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Neuroprotection by Estrogen and Progesterone in Traumatic Brain Injury and Spinal Cord Injury

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Abstract: In recent years there has been a growing body of clinical and laboratory evidence demonstrating the neuroprotective effects of estrogen and progesterone after traumatic brain injury (TBI) and spinal cord injury (SCI). In humans, women have been shown to have a lower incidence of morbidity and mortality after TBI compared with age-matched men. Similarly, numerous laboratory studies have demonstrated that estrogen and progesterone administration is associated with a mortality reduction, improvement in neurological outcomes, and a reduction in neuronal apoptosis after TBI and SCI. Here, we review the evidence that supports hormone-related neuroprotection and discuss possible underlying mechanisms. Estrogen and progesterone-mediated neuroprotection are thought to be related to their effects on hormone receptors, signaling systems, direct antioxidant effects, effects on astrocytes and microglia, modulation of the inflammatory response, effects on cerebral blood flow and metabolism, and effects on mediating glutamate excitotoxicity. Future laboratory research is needed to better determine the mechanisms underlying the hormones' neuroprotective effects, which will allow for more clinical studies. Furthermore, large randomized clinical control trials are needed to better assess their role in human neurodegenerative conditions.

Keywords: Estrogen, neuroprotection, progesterone, spinal cord injury, traumatic brain injury.

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INTRODUCTION

Although estrogen and progesterone are gonadal steroid hormones, their actions are not restricted to reproductive organs and functions. Estrogen and progesterone exert effects on various organs systems including the central and peripheral nervous systems, and they play a significant role in growth and development [1, 2]. In the past three decades, there has been increasing evidence that estrogen and progesterone are neuroactive hormones. In recent years there has been a growing body of clinical evidence that has demonstrated neuroprotective effects of estrogen and progesterone. Studies have further proposed that estrogen and progesterone serve as general neurotrophic molecules that stabilize neuronal function, support neuronal viability, and, under certain conditions, prevent neuronal death [2-4]. Interestingly, epidemiological studies have observed gender differences in the incidence of a wide range of unrelated neurological and psychiatric disorders, and it has been suggested that estrogen and progesterone among other factors contribute to this difference [5]. The mechanisms of these neuroprotective effects, however, are poorly understood. The purpose here is to review both the preclinical and clinical literature that demonstrates the neuroprotective effects of sex hormones on the central nervous system, and to describe possible mechanisms for these effects.

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NEUROPROTECTIVE EFFECTS OF ESTROGEN

Estrogen's neuroprotective effects have been shown in numerous experimental studies, including animal models of acute global and focal cerebral ischemia, spinal cord injury (SCI), traumatic brain injury (TBI), and experimental autoimmune encephalitis.

Traumatic Brain Injury

Preclinical Animal Model Studies

Clinical reports are supported by experimental preclinical evidence of the neuroprotective effects of estrogen in animal models. These laboratory studies suggest estrogen-mediated benefits in mortality, functional outcomes, and histological improvement after TBI. Using a model of impact-acceleration closed head injury, two studies have demonstrated a 100% survival in female rats compared with a 75% survival in male rats [6, 7] (Table **1**). Furthermore, selective estrogen receptor (ER) agonists were implicated as a mediator of neuroprotection after acoustic TBI in mice [8].

Emerson and colleagues reported that estrogen administration before TBI improved neurological outcome in male rats, but paradoxically worsened neurological outcomes in female rats [9]. Zlotnik and colleagues further demonstrated that injection of premarin (mixture of estrogens) in male rats

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Table 1. Neuroprotective effects of estrogen after traumatic brain injury (animal models).

Study	Animal Model	Animal Species	Estrogen Treatment Protocol	Primary End Points	Conclusions
Neese, 2010 [6]	Fluid percussion injury	Rats	Z-Bisdehydrodoisynolic acid (300μg/0.1cc/100g body weight, sc) two hours after FPI for 48 hours	1. Behavioral testing: Coordination of limb movement, memory task 2. Lesion size	Z-BDDA improved behavioral testing. There were no changes in cortical lesion size or cell death.
Meltser, 2008 [8]	Acoustic trauma	Mice	ER alpha-selective agonist – propyl (1H) pyrazole-1,3,5-triyl-trisphenol (PTT)	Auditory function	PPT pre-treatment partially protected from hearing loss after trauma.
Emerson, 1993 [9]	Fluid percussion injury	Rats	17 beta-estradiol (144 micrograms/kg intraperitoneally), 4 hours prior to injury	1. Motor function 1w after injury 2. Mortality	Male rats had significant improvement of motor function and low mortality compared with female rats.
Zlotnik, 2012 [10]	Traumatic brain injury (weight-drop)	Rats	Premarin treatment (99 ± 36 μM/l) after TBI	1. Blood glutamate levels 2. Neurological outcome	Premarin treatment followed TBI decreased blood glutamate levels and demonstrated better neurologic recovery.
Soustiel, 2005 [11]	Parietal cortex contusion by dynamic cortical deformation	Rats	Estrogen treatment for 3 days after brain injury	Histological analysis of cortical lesion	Estrogen-treated animals had a significant reduction in apoptosis compared with control animals.
Hu, 2012 [22]	Spinal cord injury using a weight-drop injury approach	Rats	17β-estradiol administration at 15 mins and 24 hrs post injury	1. Functional recovery 2. Cell death	Estrogen treatment prevented spinal cord injury-induced apoptotic cell death and enhanced functional recovery after spinal cord injury.
Naderi, 2015 [14]	Traumatic brain injury (weight-drop)	Rats	Estrogen (E2) treatment (33.3 μg/Kg) injected 30 min after TBI	1. Brain edema 2. BBB disruption	E2 reduced brain edema and blood brain barrier disruption after TBI.
Kim, 2015 [17]	Lateral fluid percussion (LFP) at 24 h after craniectomy	Rats	Estrogen sulfate (E2-SO4) (1 mg/kg BW in 1 mL/kg BW) was intravenously administered at 1 h after TBI	1. Intracranial pressure (ICP), cerebral perfusion pressure (CPP), cerebral oxygenation (pbtO2), cerebral glycolysis. 2. Brain edema and lesion size	E2-SO4 significantly decreased ICP, and increased CPP and pbtO2. Brain edema in the treatment group was reduced compared with controls. Cerebral glycolysis in the injured brain region was increased.
Schaible, 2014 [15]	Traumatic brain injury (weight-drop)	Mice	Intraperitoneal injection of 2-methoxyestradiol (2ME2) 30 min after TBI.	Histological analysis of brain damage	Early 2ME2 administration reduced secondary brain damage, likely mediated by ubiquitin proteasomes.
Day, 2013 [16]	Lateral fluid percussion (LFP	Rats	17β-estradiol (E2) treatment	Histological analysis of brain damage	E2 significantly increased neuronal survival in the ipsilateral CA 2/3 region of the hippocampus, and decreased neuronal degeneration and apoptotic cell death in both the ipsilateral cortex and CA 2/3 region of the hippocampus in a dose-dependent manner.

after TBI resulted in a significant decrease in blood glutamate concentrations and improved neurological outcomes [10]. Furthermore, Neese and colleagues demonstrated the pretreatment with Z-BDDA (estrogenic seco-steroid) resulted in a significant improvement in learning and memory tests after moderate TBI [6].

Although Neese and colleagues failed to demonstrate a reduction in cell death in the cortical lesion zone [6], other studies have demonstrated that a significant reduction in neuronal apoptosis in rats pretreated with estrogen before TBI [11]. Recent studies have supported the neuroprotective role of 17-beta estradiol in decreasing cell death in the pericontusional zone after TBI, as well as the potential neuroprotective effects of 17-alpha estradiol in an *in vivo* model of early TBI to the immature brain of male and female rats [12, 13]. Recently, several laboratory studies demonstrated a reduction in brain edema, blood-brain barrier (BBB)

disruption, and an improvement in neuronal survival when estrogen was administered after TBI [14-16] (Table **1**). Furthermore, Kim *et al.* [17] showed significant reduction in intracranial pressure (ICP) with an elevation in cerebral perfusion pressure (CPP), partial pressure of oxygen in the brain, and rate of cerebral glycolysis in the injured region of the brain.

Human Studies

Clinical reports have suggested that severe TBI is associated with some degree of hypopituitarism [18]. In one study, 116 adult patients with severe TBI were followed for 6 months after TBI [18]. In the first seven days after TBI, there is a significant decline in blood hormone concentrations of estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) [18]. This reduction in hormone levels was followed by a reduction in the acute hormone stress response and a depression of cognitive function [18]. These findings correlated with previously published observational clinical reports that female humans had both lower mortality rates and better functional outcomes after TBI compared with men [1, 19-21] (Table **3**). A clinical review of more than 72,000 patients in the 5-year cohort of the National Trauma Database demonstrated that women older than 45 year and postmenopausal women (older than 55 years) had significantly lower mortality and morbidity after moderate to severe TBI compared with age-matched men [20].

Spinal Cord Injury

There is evidence to suggest that estrogen has neuroprotective effects in the context of SCI. Estrogen treatment in SCI was shown to prevent apoptosis of neurogenic cells and improve its functional recovery in rats [22]. Similarly, estrogen treatment prevented SCI-induced apoptotic cell death and enhanced functional recovery after spinal cord injury [23]. Furthermore, pretreatment with an ER agonist attenuated TNF alpha-induced apoptosis in spinal motoneurons [8, 24]. To date, no clinical studies have investigated the neuroprotective effects of estrogen after SCI.

NEUROPROTECTIVE EFFECTS OF PROGERSTERONE

In contrast to estrogen, the neuroprotective effects of progesterone have been more extensively studied in models of TBI and SCI than in models of ischemia. Moreover, the neuroprotective role of progesterone has also been studied in laboratory models of neurodegenerative CNS diseases, including Alzheimer's disease.

Traumatic Brain Injury

Preclinical Animal Model Studies

Similar to estrogen, experimental laboratory data suggests that progesterone also has neuroprotective effects in the context of TBI [7, 25, 26] (Table **2**). In three independent studies, progesterone administration consistently and significantly decreased brain edema 24-48 hours post-TBI in both male and female rats compared to vehicle-treatment animals [27-29]. When progesterone treatment was initiated as late as 24h after TBI, it was effective in decreasing brain edema [30]. Brain edema reduction strongly correlated with plasma progesterone concentrations [29]. However, while these studies examined the extent of brain edema as a measure of neuroprotection, neither neurological outcome nor mortality rates were assessed.

It is important to emphasize that while the traumatic lesions in the frontal cortex were similar in size, female rats had less ventricular enlargement and brain edema [31]. The extent of brain edema is clinically significant because of its serious consequences, including impaired cerebral blood flow and neuronal loss, and subsequent secondary neuronal death [32-35]. Moreover, brain edema to a significant extent is associated with an increase in extracellular brain glutamate. The excessive glutamate release after TBI may be an important laboratory marker of severity of brain injury [36]. Interestingly, previously published studies showed that blood concentrations of glutamate and progesterone are inversely correlated [37, 38].

Roof and colleagues demonstrated in male rats that treatment with progesterone 1h after TBI improved spatial navigation and reduced thalamic neuronal loss after 21 days [28]. Moreover, there is evidence that progesterone administration results in a decrease in TBI-induced diffuse axonal injury [39]. Nudi *et al.* [40] demonstrated better functional recovery after TBI where progesterone treatment was followed with embryonic neural stem cells (eNSC) transplantation. Progesterone treatment after TBI significantly reduced post-injury astrocyte and microglia inflammatory responses, and reduced the pericontusional lesion and BBB macromolecular leakage [41-44] (Table **2**).

Human Studies

The promising laboratory data has resulted in the performance of randomized clinical trials, in which progesterone treatment after moderate TBI in humans was studied [45-47] (Table **3**). Wright *et al.* [46] treated 77 patients with intravenous progesterone during first 72 hours after moderate to severe TBI (Glasgow Coma Scale [GCS] between 4 and 12), and showed a reduction in mortality rate at 30 days and improved Glasgow Outcome Scale score compared with the placebo group. Furthermore, there were no any adverse side effects during the treatment protocol. Xiao and colleagues observed decreased mortality rates and improved neurological outcomes after a six-month follow up of 159 acute severe TBI patients (GCS < 8) after intramuscular progesterone injections (1 mg/kg every 12 hours for 5 days after admission to a trauma center) [47]. Aminmansour and colleagues [48] used a similar progesterone treatment protocol [47], and administered either a combination of progesterone and vitamin D, progesterone only, or placebo (n=20 in each group). The authors demonstrated an improvement in the recovery of patients with severe TBI (GCS < 8) after 3 months [48]. Shakeri and colleagues [49] observed a significant improvement in neurological outcomes up to 3 months after early treatment with progesterone (1mg/kg every 12h for 5 days intramuscularly) in patients with severe head trauma with diffuse axonal injury (5-8). Santarsieri *et al.* [50] found that serum and

Table 2 Neuroprotective effects of progesterone after traumatic brain injury (animal models).

Study	Animal Model	Animal Species	Progesterone Treatment Protocol	Primary End Points	Conclusions
Roof, 1996 [27]	Medial frontal cortex contusion by dynamic cortical deformation	Rats	Progesterone treatment 1h after injury	Brain edema	Progesterone effectively reduced brain edema when treatment was delayed until 24h after injury.
Wright, 2001 [29]	Bilateral medial frontal cortex injury	Rats	Progesterone (4 mg/kg), intraperitoneally at 1, 6, and 24 h post injury.	Brain edema	Progesterone significantly decreased cerebral edema after TBI in adult male rats.
Thomas, 1999 [56]	Spinal cord injury (Laminectomy with contusion)	Rats	Progesterone treatment	1. Functional status 2. Histologic analysis	Progesterone improved clinical and histologic outcomes compared with the control groups.
Allitt, 2015 [33]	Cortical impact acceleration-induced diffuse TBI.	Rats	Progesterone (P4) treatment	Short-term (4 days post-TBI) and long-term (8 weeks post-TBI) functional outcomes	Short-term: neural responses in supragranular layers were suppressed, and TBI-induced suppression in the granular and infragranular layers was reversed. Long-term: There were inconsistent effects of P4 on the TBI-induced hyperexcitation in supragranular layers.
Lopez-Rodriguez, 2015 [34]	Traumatic brain injury (weight-drop)	Mice	None	Correlation between levels of neuroactive steroids in the brain and plasma at 24h, 72h and 2w after injury and clinical outcomes.	Brain levels of progesterone, tetrahydro-progesterone, isopregnanolone and 17β-estradiol were decreased at 24h, 72h and 2w after TBI. Brain levels of progesterone and dehydroepiandrosterone showed a positive correlation with neurological recovery.
Peterson, 2015 [35]	Cortical contusion injury after craniotomy	Rats	Combination treatment of nicotinamide (NAM) and progesterone	1. Behavioral testing 2. Histological analysis of brain lesion	NAM and progesterone treatment resulted in significant improvements in recovery of function, and reduced lesion cavitation, degenerating neurons, and reactive astrocytes 24h after injury.
Nudi, 2015 [40]	Controlled cortical impact (medial frontal cortex)	Rats	10-mg/kg progesterone or vehicle injections 4h after injury and every 12h for 72 h after injury, followed by embryonic neural stem cells (eNSC) transplantation	Behavioral testing	Multimodal therapeutic approach after TBI improved functional recovery to a greater magnitude than either method alone.
Si, 2014 [42]	Traumatic brain injury (modified Feeney's weight-drop)	Rats	Progesterone treatment	1. Neurological outcomes 2. Histological analysis of the brain lesion	Progesterone treatment significantly reduced the post-injury inflammatory response, brain edema, and Evans blue dye extravasation, and improved neurological scores compared with control animals.
Xu, 2014 [43]	Surgical brain injury (SBI)	Rats	Low and high doses of progesterone vs. dexamethasone treatment	Histological analysis of the brain lesion	Progesterone reduced astrocyte and microglia responses, and attenuated brain edema with preservation of the blood brain barrier. Progesterone was as effective as dexamethasone in reducing brain edema and inflammation.
Geddes, 2014 [41]	Controlled cortical impact (CCI) model, pediatric model	Rats	4,8 and 16 mg/kg doses of progesterone treatment 7d after injury	Behavioral testing	Progesterone ameliorated the injury-induced neurological deficits.
Pascual, 2013 [44]	Traumatic brain injury after craniotomy	Mice	Progesterone treatment 16 mg/kg intraperitoneally	1. Neurological outcomes 2. Histological analysis of the brain lesion	Progesterone treatment reduced the size of the pericontusional lesion and blood brain barrier macromolecular leakage after TBI, and improved neurological outcomes.

Table 3. Neuroprotective effects of estrogen and progesterone in Traumatic Brain Injury (clinical studies).

Study	Study Design	Sample Size	Treatment Protocol	Primary End Points	Conclusions
Berry, 2009 [20]	Retrospective review	72,294 patients with moderate to severe TBI,	None	1. Complications after TBI 2. Mortality	Female gender was independently associated with reduced mortality and decreased complications after TBI.
Garringer, 2013 [21]	Prospective study	100 patients with severe TBI compared to healthy volunteers	None.	1. Glasgow Outcome Scale (GOS) scores 6 months after TBI 2. Mortality 3. CSF estrogen/testosterone E2/T ratios.	TBI subjects had lower CSF estradiol over time compared with controls. CSF testosterone was initially high, but declined over time. E2/T ratios were initially low compared with controls, but increased over time. A higher mean E2/T ratio was associated with a lower mortality and better GOS scores after 6 months.
Wright, 2007 [46]	Phase II, randomized, double-blind, placebo-controlled trial	100 patients with Glasgow Coma Scale (GCS) between 4 and 12	Progesterone treatment	1. Neurological outcomes 2. Mortality	Patients randomized to progesterone had a lower 30-day mortality rate. Moderate TBI survivors who received progesterone were more likely to have a moderate to good outcome than placebo controls.
Xiao, 2008 [47]	A prospective, randomized, placebo-controlled trial	159 patients with GCS ≤ 8	Progesterone treatment	1. Neurological outcomes 2. Mortality	Progesterone treatment improved neurologic outcomes for up to 6 months. The mortality rate in the progesterone group was significantly lower than in the placebo group after 6 months.
Aminmansour, 2012 [48]	Prospective, randomized controlled trial	60 patients with severe TBI, GCS < 8	Progesterone vs. progesterone-vitamin D treatment	1. Recovery rates 3 months after TBI 2. Mortality	Patients who received progesterone and vitamin D together had significantly higher recovery rates than patients who received progesterone only.
Shakeri, 2013 [49]	Prospective, randomized controlled trial	76 patients with severe TBI an DAI	Progesterone treatment (1mg/kg per 12h for 5d)	Neurologic outcomes	Progesterone significantly improved neurologic outcomes in patients with severe TBI up to 3 months after injury, especially those with GCS between 5 and 8.
Skolnick, 2014 [52]	Multinational placebo-controlled trial	1195 patients, 16 to 70 years of age, with severe TBI (GCS ≤8)	Progesterone treatment initiated within 8 hours after injury and continued for 120h.	1. GOS score at 6 months after the injury. 2. Mortality	Primary and secondary efficacy analyses showed no clinical benefit of progesterone in patients with severe TBI.
Santarsieri, 2014	Prospective study	130 adults with severe TBI.	None	Cerebrospinal fluid (CSF) progesterone and cortisol levels after TBI	CSF cortisol levels were significantly and persistently elevated during the first week after TBI, and high CSF cortisol levels were associated with poor outcome. Serum and CSF levels for both cortisol and progesterone were strongly correlated after TBI relative to controls, possibly because of blood-brain barrier disruption.
Wright, 2014 [51]	Double-blinded, multicenter clinical trial	882 patients with moderate-to-severe, or moderate acute TBI (GCS score between 4 and 12)	Progesterone treatment	1. Neurological outcome 2. Mortality	There were no significant differences between the progesterone group and the placebo group with regards to favorable outcomes.

cerebrospinal (CSF) levels of both cortisol and progesterone were strongly associated with clinical outcomes in 130 adults after severe TBI. An elevation in CSF cortisol levels was associated with poor clinical outcomes, which was attributed to blood-brain barrier disruption. As a precursor to cortisol, progesterone was thought to mediate these effects [50] (Table **3**). Recently two large, randomized, placebo-controlled and double-blinded clinical investigations by

Skolnick *et al.* [51, 52] and Wright *et al.* [51, 52] investigated the neuroprotective effects of progesterone treatment in severe and moderate-to-severe TBI. In 1195 patients (age 16 to 70) with severe TBI, Skolnick *et al.* administered intravenous progesterone within 8 hours after injury (0.71 mg/kg for the first hour and 0.5 mg/kg for the next 119 hours). Patients were followed for 6 months after injury, and no clinical benefits (improvement in Glasgow Outcome Scale and decrease mortality rate) were observed with progesterone treatment. Wright *et al.* used the same progesterone treatment protocol [51, 52], initiated within 4 hours after injury and continued during first 96 hours for severe TBI patients (GCS 4 to 12). Similar, no clinical benefit after 6 months clinical follow up was demonstrated.

Currently, several additional randomized control trials are investigating the effects of progesterone treatment in various populations with TBI. A phase II, multicenter clinical trial (NCT01336413) is studying the effects of pregnenalone, a precursor of progesterone, in treating mild TBI. This study is currently recruiting patients.

Despite the growing data of evidence in the literature of effectiveness of estrogen and progesterone in animal models of TBI, the human clinical data has not demonstrated a clinical benefit. To this end, two recent meta-analyses failed to demonstrate a reduction in mortality or clinical benefit after progesterone treatment in acute TBI compared to placebo [53, 54]. There are several possible reasons for the negative findings. First, most of the progesterone treatment protocols in the preclinical animal model studies administered progesterone in the very early time period after injury. Second, the dosing regimens in preclinical studies were different than those used in the human studies. Third, the animal models used a controlled and isolated injury, which differed from the heterogeneous and diffuse injuries often observed in humans. Finally, secondary injury associated with CNS hypoxia and organ failure is rare in healthy young animals but common in human patients with TBI [55].

Spinal Cord Injury

Progesterone may have neuroprotective effects in the context of SCI as well. Thomas and colleagues found that progesterone treatment significantly improved the neurological deficits in rats after traumatic SCI, with an associated improved histological recovery [56]. De Nicola and colleagues further demonstrated that progesterone treatment restores proliferation and differentiation of

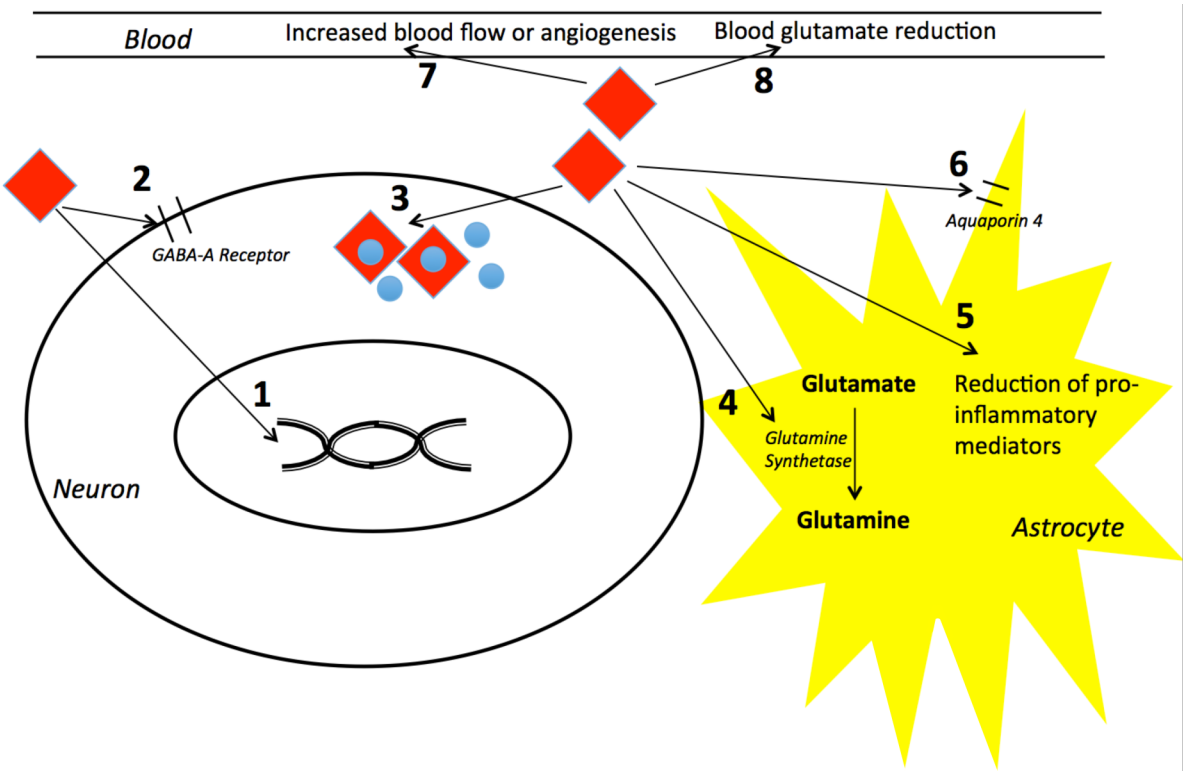

Fig. (1). Proposed mechanisms of neuroprotective effects of estrogen and progesterone on neurons, astrocytes, and the blood. Estrogen and progesterone exert its neuroprotective effects *via* complex and integrated mechanisms. Some of the mechanisms proposed include: 1) Estrogen and progesterone enter neurons by passive diffusion where they regulate gene transcription; 2) Estrogen and progesterone acts on GABA-A receptors after brain injury and increase cellular survival; 3) Estrogen and progesterone have antioxidant activity *via* intrinsic properties of scavenging oxygen free radicals; 4) Estrogen enhances the activity of the astroglial enzyme glutamine synthetase, which increases glutamate metabolism and cycling; 5) Estrogen may have effects on microglial proinflammatory mediators; 6) Progesterone modulates the expression of Aquaporin-4 channels after brain injury, resulting in decreased brain edema; 7) Estrogen may increase cerebral blood flow or promote angiogenesis; and 8) Estrogen and progesterone may scavenge blood glutamate and increase the brain to blood glutamate efflux. See text for more details.

oligodendrocytes, and prevents motor neuron degeneration after SCI in mice [57]. These results may be in part explained by a progesterone-mediated up-regulation of mRNA and protein 25-Dx in the dorsal horn of the injured spinal cord in rats [58]. There have been no clinical trials that have evaluated the effects of progesterone treatment in humans with SCI.

PROPOSED MECHANISMS OF ESTROGEN NEUROPROTECTION

Effects on Estrogen Receptors

Estrogen receptors (ERs) are expressed in a wide range of tissues, including the brain (Fig. **1**). The majority of ERs are present in the olfactory lobe, cortex, and cerebellum. Although ER-α and ER-ß are both expressed in the neuronal cell membrane of hypothalamus, the hippocampus in contrast appears to primarily express ER-ß [59]. Progesterone receptors (PRs) are also highly expressed in the adult brain. There are two classical isoforms of the PR present in brain tissue: PR-B and PR-A [3, 4].

Recently, estrogen and progesterone receptors were found on neurons and glial elements [4]. Studies have demonstrated a wide expression of classic nuclear ERs and progesterone receptors (PRs), as well as extranuclear sites present in the hypothalamus, neocortex, and other brain regions [2, 3, 60]. As estrogen and progesterone are both highly lipid-soluble, they pass the blood-brain barrier and enter the cell by passive diffusion and bind to both the nuclear and extranuclear sites. The important physiologic functions of gonadal hormones in the brain may be characterized not only by their neuroendocrine regulation, but also by their control of neuronal differentiation, cell migration and death, and synaptic organization of neurons [60, 61]. Hormonal binding on both the nuclear and extranuclear sites induces the promotion region of genes by regulating gene transcription, resulting in the formation of multi-protein units. However, they may also be integrated with other effectors and linked to different steroid signaling pathways in the cell [4, 60].

Estrogen has a plethora of cellular and extracellular effects that are mediated by ER-α and ER-ß. Estrogen classically exerts its effects by a nuclear ER-related mechanism. In this model, the steroid enters the cell by passive diffusion and binds to the nuclear ER [4]. Following a series of activation steps, the ER complex associates with an estrogen responsive element and functions as an enhancer for estrogen -containing genes. Estrogen induction of these genes may contribute to its neuroprotective effects.

The activation of ERs in the mammalian brain induces long-term genomic effects on elements such as the development and modulation of function in certain nerve cells [4, 62]. These effects of estrogen are termed “delayed”. They are dependent on genomic alterations induced by estrogen, and may include the delayed electrophysiological effects of estrogen in the hypothalamus [59], on seizure activity, on hippocampal long- term potentiation as well as general hippocampal physiology [63]. Furthermore, the delayed effects of estrogen on neuronal connectivity include estrogen-induced synaptic remodeling in the hippocampus [3, 4, 59]. Indeed, with respect to neuroprotection, estrogen promotes neurite outgrowth, sprouting of neurons, synaptogenesis, an increase in the level of the neurotransmitter acetylcholine, increased density of N-methyl-D-aspartate (NMDA) receptors, and higher expression of neurotropic factors including nerve growth factor [4, 59, 63]. These effects require a long activation time, and do not explain the rapid neuroprotective effects observed when estrogen is administered immediately prior to brain injury or immediately after inducing injury. However, these delayed effects may be important in posttraumatic brain rehabilitation. Thus, previously published studies [12, 62, 64-66] found an estrogen -mediated up regulation of the anti-apoptotic gene, bcl-2, the anti-apoptotic pro-survival factor, surviving and the neuroprotective factor, and brain derived neurotrophic factor (BDNF) following brain injury, which were associated with decreased apoptotic cell death.

Several studies have investigated the neuroprotective role of 17-beta estradiol after traumatic cerebral contusion [11, 12, 67]. They found that 17-beta estradiol protects from apoptosis in the cortical precontusional zone *via* the enhancement of ER-alpha. Furthermore, there was a decrease in caspase-3 activity *via* the activation and upregulation of nuclear and extranuclear ER-*á* mRNA induction and protein expression (bcl-2), as well as inhibited caspase-3 activation in the pericontusional zone of the brain [12]. Moreover, there is evidence that a G protein-coupled ER agonist G-1 may mediate the neuroprotective effects of estrogen against SCI, and prevent SCI-induced apoptotic cell death after estrogen pretreatment [22].

Not all the neuromodulator effects of estrogen follow the “classical” hormone receptor pathway *via* nuclear receptor occupation and activation. The rapid effects of estrogen include the modulation of excitability, which was observed in various brain regions including the hypothalamus, amygdala, striatum, cerebellum, neocortex, and hippocampus [59]. The modulation of ligand-gated ion channels or G-protein-coupled receptors by these neuroactive steroids may also directly affect nerve cell survival. Estrogen shares such modulatory interactions with neuronal membranes, which are independent of the presence and/or activation of ERs.

Effects on Signaling Systems and Neurotransmitters

McClean and colleagues showed that 17- alpha estradiol acts *via* the GABA-A receptor and induces a reduction in hippocampal volume and impaired hippocampal-dependent performance after water percussion brain injury model [13]. Another study in male rats demonstrated that pretreatment with 17-beta estradiol 4 h prior to fluid percussion injury stabilized free extracellular magnesium concentrations within 4 hours after injury, and improved motor function 1 week after TBI [6].

Antioxidant Effects

Recently, estrogen was shown to have intrinsic oxygen free radical scavenging antioxidant activity. Estrogens such as 17ß-estradiol and its derivatives ethinyl estradiol and hydroxyl estradiol equally protected cultured neurons against

oxidative cell death induced by amyloid- ß protein (A*ß*), glutamate, superoxide anions and hydrogen peroxide [5, 25, 59, 68]. In brain injury, estrogen may exert its effects by blocking the formation of oxygen free radicals and preventing cellular membrane damage induced by lipid peroxidation [30].

Effects on Astrocytes and Microglia

The indirect effects of estrogen on astrocytes and microglia may significantly contribute to the neuroprotective effects observed in many neurodegenerative disorders [4]. In organotypic cortical explants and neuronal/astrocyte co-cultures, there is evidence that the neuroprotective effects of estrogen at physiological concentrations are astrocyte-mediated. Furthermore, estrogen is known to enhance the activity of glutamine synthetase, an astrocytic enzyme that produces glutamine from glutamate. The glutamine from astrocytes is transported to neighboring neurons, where it can replenish the neuronal glutamate supply [69, 70].

Modulation of Inflammatory Response

After neuronal injury, microglia secrete pro-inflammatory mediators that may result in further secondary neuronal damage. Several studies have postulated that estrogen-mediated neuroprotection may result from its effects on the microglia's pro-inflammatory mediators, including a reduction in kappa-B phosphorylation, NF-kappa-B activation, and overexpression of inducible nitric oxide synthase (iNOS) [69, 71].

Effects on Cerebral Blood Flow and Metabolism

ERs are widely distributed in the cerebral vasculature [5]. It has been postulated that estrogen-related neuroprotection may be related to an increase in cerebral blood flow (CBF) or angiogenesis, and the formation of new collateral blood flow to ischemic regions after brain insults. This theory has been studied extensively with conflicting results. Estrogen treatment has been shown to increase CBF after middle cerebral artery occlusion (MCAO) [72] and TBI [25]. In general, the direct effects of estrogen on the wall of blood vessels may play a significant role in neuroprotection. Several mechanisms by which estrogen may induce a rapid vasomotor effect were postulated. These mechanisms include estrogen-mediated release of endothelium-derived relaxing factor, antagonism of endothelin-related vasoconstriction, direct hyperpolarizing of vascular smooth muscle, and antagonism of calcium channels in the vascular smooth muscle [25]. Taken together, the effects of estrogen on the cerebral vasculature have a predominant role in improving microcirculation, with an inconsistent effect on CBF. The improvement of microcirculation is critical to maintaining the brain to blood glutamate efflux by increasing the effective surface of glutamate exchange from the brain to the blood [73].

Effects on Glutamate, Glutamate Receptors, and Glutamate Transporters

Many acute and chronic neurological disorders, such as TBI, stroke, intracerebral hemorrhage, meningitis, brain hypoxia, and glioma are associated with pathologically elevated glutamate levels in the extracellular fluid (ECF) of the brain [74-81]. These pathologically elevated glutamate levels have been shown in both animal and human studies [82].

Teichberg and colleagues demonstrated that pathologically increased brain ECF glutamate is shifted to the plasma by glutamate transporters [73, 83]. Compared with the brain ECF, the concentration of glutamate is normally much higher in the blood. Therefore, the transport of excess glutamate from the brain ECF to the blood must work against a large concentration gradient. Reducing the blood glutamate concentration is thought to increase this gradient and facilitate the brain to blood glutamate efflux. This proposed neuroprotective mechanism reflects an exaggerated brain to blood glutamate efflux *via* mechanisms of blood glutamate reduction [73, 83].

The effect of plasma estrogen and progesterone levels on glutamate was evaluated along the course of female menstrual cycle [37]. There are predictable changes in plasma levels of estrogen and progesterone during the course of the menstrual cycle [84-90]. At the start of the menstrual cycle, plasma estrogen and progesterone concentrations are low. Plasma estrogen concentrations begin to rise on the fifth or sixth day of the cycle, with maximum values observed a day or two prior to ovulation on day 14. There is a sharp drop after 3 days at maximal levels, followed by a secondary rise in plasma estrogen between days 18 and 24, decreasing again to its original level at the end of the cycle [84-90].

In another study, the physiological rise of estrogen and progesterone was accompanied with a significant reduction in blood glutamate levels [38]. Moreover, Stover and Kempsky found that women have lower glutamate levels in the blood compared with men during craniotomies [83]. In animal studies, injecting premarin (estrogens mixture) in naïve and head-injured rats led to sustained decreases in blood glutamate levels, and was associated with a significant neuroprotective effect.

It is important to note that studies have demonstrated that estrogen increased the expression of the astrocytic glutamate uptake transporters GLAST (glutamate and aspartate transporters) and GLT-1 (glutamate transporter) [69, 91]. This effect may be an important mediator of neuroprotection by reducing the excitotoxic ECF concentrations of glutamate, which may attenuate glutaminergic receptor activation [92].

The removal of excess glutamate in the ECF of the brain is thought to attenuate the glutamate-mediated excitotoxicity that significantly impacts neurological outcomes [36]. The neuroprotective effects of 17-beta-estrodial were investigated in a rat model of glutamate neurotoxicity in which glutamate was infused in the cortex through a microdialysis probe. Prior to increasing brain glutamate, the rats were administered intravenous 17- beta-estradiol. The study demonstrated that pretreatment with beta-estradiol resulted in a significant reduction in the size of the glutamate-mediated lesion [93].

PROPOSED MECHANISMS OF PROGESTROGEN NEUROPROTECTION

The neuroprotective effects that characterize progesterone may be similar that of estrogen [94], and are also thought to be mediated by several mechanisms [95].

Effects on Progesterone and Other Receptors

The effects of progesterone on neurotrophin brain derived neurotrophic factor (BDNF) expression may be related to transcriptional activation through the progesterone receptor (PR)-A and PR-B receptor-signaling pathway. Some authors have argued that progesterone acts by activating specific neuroprotective signaling pathways, and by increasing the expression of anti-apoptotic proteins [96]. In contrast, others suggest that the neuroprotective effects of progesterone may be related to its regulation of cellular events *via* progesterone's interaction with the PR or GABA receptor. To this end, metabolites of progesterone bind to the GABA-receptor complex, increase the effects of GABA on its receptor, and subsequently increase the survival of the cell [25, 95].

Effects on AQP4 Channel Expression

Aquaporin-4 (AQP4) is a water-permeable channel that may play a significant role in regulating cerebral edema. After TBI, it was demonstrated that progesterone decreased cerebral edema by modulating the expression AQP4 72 hours after brain injury in a rat model [97].

Antioxidant Effects

Like estrogen, the antioxidant effects of progesterone may contribute to increasing neuronal survival after injury [25, 27]. Progesterone might reduce the inflammatory response after an acute insult, and progesterone pretreatment has been shown to reduce levels of inflammatory mediators such as glial fibrillary acidic protein (GFAP), complement factor C3, and nuclear factor kappa beta (NFκB) after cortical brain injury [98]. As with estrogen, progesterone treatment after brain injury resulted in reduced lipid peroxidation in male rats [25].

Effects on Glutamate, Glutamate Receptors, and Glutamate Transporters

As discussed in detail above in the context of estrogen, the plasma levels of progesterone fluctuate during the female menstrual cycle and are inversely correlated with blood glutamate levels [84, 90, 99]. Tsesis and colleagues investigated whether blood glutamate levels are influenced by changes in estrogen and progesterone levels observed during normal pregnancy in humans [38]. Estrogen and progesterone levels are known to increase exponentially during pregnancy. Elevated female hormones lead to a significant decrease in blood glutamate concentrations in the second and third trimesters of pregnancy compared to the first trimester. Despite higher levels of estrogen and progesterone during the third trimester compared with the second trimester, blood glutamate was not reduced additionally in the third trimester [38]. This seems to suggest that once the maximum glutamate-lowering capacity of estrogen and progesterone are reached, increasing estrogen and progesterone levels further do not result in more glutamate reduction.

Additionally, *in-vitro* studies have demonstrated that progesterone reduces the neuronal response to excitatory amino acids and prevents glutamate neurotoxicity [25]. The activation of ionotropic glutamate receptors after TBI directly results in depolarizing currents, unbalanced calcium influx through the L-type calcium channels, resulting in neuronal apoptosis [100]. Luoma and colleagues demonstrated that progesterone might block calcium entry through L-type calcium channels and prevent subsequent calcium-induced neurotoxicity [101]. Collectively these data provide evidence that progesterone, with or without estrogen, promotes neuronal survival. The observed progesterone-induced neuroprotection seems to be mediated by several complex and integrated mechanisms.

FUTURE INVESTIGATIONS AND CONCLUSIONS

Over the past three decades, there has been much evidence that supports the neuroprotective role of estrogen and progesterone. Although several mechanisms have been suggested, the precise underling mechanisms hormone-related neuroprotection is still largely undetermined. Laboratory investigations that aim to elucidate the underlying mechanisms may result in novel, targeted treatments. Furthermore, very few small clinical studies regarding progesterone neuroprotection have been published in recent years, and none have studied estrogen treatment in the context of TBI. The optimal hormone dose and length of therapy in humans is currently unknown. As estrogen and progesterone treatment are generally considered safe, large clinical multicenter, prospective, randomized control studies should be done to better assess their clinical role in human brain neuroprotection after an acute traumatic insult.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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