemical reaction, such as pH-dependent hydrolysis) in the body [108].

An important development in achieving a true CNS-selective estrogen therapy has been achieved by our laboratory [101] and was derived from our previous discovery of a novel antioxidant cycle for estrogens we call the Prokai antioxidant cycle for simple phenolic antioxidants [98–100], and detailed in Section 2. We recognized that 10β,17β-dihydroxyestr-1,4-dien-3-one (DHED, Figures 5 and 6), which is chemically a para-quinol (not to be mistaken for quinones involved in E2-induced carcinogenesis through redox cycling [99]), can be reductively rearomatized to the parent E2 and, thus, could serve as a bioprecursor prodrug for E2. The lesser-known bioprecursor prodrugs do not carry auxiliary promoiety(ies) [108] because the bioreversible chemical manipulation is carried out within the drug molecule itself [101, 110]. Therefore, creation of bioprecursor prodrugs, such as DHED, requires significantly greater innovation than that of simple prodrugs; moreover, potential toxicity issues that may arise from the release of the “promoiety(ies)” from simple prodrugs is eliminated with bioprecursor prodrugs. Indeed, we have established that CNS-specific and NADPH-dependent
dehydrogenase/reductase metabolized DHED to E2 (Figure 6), while prodrug activation did not occur in the periphery. This is an unprecedented and distinguishing feature of DHED in the context of translational research [101]. With a series of in vitro and in vivo studies, we showed that with DHED, for the first time, E2’s actions can be restricted to the brain independently of the route and duration of DHED administration and, therefore, it can be used (at least in preclinical settings) for the efficacious treatment of estrogen-responsive and centrally regulated maladies and injuries, including neurodegeneration brought about by ischemic stroke, without hormonal burdens for the rest of the body [101].

The transient middle cerebral artery occlusion (tMCAO) model followed by reperfusion is one of the most frequently used preclinical animal models for testing an agent for its ability to act as a neuroprotectant, that is, to reduce infarct volume and aid in functional recovery [20, 24, 101, 111]. As Figure 7 shows, a dose-dependent reduction of infarct volumes and neurological deficits was observed in DHED-treated animals. Moreover, about 10-times higher systemic E2 (i.e., the parent drug that is formed in the brain from DHED) dose was needed to achieve the same neuroprotection indicating the profound ability of the bioprecursor prodrug to enter into the brain from the circulation and, then, produce E2 within the brain, and only in

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**Figure 6.** Schematic illustration of DHED bioprecursor prodrug’s CNS-selective enzymatic metabolism to E2 via an NADPH-dependent reductase.

**Figure 7.** Dose-dependent (A) brain infarct volumes and (B) neurological deficit (ND) scores in rats treated with DHED 1 h before tMCAO followed by 24-h reperfusion [101]. The control groups received E2 (200 μg/kg, s.c., approximately representing ED50, equivalent to 50% of the maximum effect) or vehicle alone. ©Reproduced with permission by the American Association for the Advancement of Science.
the brain. In the context of translational research, it is noteworthy that the capacity to generate E2 from DHED is not lost in an injured brain, as neuroprotection was highly preserved post-stroke and, again, no hormonal exposure to the rest of the body was observed [101].

4. Conclusion

As neurotrauma and neurodegeneration in the CNS are complex and multifactorial in nature requiring therefore broad-spectrum therapeutic interventions, E2 is an attractive lead agent to address unmet medical needs in this field. The powerful antioxidant action of E2 against oxidative stress owing to its phenolic A-ring is unique among neurosteroids with potential neuroprotective roles; therefore, non-genomic mechanisms contribute significantly to the overall neuroprotection. This chapter presented an overview of our current knowledge on the well-orchestrated genomic and non-genomic events by which E2 could beneficially counteract the initiation and/or progression of neuronal cell death. However, there has been incongruency between basic science and clinical studies in terms of estrogen therapy impacting the brain because most researchers ignore the requirement to confine E2’s actions into the CNS upon systemic administration to ensure therapeutic safety and efficacy. We highlighted here a novel and unique bioprecursor prodrug approach our laboratory pioneered for brain-selective delivery of E2 without exposing the rest of the body to unwanted hormonal burden.

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Conflict of interest

The authors are inventors in the patents covering the use of 10β,17β-dihydroxyestra-1,4-dien-3-one (DHED) as a CNS-selective bioprecursor prodrug of 17β-estradiol and are co-founders of AgyPharma LLC with equity in the company that licensed the patents.

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References


[33] Datto JP, Yang J, Dietrich WD, Pearse DD. Does being female provide a neuroprotective advantage following spinal cord injury? Neural Regeneration Research. 2015;10:1533-1536. DOI: 10.4103/1673-5374.165213


[107] Zhang F, Chen Y, Pisha E, Shen L, Xiong Y, van Breemen RB, Bolton JL. The major metabolite of equilin, 4-hydroxyequilin, autoxidizes to an o-quinone which isomerizes to the potent cytotoxin 4-hydroxyequilenin-o-quinone. Chemical Research in Toxicology. 1999;12:204-213. DOI: 10.1021/tx980217v


