Chapter

Neuroactive Steroids in Hypoxic–Ischemic Brain Injury: Overview and Future Directions

Nicolas Toro-Urrego, Marco Avila-Rodriguez, María Inés Herrera, Andrea Aguilar, Lucas Udovin and Juan P. Luaces

Abstract

Hypoxic–ischemic brain injury is a number one cause of long-term neurologic disability and death worldwide. This public health burden is mainly characterized by a decrease in oxygen concentration and blood flow to the tissues, which lead to an inefficient supply of nutrients to the brain. This condition induces cell death by energy depletion and increases free radical generation and inflammation. Hypoxic–ischemic brain injury may occur in ischemic-stroke and over perinatal asphyxia, being both leading causes of morbidity in adults and children, respectively. Currently, there are no effective pharmaceutical strategies to prevent the triggering of secondary injury cascades, including oxidative stress and metabolic dysfunction. Neuroactive steroids like selective estrogen receptor modulators, SERMs, and selective tissue estrogenic activity regulators, STEARs, exert several neuroprotective effects. These encompass mitochondrial survival, a decrease in reactive oxygen species, and maintenance of cell viability, among others. In this context, these neurosteroids constitute promising molecules, which could modify brain response to injury. Here we show an updated overview of the underlying mechanisms of hypoxic–ischemic brain injury. We also highlight the neuroprotective effects of neurosteroids and their future directions.

Keywords: neuroactive steroids, hypoxia-ischemia, brain injury, oxidative stress, metabolic dysfunction

1. Introduction

Hypoxic–ischemic (HI) brain injury is a major cause of long-term neurologic disability and death worldwide. Brain damage caused by hypoxia-ischemia responds to a wide variety of factors, being the central nervous system (CNS) especially susceptible to changes in energy levels, mainly glucose concentrations and oxygen [1]. The brain has a 25% glucose and 20% oxygen consumption of total body weight [2, 3]. This high energy demand is attributed to the functions performed by brain cells such as synaptic activity, neurotransmitter recycling and ion transport [2]. Thus, ensuring correct brain metabolism results in optimal neuronal functioning. HI brain injury is mainly characterized by a decrease in the concentration of oxygen and blood flow, which causes an insufficient supply of nutrients to the brain. These pathological conditions lead to cell death due to

the increase in free radical production and depletion of ATP [4]. This phenomenon is observed both in perinatal asphyxia (PA) and in ischemic stroke (IS) [5–7]. Around 15 to 20% of infants that suffer PA will die in the postnatal period and further 25% will develop severe and long-lasting neurological impairments such as cerebral palsy, epilepsy and neurodevelopmental disorders [8], also representing one of the main causes of morbidity in children and adults in the world [9, 10]. Similarly, at a structural level HI injury mainly affects the layers II, III and VI of the cortex, CA1 and CA3 hippocampal areas, striatum and cerebellum [11]. Therefore, the understanding of the underlying mechanisms of this pathology is essential for the establishment of efficient treatments.

Several neuroprotective strategies have been tested, including Selective Estrogen Receptor Modulators (SERMs) and Selective Tissue Estrogenic Activity Regulators (STEARs), which have shown the same benefits as estrogen, including the decrease of reactive oxygen species (ROS), maintenance of cell viability, mitochondrial survival, among others; without its negative side effects [12–14]. However, there are no effective pharmaceutical strategies to prevent the triggering of secondary injury cascades, including oxidative stress and metabolic dysfunction. In this sense, the present chapter summarizes the underlying mechanisms of HI brain injury and compiles several neuroprotective strategies, including SERMs and STEARs.

2. Mechanisms of brain damage in hypoxia-ischemia

Hypoxia is a condition that affects mainly the brain, and it is characterized by a low concentration of oxygen, affecting the proper functioning of the organs and tissues exposed to it. This insult causes a variety of responses in the brain. An initial response occurs immediately after the insult and is associated with a depletion of ATP, glucose and phosphocreatine inside the brain. This immediate reaction determines the patient's outcome against injury, which in turn triggers a secondary response that occurs several hours later. A temporary energy recovery takes place almost to the initial physiological levels, providing a treatment window between 1 and 6 hours following injury [8, 15, 16]. A third phase of persistent effects lasts for several years [17]. In general terms, global hypoxia affects the cerebral cortex, the sensorimotor cortex, the talamo and the basal ganglia, causing damage in deep gray matter [18].While the complete pathogenic pathways of HI are not fully described, some mechanisms like apoptosis, increased glutamate, calcium overload, mitochondrial dysfunction and oxidative stress have been proposed to contribute to generate neuronal damage [19].

Primary response depends on the energetic failure, which is characterized by the reduction of the energy supply, generating the accumulation of Reactive Oxygen Species (ROS) via lactate production augment, making the cell susceptible to oxidative stress and mitochondrial dysfunction [18]. Besides this, restricted cerebral blood flow causes a switch to anaerobic respiration, reducing ATP and phosphocreatine, and increasing lactic acid production [16]. Low levels of ATP derived from this energetical failure affect the integrity of the cell membrane. Calcium enters easily to the cell causing the membrane depolarization, blocking calcium storage in the cell, which in turn accumulates in the extracellular space. In addition, the ion flux of sodium/potassium is altered by the Na+/K+ pump dysfunction [20]. The second phase of injury is related to the recovery of blood flow and the reestablishment of brain metabolism, characterized by an inflammatory response, excitotoxicity and oxidative stress, being the main responsible for the brain cells death after hypoxia [7, 18].

2.1 Second phase of injury

Apoptosis as necrosis are the death pathways of the cell. They are present in brain damage caused by hypoxia, being apoptosis the most common death pathway

in the young brain unchained by mitochondrial failure [21]. Apoptosis can follow two pathways, being the extrinsic triggered by external signals like the tumor necrosis factor alpha (TNF-α), Fatty acid synthase (FAS), and the intrinsic path mediated by internal factors such as DNA damage or cell stress [22]. The extrinsic pathway is involved in the action of caspase 8 and 10, which activate caspase effectors directly, interacting with the intrinsic pathway, and triggering a permeabilization of the mitochondrial membrane [23].

The Intrinsic pathway is mediated by the release of apoptotic factors such as cytochrome-c, Serine protease HTRA2, mitochondrial (Omi/HtrA2), apoptosis inducing factor (AIF), endonuclease G (endoG), Second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac / Diablo) after permeabilization of the membrane. These apoptotic factors can trigger cell death processes that can be mediated by caspase-dependent pathways. Each of these factors has a role in programmed death. Cytochrome c interacts with Apoptosis protease-activating factor-1 (Apaf-1), creating the apoptosome. Smac/Diablo interacts with apoptosis inhibitors, AIF and endoG act through a caspase- dependent pathway. These are translocated to the nucleus, causing nuclear fragmentation [24, 25]. Hence, the permeabilization of the mitochondrial membrane has been proposed as a marker of a point of no return in hypoxic injury.

2.2 Excitotoxicity

HI injury triggers responses at both the systemic and cellular levels. When the energy supply is interrupted, excitotoxicity occurs through an uncontrolled release of excitatory neurotransmitters such as glutamate, causing an acute cascade damaging neurons and glial cells at cytoplasmic and mitochondrial levels, and also causing disruption of the BBB [23]. Glutamate activates NMDA receptors, causing the accumulation of Ca ++ and nitric oxide (NO), which in turn cause production of ROS. The increased levels of intracellular calcium in neurons and glial cells in turn results in the activation of calcium-dependent proteases, reactive oxygen species (ROS) production, mitochondrial dysfunction, oxidative stress, cytotoxic edema, lipases and deoxyribonuclease (DNase), and the stimulation of pro-cell death pathways [23, 26, 27].

2.3 Oxidative stress

The balance between the oxidant and the antioxidant levels of the cell is called redox homeostasis. An imbalance in favor of the intracellular level of oxidants results in what is known as oxidative stress. This deregulation occurs mainly in two free radicals, the reactive oxygen species (ROS), and the reactive nitrogen species (RNS) [28, 29]. Oxidative stress plays a major role in the pathophysiology of HI, due to the significant damage to nucleic acids (DNA degeneration), lipids (lipid oxidation), proteins and different organelles such as the mitochondria [7]. There are different sources of free radicals (ROS and RNS) following HI, including mitochondrial electron transport chain (ETC), xanthine oxidase (XO), NADPH oxidases (NOX) and nitric oxide synthase (NOS), and arachidonic acid (12/15 lipoxygenase) [26, 28].

2.4 Mitochondria

Mitochondria plays a vital role in survival of the different cells of the CNS [30]. It is composed of two membranes, one internal and one external, each with different functions. Within these membranes is the matrix. There are enzymes responsible for the main metabolic processes to produce ATP, such as the Krebs cycle, β-oxidation, as well as the metabolism of aminoacids [31]. Additionally, the mitochondria is involved in moderating processes of death (apoptosis) and biogenesis or

cell proliferation [31, 32], also in critical processes such the maintenance of neuronal homeostasis, including autophagy, elimination of toxic metabolites like ROS, and calcium homeostasis [26, 30, 31, 33].

Neonatal brain has increased vulnerability to damage by oxidative stress when compared with the adult brain, in part due to lower levels of antioxidants [34]. In adult brain, superoxide dismutase (SOD) 1 can scavenge ROS generating hydrogen peroxide (H2 O2), thus allowing further breakdown by catalases to H2 O. In contrast, neonatal SOD1, although expressed, can exacerbate brain injury caused by HI possibly due to the absence or downregulation of enzymes such as catalase and glutathione peroxidase 1, required downstream of SOD1 [35].

Mitochondria plays a key role in HI injury since the disturbances in energy metabolism trigger a number of pathophysiological responses converging at mitochondrial levels, such as the control of energy metabolism, production of ROS, and the release of apoptotic factors into the cytoplasm [36]. Mitochondria constitutes an important regulator of cell death due to its ability to release proapoptotic proteins following mitochondrial permeabilization. Apoptosis can occur through an intrinsic pathway, where DNA damage or cellular stressors activate apoptosis, or an extrinsic pathway, following activation of death receptors [36].

2.5 Cardiolipin peroxidation

Another consequence of cell death caused by ROS-induced oxidative stress is the peroxidation of a mitochondrial lipid, cardiolipin [37], one of the most critical targets in the components of the evolution of HI injury. This is a unique phospholipid, which is found mostly in the inner mitochondrial membrane, where it has a very close association with the components of oxidative phosphorylation [37, 38]. Cardiolipin plays a crucial role in the insertion into the membrane and the function of cytochrome C, cytochrome C oxidase and other phosphorylation complexes. This is required, therefore, for an optimal functioning of complexes I (NADH: ubiquinone reductase), complex III (NADH: ubiquinone cytochrome C oxidoreductase), complex IV (cytochrome C oxidase) and complex V (ATP synthase) [39].

When HI occurs, enzymatic and non-enzymatic processes induce lipid peroxidation. The non-enzymatic process is triggered by the interaction of ROS with the fatty acyds of the membranes, and the enzymatic process include the activation of lipoxygenases (LOX), cyclooxygenases (COX), phospholipase A2 (PLA2) and Cyt C [40, 41], which leads to an alteration in the structure of this phospholipid responsible for mitochondrial dysfunction. Hence, the release of cytochrome c depends on the integrity of itself. This severe sensitivity to ROS is due to its high content of fatty acids [39].

2.6 Inflammation in HI

Accompanied by the reactions mentioned above, there is a role played by different glial cells in the injury caused by hypoxia, mainly in inflammation. This injury initially triggers an immediate response in neuroglial cells, which contribute to the damage mechanisms mentioned above, due to the secretion of a large amount of proinflammatory cytokines and ROS.

2.7 Astrocytes

In the last 20 years, astrocytes have been granted multiple functions, such as providing support, helping in the maintenance of the cerebral microenvironment for an appropriate function, regulating the blood flow in the brain, which are

essential for the adequate functioning of neurons [2, 42]. Another important astrocytic function is the contribution to brain metabolism [43]. Astrocytes takes glucose from blood vessels and provide energy metabolites to neurons [44]. In addition, through the lactate shuttle, astrocytes provide lactate to the neurons as a substrate for the citric acid cycle and can therefore supply their energy requirements [45].

However, the role of astrocytes in injuries such as hypoxia are not fully elucidated. Astrocytes as microglia, when subjected to insults such as hypoxia, may act differently depending on the severity of the injury. Immediately after hypoxia, astrocytes enter in an activated state, which eventually ends in a glial scar [46, 47]. Astrocytes plays important roles in the brain during HI. Because of the tight connection with brain capillaries, astrocytes suffer damage firstly after ischemia, and then, damaged astrocytes kill neighboring neurons. The number of apoptotic astrocytes increases gradually as the extension of ischemic time, which leads to further expand of cerebral infarction area [48].

Astrocytes can exacerbate cytotoxicity death due to secrete inflammatory cytokines such as IL-1, IL-6, interferon-γ, and TNF-α; and can also help the migration of immune cells to the CNS by the secretion of chemokines [49]. Likewise, there is also a protective effect exerted by astrocytes, which play an important role in tolerance to cerebral ischemic injury [50] and inflammation [50, 51].

2.8 Microglia and endothelial cells

Microglia, the immune cells of the CNS, are the first to be activated after hypoxia. They migrate to the place of injury and change their morphology to an amoeboid-lice functional cell, acting in conjunction with monocytes and macrophages [49, 52, 53]. Microglia M1 release proinflammatory agents to the environment such as ROS, cytokines ((IL) -1β, IL-6, tumor necrosis factor-alpha (TNF-α)), glutamate, nitric oxide, creating a cytotoxic environment triggering cell death [49, 52, 53].

The extent of injury noted in HI is not only determined by the biochemical cascades that trigger the apoptosis-necrosis continuum of cell death in the brain parenchyma, but also by the pro-inflammatory factors of the Blood Brain Barrier (BBB), such as the endothelial cells [54]. Endothelial cells can sense variation in the Parcial Oxygen Pressure (PO2) through different mechano-sensors. Then, they can adapt their metabolism to maintain ATP production, switching into an hypoxic metabolism. In this way, endothelial cells augment the production of ROS by making the respiratory chain slower, reduce the cytochrome-c capacity in order to trap O2, and alter the cellular redox potential [54, 55]. In cerebrovascular endothelial cells (cEND) OGD augmented the mRNA expression of IL-1 alpha, IL- 6 glut-1 transporter and total nitric oxide concentration increasing significantly the permeability of the cEND monolayer [56].

2.9 Selective vulnerability of the brain to HI

The pathophysiology of HI is complex. The damage on the developing brain is determined by several factors: timing of asphyxia, intensity, severity of HI and immaturity of the brain. Beside this, different areas of the brain and different cell types present a selective vulnerability to this injury [18].

The immaturity of brain represents a significant factor in the outcome of HI brain injury. Although risk factors of HI in term newborns are similar to those observed in preterm newborns, the immature brain in the last ones, especially those with a very low birth weight, is highly vulnerable to injury [18]. This, due to hypoperfusion caused by the defectively functioning lungs and hearts in preterm newborns, and the poor auto-regulatory capacity the immature brain possess [57].

HI injury induces white matter injury with noticeable oligodendroglia loss, due to the poorly vascularization in white matter compared with cerebral cortex. This injury, known as periventricular leukomalacia (PLV), triggers cognitive, sensory, and motor impairment in preterm infants. Abnormalities of cortical gray matter and hippocampus are also found in the immature brain [18].

In addition, in the developing brain there is a spectrum of lesions caused by HI. Alongside PVL, periventricular hemorrhagic infarction in association with geminal matrix (ganglionic eminence) hemorrhage, with or without intraventricular hemorrhage, or thalamocortical injury (**Table 1**) [58].

The developing brain exhibits selective vulnerability. As it was mentioned above, certain cells and regions appear vulnerable depending on the severity and timing of injury. Projection neurons, especially in the deep gray nuclei, are at greatest risk during ischemic insults in the term brain [18]. Subplate neurons are the earliest and the most transient cell population of the neocortex. The subplate zone peaks at the onset of the developmental window of vulnerability to PVL (GW 24) and undergoes dissolution during the third trimester. Subplate neurons are largely absent at 6 months of postnatal age. HI injury leads to moderate to near-complete subplate neuron cell death, whereas most cortical neurons are intact. This selective vulnerability may be due to early cellular maturation and a developmentally related increase in glutamate receptor expression, including NMDA receptor 1, kainate and AMPA receptors [59]. On the other hand, in the preterm brain, subplate neurons and oligodendrocytes (OL) precursors are most vulnerable. Consequent abnormal thalamocortical connectivity may explain the somatosensory and visual impairment seen in prematurely born infants suffering HI brain injury [60, 61]. OL progenitors appear to be the most vulnerable, showing impaired maturation and development following injury.

Hemorrhagic lesions
Germinal matrix (ganglionic eminence) (frequently associated with PVL)
Limited (grade I†)
With intraventricular hemorrhage (grade II)
With ventricular expansion (grade III)
With PHI (grade IV)
Subpial
Cerebellar
Subarachnoid space (temporal lobe and cerebellum)
White-matter lesions
Periventricular leukomalacia (PVL)
With focal necrosis
With diffuse white-matter gliosis only
Periventricular hemorrhagic infarction (PHI)
Combined gray- and white-matter lesions
Single cerebral artery–distribution infarcts (porencephaly)
Hydranencephaly (bilateral large hemispheric infarcts)
Multicystic encephalomalacia
Gray-matter lesions
Thalamic and basal ganglionic injury (“status marmoratus”)
Neuronal necrosis in basis pontis and subiculum (pontosubicular necrosis)
Mobius syndrome (brainstem neuronal loss and gliosis)
Cerebellar infarct

PHI = periventricular hemorrhagic infarction; PVL = periventricular leukomalacia.
†Grade refers to clinical severity assigned based on transfontanelle ultrasonography or other neuroimaging. Adapted from [58].

Table 1.
Lesions caused by HI in the developing brain.

2.10 Oligodendrocytes and astrocytes

Oligodendrocytes, the myelin-forming glia that ensheath axons in the CNS, exhibit four sequential stages of maturation. Oligodendroglial progenitors, the pre-OL (or late oligodendroglial progenitor), the immature OL, and the mature myelin-producing OL [60], are extremely susceptible to HI. The injury involves maturational delays in oligodendrocyte population inducing oxidative stress. Following HI, OLs fail to fully mature, leading to persistent aberrations in myelin ultrastructure, which are associated with permanent disability and neurodevelopmental impairment [62].

Astrocytes are the predominant glial population in the CNS. They play a crucial role in HI as mentioned above. However, sustained HI brain injury can lead to decreased astrocytic function and, thereby, greatly decreased neuronal regeneration [60].

2.11 Blood–brain barrier and vascular fragility

The brain evidences a high requirement of oxygenated blood. This demand has resulted in the development of specific cerebral blood vessel networks with arteriovenous hierarchy. The Blood–Brain Barrier (BBB) is a specific and unique component of the cerebrovascular network. It is a highly specialized biochemical and structural barrier at the interface between blood and brain. BBB is involved in preserving ionic homeostasis within cerebral microenvironment and regulating the entry of molecules into the brain [63].

HI injury in neonatal brain induces an increase in BBB permeability, affecting important cellular and functional components of this vessel network such as pericytes, the tight junctions of endothelial cells and astrocytes [60, 63, 64].

Delicate and thin vessels in the developing brain may not sustain the lack of blood flow to compensate the requirements of oxygen and nutrients that the brain needs, due to the underdeveloped distal arterial network and an immature cerebral auto regulatory capacity. Peripheral arteries in the growing brain lack collateral vessels and exhibit limited vasodilatory function in response to the hypoxic–ischemic event, resulting more susceptible to HI injury [60].

3. Experimental models

In vivo and in vitro models are used for studying hypoxia (**Table 2**). In the most used animal model, a unilateral ligation of the carotid artery (UCCAO) is performed, followed by an exposure to an oxygen atmosphere of 8% for 1–3 hours, mainly developed in rodents [65]. This reproduces the anatomical damage caused by HI in neonates, with gray matter damage in the hippocampus, thalamus and basal ganglia, as well as in white matter [65, 66]. Similarly, it reproduces metabolic damage in parameters such as: cerebral acidosis, decreased cerebral blood flow, and decreased glucose uptake [52] and has the ability to show the neuroprotective effect of different therapeutic approaches like hypothermia [67, 68]. Bilateral ligation of the carotid artery is also used to accentuate white matter damage [69, 70] .

In another animal model, ligation of the common carotid is excluded and hypoxic damage is performed by oxygen deprivation. This experimental paradigm is used to describe milder lesions and to investigate the biochemical alterations of the brain [52]. On the other hand, this model has been used in larger animals such as primates, sheep, pigs and rabbits in order to better replicate the conditions of a human fetus with HI, with the disadvantage of not being able to perform behavioral tests and not having a methodological archetype between experiments [52, 71–73].

3.1 In vitro approaches

The different methodological limitations of in vivo models make in vitro models relevant. In order to replicate the conditions that occur in the presence of a deprivation or decrease in glucose and oxygen levels such as those present in HI, several studies have proposed a model of oxygen and glucose deprivation (OGD) (**Table 2**). This experimental model has the ability to adjust to specific research needs and the versatility of being able to use different cell lines, making possible the study of the bases of the molecular and biochemical mechanisms of HI injury. However, methodological differences have been found in the implementation of this model, especially in the exposure time of hypoxia and reoxygenation. [74–81], making this model dependent on the specific conditions of the tissue or cells used [7].

Another methodological approach used to study the effects of hypoxia in vitro include chemical hypoxia-mimetic agents (HMAs) (**Table 2**). These are based on producing at molecular level the effects caused by low concentration of oxygen, mainly those involved in the expression of Hypoxia-inducible factor-1 (HIF-1) [82, 83]. The activation of this factor depends on oxygen concentration, and HIF-1 is involved in several cellular processes that trigger hypoxia [84–89].

Reference	Species	Animal model	Outcomes
Large animal models			
[73]	*Macaca nemestrina*, near term	UCO	Poor weight gain and cerebellar growth, abnormal brain DTI, behavioral impairment, 43% develop CP.
[90, 91]	Fetal sheep, near term	Bilateral CCAO	Shorter HI (<30 min): selective neuronal loss. Longer HI: cortical necrosis. Post-HI EEG suppression related to insult severity and pathology; prevented by hypothermia.
[92]	Fetal sheep, midgestation	Bilateral CCAO	Necrosis of subcortical white matter, neuronal loss in thalamus and striatum similar to near term fetus. Little loss of final EEG amplitude.
[93]	Fetal sheep, midgestation and near Term	UCO	Hippocampal neuronal loss only in near term group. Degree of injury associated with the severity of hypotension during UCO.
[94]	Pigs, <24 h old	CCAO + hypoxia	Secondary energy failure. Energy metabolism ameliorated by hypothermia (35°C for 12 h) at 24 h–48 h.
[95]	Pigs, P9	Hypotension + hypoxia	~60% fall in CBF, reduced cerebral O2 uptake, phosphorylated metabolites and pH and increased inorganic phosphate.
[71]	Rabbits, 21–22d gestation	Uterine ischemia	P1 pups: overt posture and tone after ischemia >37 min, correlates with microgliosis in basal ganglia and thalamus. MRI: WMI in IC.
Rodent models with global hypoxic or excitotoxic component			
[96]	Mice at E8, P0 or P5	Ibotenate, i.c.v.	Laminar neuronal depopulation of layer V–VIa. P5: neuronal loss in all cortical layers, formation of porencephalic cysts.

Reference	Species	Animal model	Outcomes
[97]	Pregnant Sprague–Dawley rats, embryonic	Hypoxia E5-E20	White matter cysts in offspring P0–P7, increased lipid peroxidation, WMI and macrophages.
Rodent models with hypoxia-ischemia			
[98, 99]	Sprague Dawley rats, P1–P3	CCAL + hypoxia	Selective vulnerability of late OL progenitors, independent of age. Death of sub-plate neurons, motor deficits, altered thalamocortical connections to somatosensory and visual cortex normal.
[65]	Sprague–Dawley rats, P7	CCAL + hypoxia	Unilateral ischemic injury in the cortex, hippocampus, basal ganglia in >90% of survivors.
[100]	Wistar rat, P7	LPS, 4 h prior to CCAL + hypoxia	Blocking lymphocyte trafficking reduced brain inflammation, BBB damage, and improved LPS-induced HI brain injury. No effect with pure HI.
[101]	C57Bl/6 WT, Tg SOD1, GPx1 over-expressing P7 mice	CCAL + hypoxia	Reduced injury in GPx1-Tg mice but not in SOD1-Tg or GPx1/SOD1. NOS inhibition did not improve outcome in SOD-Tg.
[102, 103]	C57BL/6 WT and Gal-3 KO, P9	CCAL + hypoxia	Increased BBB permeability 2–24 h, reduced BBB protein expression. Infarct volume reduction in Gal-3 KO mice.
[104]	C57BL/6 J and TRIF KO mice, P8–9	Poly I:C, 14 h prior to CCAL + hypoxia	Increased infarct volume and WMI, prevented in TRIF KO. Injury linked to inflammatory response & decrease in M2-like microglia.
Focal ischemia rodent models			
[105]	Wistar rat, P7	Permanent MCAO +1 h CCAO	Infarcts in frontoparietal cortex at 3-month recovery. DNA fragmentation from 6 to 96 h.
[106–108]	Sprague Dawley rats, P7	Transient MCAO, 3 h	Severe unilateral perfusion deficits, restoration of CBF upon suture removal. Decreased ADC associated with brain injury at 24 h reperfusion. Demonstrated endogenous neuroprotective role of microglial cells after acute injury.
[109]	Sprague Dawley rats, P10	Transient MCAO, 1.5 h	Time resolved cell-type specific increase in HIF-1a and VEGF expression, gliosis.
[110]	C57/Bl6 mice, CD36 KO and WT, P9	Transient MCAO, 1.5 h and 3 h	Focal ischemia–reperfusion, increased injury and caspase-3 cleavage associated with apoptotic neuronal debris in CD36 KO. Effects independent of NFκB activation.
In vitro models			
Reference	Cell line	Experimental model	Outcomes
[74]	PC12 cells	48 h OGD/ 2 h reperfusion	Significant morphological cell changes
[75]	Primary cortical astrocyte	6 h OGD/ 0, 12, 24, 48 h reperfusion	Significantly increased 2- NBDG uptake by about 1.2 to 2.5 times in cells compared to control

Reference	Species	Animal model	Outcomes
[76]	Primary cerebral cortex neurons	3 h OGD/ 48 h Reperfusion	Damage to neuronal viability, dendrite branch number in neurons deceased significantly
[79]	Primary astrocyte	3, 5, 7 h OGD/ 24 h Reoxygenation	Increases in HMGB1 and TNF-a, induced phosphorylation of PI3K, promoted nuclear translocation of NF-kB
[111]	Primary cortical neurons	2 h OGD	Suppressed significantly cortical neurons proliferation
[112]	SH-SY-5Y cells	6 h OGD/ 1 h reoxygenation	Caused significant mitochondrial fragmentation, excessive mitochondrial fission
[77]	Primary Cortical Neuron	OGD	Decrease in neurite outgrowth
[78]	Neural progenitor cell	6 h OGD	Increased apoptosis
[113]	Mouse hippocampal neurons HT22	4 h OGD/ 24 h Reoxygenation	miR-144-3p expression was significantly downregulated in neurons following OGD/R treatment
[81]	Neuro 2a cells	4 h OGD/ 12 h Reoxygenation	Inhibited cell viability and cell proliferation, reduced phosphorylation levels of p38 MAPK and ERK1/2
[114]	SH-SY5Y cells and primary murine cortical neurons,	4 h OGD	OGDR-induced mitochondrial depolarization, reactive oxygen species production, lipid peroxidation and DNA damages
[115]	Primary astrocytes and microglial cells	2 h OGD/ 48 h Reoxygenation	Induced abnormally opened hemichannels with increased ATP release and EtBr uptake but reduced GJIC permeability. Astrocytic Cx43, hemichannels, and GJIC play critical roles in OGD/R injury-induced neuroinflammatory responses.
[116]	Primary astrocytes	4 h OGD/ 3 h, 6 h, 12 h, 24 h reoxygenation	Expression of Ski was proved to be up-regulated
[117]	Primary hippocampal neurons	2 h OGD/ 24 h reperfusion	Caspase-3 activity and expression increased in the first 24 h,
HMAs models			
Reference	Cell line/species	Experimental model	Outcomes
[82]	Multiple myeloma cell line U266	CoCl2	CoCl2-mediated hypoxia affects the expression profiles of genes that are functionally related to apoptosis and angiogenesis
[83]	Myeloid leukemic cell lines NB4 and U937	CoCl2 and DFO	Apoptosis with a loss of mitochondrial transmembrane potentials, activation of caspase-3/8 and cleavage of anti-apoptotic protein Mcl-1
[118]	U251 human glioblastoma cell line	CoCl2	Increases HIF-1a gene expression
[119]	Glioblastoma cell lines U373MG and DBTRG05MG	DFO	Activation of factors associated with ECM degradation and invasion of glioma cells

Reference	Species	Animal model	Outcomes
[120]	C57BL/6 mice	DFO	DFO up-regulated the expression of vascular endothelial growth factor (VEGF), HIF-1α protein and growth associated protein 43 (GAP43) and down-regulated the expression of divalent metal transporter with iron-responsive element (DMT1 + IRE), α-synuclein, and transferrin receptor (TFR)
[121]	Hippocampal neurons	DFO pretreatment/3 h OGD	45% reduction in cell death
[122]	Sprague–Dawley rats	Subarachnoid hemorrhage/DFO treatment	DFO-induced increase in HIF-1 protein level and activity exerts significant attenuation of BA vasospasm
[123]	Hippocampal cultures	Ppreconditioning CoCl2, DFO or dimethyloxylalyglycine (DMOG), 3 h OGD	Cobalt induced the transcription of the cytokine erythropoietin. Cobalt and DFO, enhanced survival of neurons. DMOG exacerbates OGD-induced neuronal death
[124]	Sprague–Dawley rats	CCA/DFO treatment	Neural-protective and angiogenesis effects through regulating the levels of HIF-1α
[125]	Adipose-derived stem cells	DFO preconditioning	Restored neovascularization potential of ADSCs
[126]	Sprague – Dawley rats	MCA/DFO treatment	Preserved brain volumes, upregulation of HIF1a
[127]	Wistar rats	MCAO/ DFO + Erythropoietin treatment	Reduced the number of cleaved caspase 3-positive cells in the ipsilateral cerebral cortex.

Modified from [7].

Table 2.
Experimental models for HI.

4. Neuroactive steroids

Neuroactive Steroids were defined by Baulieu [128] as steroids synthesized in the nervous system capable of inducing neuronal excitability [129]. Compounds as dehydroepiandrosterone, androstenedione, and deoxycorticosterone meet the requirements to be categorized as neuroactive steroids. Interestingly, neuroactive steroids induce responses on GABA receptors and modulate the activity of 5α and 3α reductases affecting steroid synthesis [130–132]. In this regard, neuroactive steroids can be exogenously synthesized and produce similar effects on the CNS. In the current definition neuroactive steroids are molecules capable of inducing several effects on CNS including ion channel modulation, voltage-dependent calcium channels activation and AMPA-NMDA receptors activation [133–135]. Besides the neuroactive properties of steroids, there are a plethora of protective functions characterized on neurons, astrocyte and microglia [136–139]. The effects of neuroactive steroids on neurons include the increase of dendritic spines, viability, antioxidant capacity [140, 141]. On astrocytes, neuroactive steroids improve the mitochondrial function, modulate the synthesis of antioxidant molecules and growth factors and pro-survival factors as Bcl-2 [142–145]. Finally, on microglia, the effects include the modulation of immune response via regulation of the synthesis and secretion of cytokines and inflammatory mediators [139].

Neuroactive steroids may induce both genomic and non-genomic mechanisms associated with its protective effects [146]. The genomic mechanisms involve the modulation of pro-survival genes, anti-inflammatory [147] and anti-apoptotic functions [148]. For example, the activation of signaling pathways like Akt-PI3K and MAPK, and the upregulation of the anti-apoptotic mediators like Bcl-2 and antioxidant enzymes like SOD and GPx [149] are under control of Neuroactive steroids. Other mechanisms include the downregulation of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [150]. The non-genomic effects include the antioxidant properties of some neurosteroids, especially the ones that include an A-phenolic ring in their chemical structure [151]. Interestingly, some neuroactive steroids are capable of exerting its effects through G-protein coupled receptors, for example via GPR30 receptor [152]. Until now, there is a large body of evidence demonstrating the beneficial effects of neuroactive steroids following ischemia/reperfusion and traumatic brain injury (TBI) in animal models (Liu et al., 2005; O'Connor et al., 2005) as well as steroid-demonstrated effectiveness in glucose deprivation and oxygen–glucose deprivation in in vitro models [148]. Despite this evidence, the direct use of estrogens is not fully recommended and still represents a potential risk for human health [153, 154] (For further evidence, see **Table 3**). In fact, it has been documented that the use of estrogen and progesterone increases the risk to develop breast and uterus cancer, as well as, vascular diseases, brain hemorrhage and clotting disorders [155–159]. To circumvent these issues, selective compounds that mimic the protective action of neuroactive steroid without the side effects were developed. These compounds were defined as selective estrogen receptor modulators (SERMs) and selective tissue-specific estrogenic activity regulators (STEARs). SERMs and STEARs exert their actions as estrogenic agonists or antagonists depending on the target organ [146, 160]. Tissue selective properties of SERM and STEAR are currently under investigation (**Figure 1**).

Reference	Type of study	Outcomes
[197]9	Human Psychiatric study	The evidence summarized supports the idea that MDD and PPD are psychiatric disorders involving neurosteroids and GABAergic dysfunction
[198]	Comparative human and animal studies	The study shows potential mechanisms that underlie sex-related differences in behavior and its implications for stress-related illnesses.
[199]	Animal and human studies	The negative cognitive consequences of sleep deprivation may arise from the effort of the brain to counteract the detrimental effect of sleep loss via compensatory mechanisms
[200]	Animal (neonatal foal) study	Progesterone might be a promissory marker for identifying continuous endogenous production of neuroactive steroids in foals with suspected NMS and other diseases
[201]	Human study	Individual domains of cognitive can be considered as an endophenotype of psychosis. It is possible that higher levels of cortisol and testosterone in siblings are consistent with high-risk states for psychosis
[202]	Animal model	Exposure to neuroactive steroids induced a sustained elevation in tonic current in Fmr1 KO mice. Neuroactive steroids may act to reverse the deficits of tonic inhibition seen in FXS, and thereby reduce aberrant neuronal hyperexcitability associated to this disorder
[203]	Peripartum depressed women	Cortical GABA+/Cr concentrations are associated with postpartum RSFC. It is possible that allopregnanolone may be associated with postpartum intra-DMPFC connectivity.

Reference	Type of study	Outcomes
[204]	Animal and human studies	Nervous diabetic complications show sex dimorphic features. In this regard, sex-oriented therapies with neuroactive steroids might be aimed to counteract nervous damage observed in diabetic pathology.
[205]	Animal and human studies	Neuroactive steroids under pathological conditions may alter their levels involving sex differencies in the outcome. Neuroactive steroid may be considered as neuroprotective factors to be deeply investigated.
[206]	Animal and human studies	Some studies point to a lag between neuroactive steroid dysregulation and subsequent symptoms. The study also consider key interactions with other aspects of neuroactive steroid physiology, such as synthetic enzymes or receptor plasticity.
[207]	Animal and human studies	There is a very close link among neuroactive steroids and the control of metabolic axis to understand the biological basis of many pathologies based on metabolic alterations, for example the metabolic syndrome, obesity or diabetes.
[208]	Women study	Women at both extremes of the weight spectrum have low mean serum allopregnanolone. Neuroactive steroids such as allopregnanolone may be potential therapeutic targets for depression and anxiety in traditionally treatment-resistant groups.
[209]	Animal and human studies	Low levels of neuroactive steroids could have a part in development of depression, neuro-inflammation, multiple sclerosis, experimental autoimmune encephalitis, epilepsy, and schizophrenia. On the other hand, stress and attention deficit disorder could occur during high levels.
[210]	Animal and human studies	Several Compounds have completed a phase 1 single ascending dose (SAD) and multiple ascending dose (MAD) clinical trial and is currently being studied in parallel phase 2 clinical trials for the treatment of postpartum depression (PPD), major depressive disorder (MDD), and essential tremor (ET).
[211]	Animal model	DHEAS and progesterone were good predictors of HPA Axis dysfunction and outcome in hospitalized foals.
[212]	Clinical study	The first-episode antipsychotic-naive schizophrenic patients showed a significantly higher blood level of DHEA-S compared with healthy controls. On the other hand, serum DHEA-S level has an inverse relationship with aggression and may serve as a biological adaptive mechanism to antagonize the neuronal damage caused by cortisol.
[213]	Animal and human studies	Clinical trials designed to test neuroactive steroid therapeutics in PTSD may benefit from such considerations. However it is needed to validate clinically accessible methods for identifying specific neuroactive steroid system abnormalities at the individual level.
[214]	Animal and human studies	Strain variation in neuroactive steroid levels correlated with numerous behavioral phenotypes of anxiety sensitivity accessed in GeneNetwork, consistent with evidence that neuroactive steroids modulate anxiety-like behavior.
[215]	Aged human study	We observed a significant difference in plasma concentration of cortisol and estradiol between experimental groups. In the AIS group, higher levels of these neuroactive steroids were associated with more pronounced neurological, cognitive and functional deficits in women compared to men.

Table 3.
Neuroactive steroids used in experimental models and clinical studies.

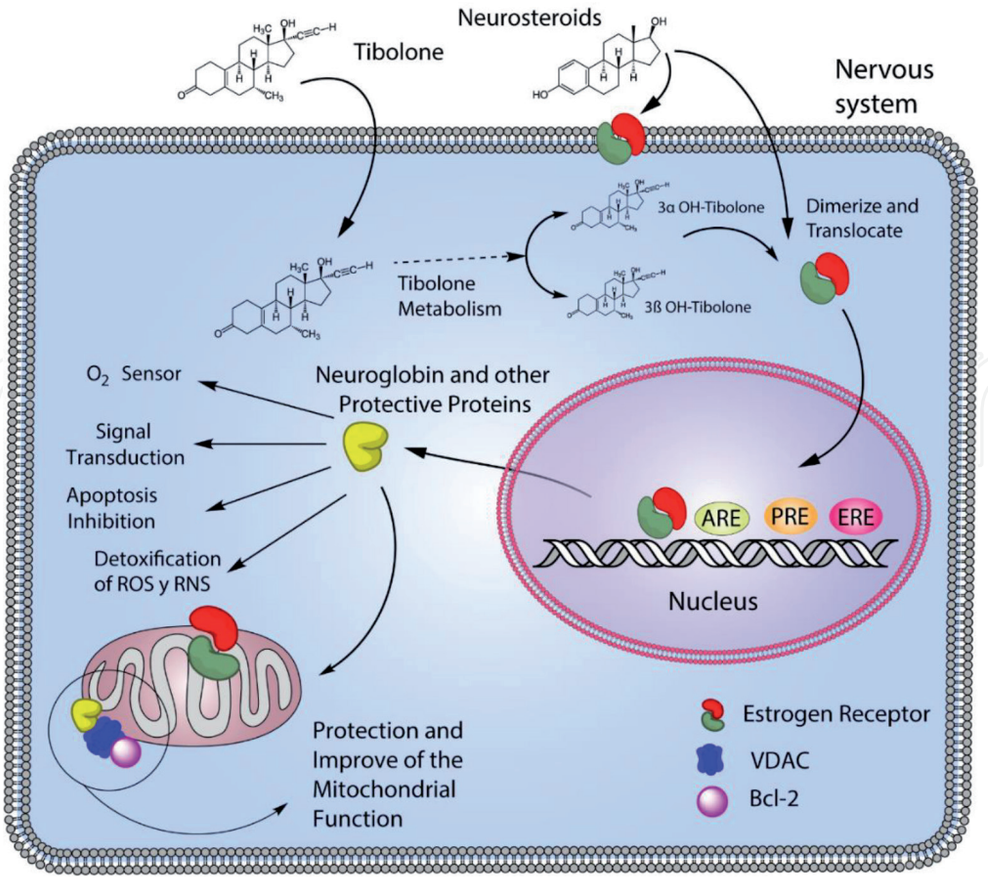

Figure 1.
Potential Neurosteroids action mechanism. The effects of neurosteroids on neurons include the increase of dendritic spines, viability, and antioxidant capacity. The action mechanism is associated to classical (canonical) transduction pathway that includes the transactivation of estrogen receptor to dimerize and promote the transcription of estrogen response elements ERE. For tibolone, it is described the classical transduction pathway but also the transactivation of androgen response elements ARE and progesterone response elements PRE. It is possible that all together response elements explain the beneficial and protective properties of tibolone. Interestingly, the protective properties also has been observed on astrocytes and microglia.

4.1 Selective estrogen receptor modulators

The activation or partial activation of Estrogen receptors (ER) trigger critical signal pathways due to complex molecular mechanisms. ER interact with several endogens and exogenous ligands promoting structural changes with the subsequent transactivation of estrogen response elements (ERE) in the DNA. ER interact also with co-activators, co-repressors and chaperones, affecting the way that the tissues exert their estrogenic response [161, 162]. ER show structural components that may be involved in their particular action mechanism. One of the most striking domain is the ligand binding domain (LBD) that interacts with specific ligands [163] (Cano et al., 2006). It is believed that the high or low affinity of the ligand with LBD plays a central role in the function of ER. Ligand interaction with LBD induces conformational changes that lead to specific bind to activators with co-activators and co-repressors modulating the estrogenic response [161, 164]. In this context, the conformational change is predetermined in part by the chemical nature of the ligand and its interaction with ER [165]. SERMs are capable of exploiting this advantage. A clear example is tamoxifen, a selective compound with estrogenic activity in the liver, but anti-estrogenic activity in breast tissue [166]. These compounds have been widely used in clinics for the treatment of breast cancer and as hormonal replacement therapy (HRT) strategies [167]. SERMs are defined as compounds that are capable of binding ER and produce several responses, ranging from a pure estrogenic agonism

to an anti-estrogen activity [146]. SERMs may protect nervous tissue following spinal cord and traumatic brain injuries [168, 169]. Gonzales-Burgos et al. (2012) demonstrated that SERMs increase the number of dendritic spines in hippocampal neurons [170]. Raloxifene, a second-generation SERM, demonstrated to improve sensory motor and working memory deficits following TBI [168], suggesting that SERMs may act as potential therapeutic compounds after CNS injury.

SERMs action mechanisms include the activation of transcription factors such as NF-κB through the PI3K-P38-ERK1/2 pathway [146]. SERMs also induce the production of antioxidant enzymes such as manganese superoxide dismutase (MnSOD) [171] and the endothelial nitric oxide synthase (eNOS) [172]. Interestingly, SERMs may induce the upregulation of anti-apoptotic proteins such as Bcl-2 [173]. Altogether, the activation of these multifactorial protective signaling cascades may improve the outcome of highly heterogeneous pathologies like TBI and HI Brain Injury (HIBI). Currently, SERM are used as primary treatments to counter osteoporosis and some kind of cancer. Compounds like raloxifen (Evista ®) and tamoxifen (Nolvadex®) are routinely prescribed for thousand women [174, 175]. Several reports have described the protective effects of SERMs on the CNS [176–178]. It is well known that tamoxifen is capable of preserving pyramidal neurons following penetrant lesion [179]. Furthermore, raloxifen exerts protective functions by increasing glutamate reuptake via induction of GLT-1 expression on primary astrocytes [180]. However, the complete action mechanism of several SERMs needs to be fully elucidated, due in part, to the complex agonist–antagonist action [181].

4.2 Selective tissue estrogenic activity regulators

The pharmacologic necessity to develop estrogenic safe compounds against climacteric symptoms in post-menopause women lead to synthesize a distinctive compound with selective estrogenic properties. As a result, STEARs are compounds capable of inducing an estrogenic, progestogenic and androgenic response. The most used STEAR compound is tibolone [160]- Tibolone has become a well-known treatment for climacteric symptoms than other HRT compounds, especially in women suffering low libido, persistent fatigue and blunted motivation [172, 182]. Tibolone has been used in the prevention of cardiovascular diseases and osteoporosis [183, 184] Tibolone exhibits weak estrogenic, progestogenic and androgenic properties [160, 183, 185].

The selective action mechanism of tibolone and STEARs is currently under investigation. However, it is well known that tibolone acts as a pro-drug that has complex effects due to its particular mode of action on different steroid receptors. It has been demonstrated that the body metabolized tibolone via two-phase reacts to produce three different metabolites [186]: two hydroxyl-metabolites (3-alpha-hydroxy- and 3-beta-hydroxy tibolone) as a result of 3-alpha and 3-beta hydroxysteroid dehydrogenase enzymes (3α-HSD and 3ß-HSD), and one isomer (delta-4 tibolone) synthesized by 3-beta-hydroxysteroid dehydrogenase [160, 183, 185].

Interestingly, 3α-HSD is predominantly expressed in the liver, whereas 3ß-HSD is expressed in adrenal glands, ovary and placental tissue [160, 183, 185]. Tibolone metabolism is under liver control by α-ketoreductases including hepatic AKR1C1 and AKR1C2 [186]. STEARs like tibolone might be metabolized by the brain, due to brain cells, for example, astrocytes fully expressing all the needed enzymes to carry out the biochemical steps. Kloobsterboer et al. 2017 demonstrated in primates (cynomolgus) the occurrence of 3α OH tibolone and 3β OH tibolone metabolites in the brain. They also detected sulfated tibolone metabolites (inactive chemical compounds) in the brain and plasma. Each metabolite has different features. For example, tibolone perse and delta-4 tibolone are agonists for progesterone receptor PR and androgen

receptor AR [185], while 3-alpha and 3-beta hydroxy metabolites are agonists for ER, but antagonists for PR and AR [185, 187]. This tibolone-steroid receptor interaction and other regulatory mechanisms might explain the tissue-selective effects of tibolone [160, 186]. Belenichev et al. (2012) used cortical neurons from neonatal rats to evaluate the neuroprotective activity of tibolone in a model of glutathione depletion that produces oxidative stress and mitochondrial dysfunction. These authors found that tibolone prevented mitochondrial dysfunction and neuronal cell death. Additional studies account for the protective effects of tibolone in an ovariectomized rat model following cerebral ischemia injury [188]. Tibolone has also shown anti-inflammatory effects tested in cardiovascular animal models [184].

Kloosterboer et al. 2007 propose an additional action mechanism of tibolone and STEARs that involves the control of sulfatase and sulfotransferase tissue-specific activity [189]. Since sulfatase and sulfotransferase activity is tissue-specific, it is possible that tibolone exerts its function according to cell type specificity and modulating nuclear receptors activity in the tissues [190]. For instance, it is needed to further investigate the tissue-specific role of tibolone in CNS, for example, in neurons, astrocytes, and microglia. Interestingly, tibolone protects the mitochondrial activity by the preservation of the mitochondrial membrane potential and by increasing the levels of proteins that control the opening of the mitochondrial permeability transition pore (mPTP), such as Bcl-2. Avila-Rodriguez et al. (2014) demonstrated that tibolone protects the mitochondria of T98G glial cells from glucose deprivation [141].

De Marinis' research group recently described and characterized a particular globin belonging to CNS called neuroglobin (Ngb1). Neuroglobin is under control of estrogenic response. In fact, the use of estradiol in several cellular models demonstrated the increase of neuroglobin levels [191–193]. Currently, it is known that neuroglobin is an 18 kDa protein that binds molecular oxygen with more affinity than hemoglobin, probably, increasing the availability of oxygen in the neural tissue [194]. Neuroglobin is expressed in neurons under basal conditions and is also expressed in astrocytes and microglia after brain injury [194]. Avila-Rodriguez et al. 2016 demonstrated that tibolone is capable of increasing the expression of neuroglobin producing a protective effect in a glucose deprivation astrocyte-like model. The action mechanism of tibolone may be associated with ERß receptor as demonstrated by several studies [191, 193].

Other studies demonstrated the protective effect of tibolone against lipid peroxidation and protein oxidation [195]. Tibolone is capable of increasing the density of dendritic spines in hippocampal neurons, indicating a potential role in synaptic plasticity and memory [196]. Guzmán et al. (2007), also showed that tibolone metabolites exert estrogenic activity on human astrocytes and oligodendrocytes-like cell lines [187]. Tibolone may become a promissory option to counter the detrimental effects of TBI and hypoxic injury due to its pleiotropic beneficial properties.

4.3 Selective tissue-specific estrogenic activity regulators and neuroglobin

Pathologic conditions like hypoxia and glucose deprivation, which may lead to neuroinflammation, reduce the expression of ER-α and increase the expression of ER-ß [216]. In this regard, De Marinis et al. (2013) showed that hypoxia may induce the production of pro-inflammatory mediators like IL-6, and INF-γ [193]. Interestingly, estrogen is capable of diminishing the secretion of those pro-inflammatory mediators. It was demonstrated in a pro-oxidant model induced by H2O2 and stimulated via lipopolysaccharide (LPS). Later, it was demonstrated that the anti-inflammatory effect was mediated by NF-κB modulation and ER-ß activation [191, 193]. Therefore, it is reasonable to assume that the activation of ER-ß in hypoxic and glucose deprivation models may be considered as beneficial for brain tissues. Tibolone is capable of inducing the activation of ERß and increasing neuroglobin expression. Avila-Rodriguez

et al. (2016) demonstrated that neuroglobin expression depends on ER-ß activation and tibolone favors both mechanisms [217]. Originally, neuroglobin was reported in neurons but later it was detected in other cell types such as astrocytes [218]. Interestingly, neuroglobin has been associated with neuroprotective effects on several injury models including middle cerebral artery occlusion (MCAO), focal cerebral ischemia, ß-amyloid induced toxicity, oxygen and glucose deprivation [217, 219–221].

Neuroglobin may mediate the response against hypoxia by inducing signal pathways. It has also been documented as a reactive oxygen radical scavenger with NADH oxidase activity to favor anaerobic glycolytic metabolism [217]. Controversial studies based on low levels of neuroglobin and low relative oxygen affinity propose that neuroglobin may exert or participate in collateral roles other than solely oxygen store [217, 222] (See **Figure 2** for further illustration). Additionally, photoactivation (NADH/FMN) experiments demonstrated that neuroglobin participates in the ROS and RNS elimination, suggesting a critical role in removing dangerous highly reactive species [223]. The change in the hexaco-ordinated state of neuroglobin according to normoxic or hypoxic conditions also suggests oxygen sensor capabilities [222]. Proper neuroglobin activity protects neurons and astrocytes against cell death [191]. In this regard, overexpression or induction of neuroglobin may be considered as potential neuroprotective therapies. Interestingly, STEARs such as tibolone are capable of increasing and inducing neuroglobin activity, which have been proposed as potential action mechanisms in

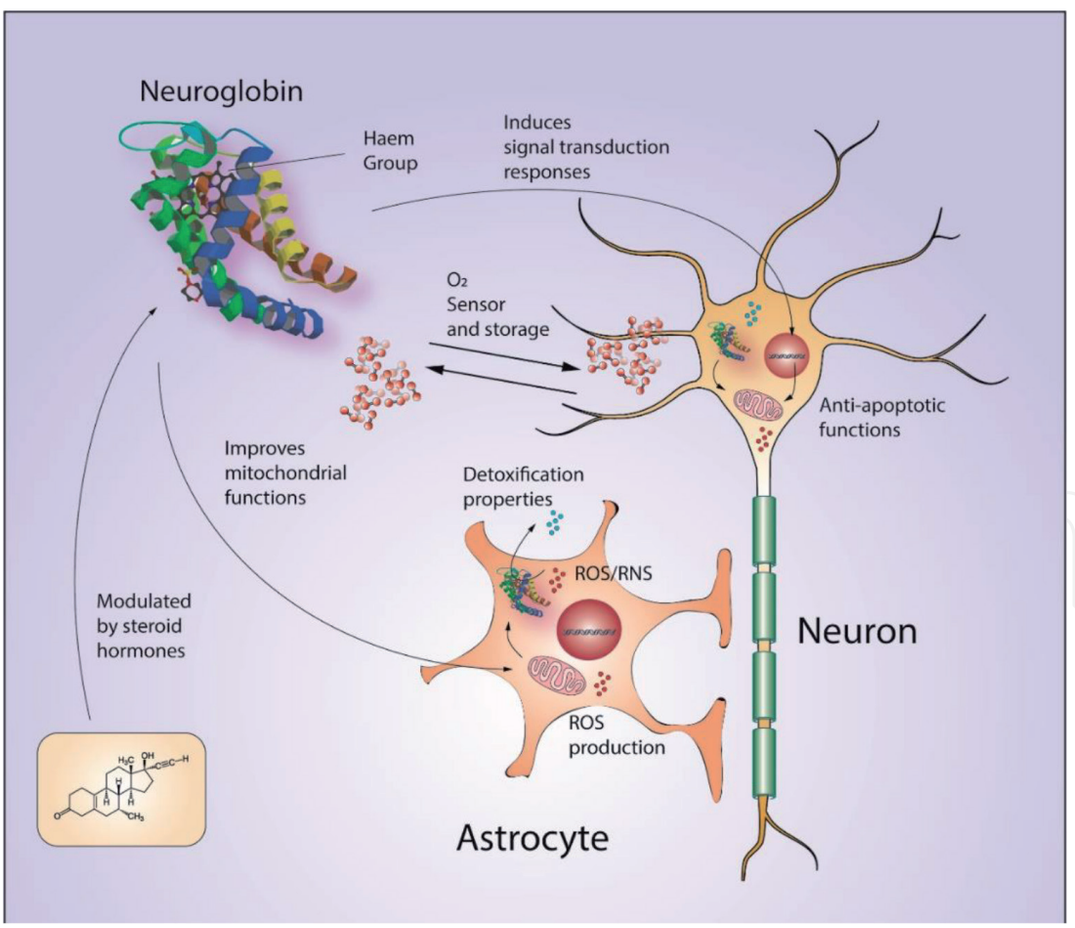

Figure 2.
Neuroglobin exerts interesting beneficial properties. Neuroglobin includes in its protein structure a particular prosthetic haem group to store oxygen. However, it is reported for neuroglobin additional protective functions that include oxygen sensor capabilities and detoxification properties (against reactive oxygen species and reactive nitrogen species). Evidence shows that the protective functions of neuroglobin may be induced via signal transduction mediators including steroid hormones and neurosteroids. For example, some neurosteroids increase neuroglobin production improving mitochondrial functions and inducing anti-apoptotic mechanisms.

brain tissue [191, 217, 222]. According to computations studies and simulations, it has been proposed the neuroglobin may interact with cytochrome c. This apparent interaction may explain the electronic transfer between neuroglobin (ferrous) and cytochrome c (ferric) [191, 224]. Potentially, neuroglobin may modulate cytoplasmic cytochrome c, resulting in diminished apoptotic processes in injured tissues. Surprisingly, De Marinis et al. (2013) showed that neuroglobin hijacks cytochrome c in a neuroblastoma cell model injured via hydrogen peroxide [191]. The estrogenic induction of neuroglobin (and eventually by tibolone) increased neuroglobin expression and diminished the apoptotic cell death mechanism [191].

5. Neuroprotective properties of estrogen and its derivates on brain injury

A derivate of estrogen, 17β-estradiol, is a female sex hormone and neuroactive steroid (NAS) related to the development of secondary sexual characteristics, fat storage and regulation of menstrual cycle [225]. 17β-estradiol, showed beneficial effects in verbal and visual memory performance, which was originally administered as a hormone replacement therapy in order to ameliorate climacteric symptoms [226]. The activity of 17β-estradiol depends on its union with ERs [43, 226, 227]. These receptors are classified in two subtypes: estrogen receptor-beta (ER-β) and estrogen receptor-alpha (ER-α). ERα has its locus in 6 chromosome, while the locus for the Erβ is in the 14 chromosome [226]. These ERs are transcription factors which present the peculiarity of being activated by a ligand. ER-α and ER-β have a similar structure, with a DNA-binding domain and a ligand-binding domain [228]. 17β-estradiol binds to ERs and induces the activation and the homodimerization or heterodimerization of these receptors. Then, the ERs bind to estrogen-responsive elements (EREs) in the promoter region of specific genes through the DNA-binding domain, recruiting transcriptional co-activators and co-repressors [228, 229]. Classical ERs may also regulate gene transcription by acting as transcriptional partners at non-ERE sites, such as activating protein 1 (AP1) sites [230]. 17β-estradiol can bind to membrane-associated non-classical ERs, such as G protein-coupled ERs (GPERs). GPER30, a member of the G protein-coupled receptor superfamily, regulates the activity of extracellular signal-regulated kinases (ERKs) and the phosphoinositide 3-kinase (PI3K) signaling pathway. This union allows the interaction with the signaling of other neuroprotective molecules [228, 231]. Another membrane-associated non-classical ER is Gαq protein-coupled membrane ER (Gq-mER), which was originally identified in hypothalamic neurons, modulating μ-opioid and GABA neurotransmission [228, 232].

These findings have led to research on the neuroprotective properties of estrogen and its derivates in brain injury. In HI brain injury 17β- estradiol has shown several neuroprotective effects, such as: reducing reactive gliosis, decreasing oxidative stress, ameliorating the release of pro inflammatory molecules, preventing cell death and mitochondrial dysfunction, releasing neurotrophic factors [7]. It has also been reported that 17β- estradiol produced significant protection against OGD-induced cell death in primary oligodendrocytes and against oxidative stress, having a potential role in attenuation of HI and oxidative injury [233]. In addition, in neonate rats subjected to HI, three doses of 17β-estradiol (using repeated dosing paradigm) provided approximately 70% protection of the hippocampus, basal ganglia, and amygdala. These results suggest 17β-estradiol acts as a potent neuroprotective agent against HI-induced damage to the developing brain, and that pretreating infants at risk for hypoxic ischemic injury may be advisable [234]. Moreover, treatment with estradiol after PA augmented the expression of IGF-1 and its receptor (IGF-IR). The PI3K/Akt/GSK3 signaling pathway was activated as an increase

in Akt and GSK3 phosphorylation [235]. However, it has been found that male sex is a well-established epidemiological risk factor for poor neurodevelopmental outcome after PA. While the mechanisms responsible for this gender difference are unknown, growing evidence has identified neuro-inflammation, oxidative stress and cell death pathways as key players in these differences [236].

Using a mice model of MCAO with a mutant form of ER-α, neuroprotection was absent, showing that protective properties depend on Er-α [237]. Similarly, after emulating hypoxia in the neuroblastoma cell line SH-SY5Y by using CoCl2 (250 μg/mL), an hypoxic mimetic agent, treatment with 17β-estradiol (250 nM) exerted neuroprotection.

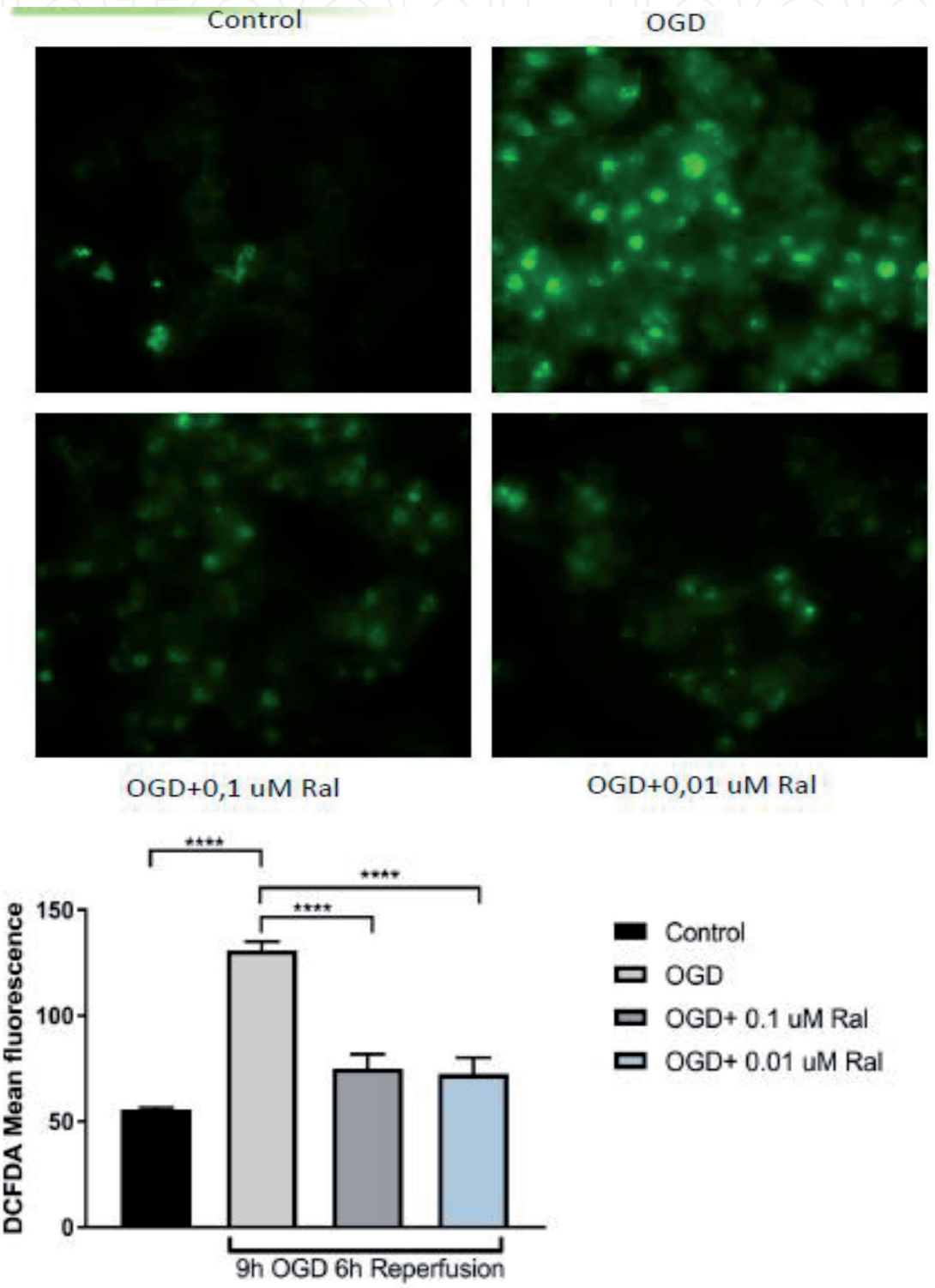

Figure 3.
Ros production.

Afterwards, using ER-α and ER-β agonist (PPT and DPN, respectively) without 17β-estradiol treatment, results showed neuroprotection was mimicked by PPT and suggested that ER-α regulates this protective effect [235]. Likewise, in a model of astrocytic cells it was found that estradiol improved in one of the HI conditions, parameters such as cell viability, mitochondrial membrane potential, reduced ROS production and prevented the loss of mitochondrial mass [38]. Nevertheless, estrogen use can have detrimental effects like the augment in the incidence of breast and uterus cancer [12–14]. In order to maintain the benefits and avoid these side effects, other drugs have been developed, mainly SERMs and STEARs [12–14]. The mechanism of regulation of the SERMs that determines either if they act as agonist or antagonist in an specific cell type depends on the predominant subtype of estrogen receptor alpha or beta. In addition, the co-activators, co- factors and helper proteins of each cell will determine the kind of the response of the tissue exposed to SERMs [238, 239].

In a MCAO rat model, neurogenesis in the ipsilateral subventricular zone (SVZ) after ischemia was significantly higher in estrogen and raloxifene-treated animals compared to rats treated with placebo. Otherwhise, tamoxifen did not show this enhancing effect on neurogenesis. However, both SERMs tamoxifen and raloxifene as well as estrogen, significantly reversed the spine density loss observed in the ischemic cortex at day-5 post ischemia [240]. On the other hand, tibolone action is given by the metabolization of the tibolone to three different metabolites (delta-4 tibolone; alpha-hydroxy tibolone and 3- beta-hydroxy tibolone). Each of them produces different responses. Delta-4 tibolone is an agonist to the androgen receptor and the progesterone receptor, meanwhile alpha-hidroxy and beta-hidroxy tibolone are antagonists of those receptors but agonists of the ER [241]. Keeping this in mind, Avila-Rodriguez et al. (2014) found out that tibolone ameliorates the effects of the GD on an in vitro model of astrocytes, making this molecules interesting for further research in a OGD model [12]. For this reason, in recent years we have been working on the implementation of these neuroprotection strategies in an astrocyte model using Raloxifene as a neuroprotector in the OGD model. **Figures 3** and **4** show the

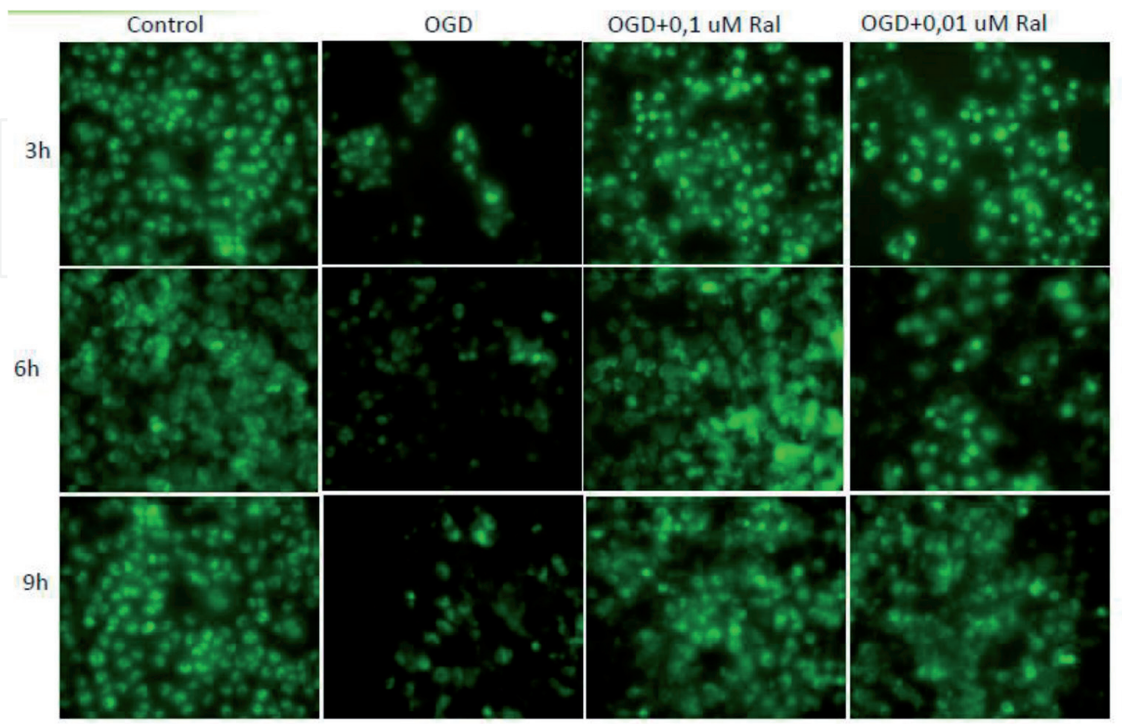

Figure 4.
Mitochondrial mass.

deleterious effect caused by glucose and oxygen deprivation, both in the production of ROS and in the loss of mitochondrial mass, respectively, and how this neuroactive steorid may decrease damage in different concentrations (unpublished data).

6. Conclusion

The different pathologies in which the HI events and with these, the oxygen and glucose deprivation are present, have been shown to exert a high impact on society. Over the years, a multitude of efforts have been directed towards the search for effective treatments that counteract the damage caused by these conditions. The different neuroprotection targets try to combat specific points of damage caused by hypoxia, including oxidative stress, dysregulation of the cell cycle and energy homeostasis [242]. Both in the initial damage phase and in the final one, the different neuroprotective agents may have anti-inflammatory, antioxidant, anti-excitotoxicity or anti-apoptotic capacities [243]. However, due to the complex network of factors that influence these pathologies, such as the cellular interactions (molecular, biochemical, protein, etc.) inherent to the CNS, as well as the gender-dependent response [236] to the use of these neuroprotective agents, the success in the treatments has not been optimal [7]. Estradiol treatment not only prevents neuronal damage, but may also limit the neurodegenerative modifications induced by HI in the early stage of development. The development of SERMs and STEARs brings with it a range of possibilities for the treatment of HI, due to its advantages, focused on the nervous system without having side effects. However, it is necessary to develop new generations of these compounds to improve their neuroprotective effects. Further research is necessary to provide new alternatives in the implementation of new therapeutic strategies and novel approaches.

Author details

Nicolas Toro-Urrego[1*], Marco Avila-Rodriguez[2], María Inés Herrera[1,3], Andrea Aguilar[1], Lucas Udovin[1] and Juan P. Luaces[1]

1 Instituto de Investigaciones Cardiológicas, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, ININCA, UBA-CONICET, Buenos Aires, Argentina

2 Facultad de Ciencias de la Salud, Departamento de Ciencias Clínicas, Universidad del Tolima, Tolima, Colombia

3 Facultad de Psicología y Psicopedagogía, Centro de Investigaciones en Psicología y Psicopedagogía, Universidad Católica Argentina, Buenos Aires, Argentina

*Address all correspondence to: nicolas.toro3@gmail.com

*Share authorship.

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