



Review

Endogenous neuroprotective factors: neurosteroids

Katarzyna Wojtal¹, Michał K. Trojnar¹, Stanisław J. Czuczwar^{1,2}

¹Department of Pathophysiology, Medical University, Jaczewskiego 8, PL 20-090 Lublin, Poland

²Department of Physiopathology, Institute of Agricultural Medicine, Jaczewskiego 2, PL 20-950 Lublin, Poland

Correspondence: Stanisław J. Czuczwar, e-mail: czuczwar@poczta.onet.pl

Abstract:

Neurosteroids are a group of steroid hormones synthesized by the brain in the presence of steroidogenic enzymes. Specific neurosteroids modulate function of several receptors, and also regulate growth of neurons, myelination and synaptogenesis in the central nervous system. Some neurosteroids have been shown to display neuroprotective properties, which may have important implications for their potential use in the treatment of various neuropathologies such as: age-dependent dementia, stroke, epilepsy, spinal cord injury, Alzheimer's disease (AD), Parkinson's disease (PD) and Niemann-Pick type C disease (NP-C). This paper focuses on neuroprotection afforded by neurosteroids.

Key words:

neurosteroids, neuroprotection

Abbreviations: 3 α -HSOR – 3 α -hydroxysteroid oxidoreductase, 3 β -HSD – 3 β -hydroxysteroid dehydrogenase-isomerase, 3 α ,5 α / β -TH PROG – 3 α ,5 α / β -tetrahydroprogesterone, 5 α / β -DH PROG – 5 α / β -dihydroprogesterone, 17 β -HSD – 17 β -hydroxysteroid dehydrogenase, AD – Alzheimer's disease, CHAT – choline acetyltransferase, DA – dopaminergic, DHEA – dehydroepiandrosterone, DHEAS – dehydroepiandrosterone sulfate, EPIA – epiandrosterone, GABA – γ -amino-butyric acid, MCAO – middle cerebral artery occlusion, MPA – medroxyprogesterone acetate, MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, mRNA – messenger ribonucleic acid, Na⁺-K⁺-ATP-ase – sodium-potassium adenosine triphosphatase, NMDA – N-methyl-D-aspartate, NP-C – Niemann-Pick type C disease, P450SCC – cytochrome P450 cholesterol side-chain cleavage, PD – Parkinson's disease, PREG – pregnenolone, PREGS – pregnenolone sulfate, PROG – progesterone

Introduction

Neurodegenerative diseases are characterized by progressive dysfunction and death of neurons. Neurode-

generation may occur by apoptosis, necrosis or both. It is believed that there are many different mechanisms and neurochemical modulators responsible for the central nervous system damage, which may overlap temporarily. Among the most important factors contributing to neuronal cell death are: genetic factors, glutamate-mediated excitotoxicity leading to disturbances in intracellular calcium and sodium metabolism, mitochondrial dysfunction, oxidative stress, growth factor withdrawal, cytokines and toxins [29, 30]. Intensive research carried out in recent years has shown that some steroids synthesized in the nervous system, called 'neurosteroids', display beneficial neuroprotective properties, which may be of particular importance in the treatment of diseases where neurodegeneration is predominant including age-dependent dementia, stroke, epilepsy, spinal cord injury, Alzheimer's disease (AD), Parkinson's disease (PD) and Niemann-Pick type C disease (NP-C).

Neurosteroids, according to the denomination proposed by Baulieu, are steroid hormones that are syn-

thesized in the central and peripheral nervous system either *de novo* from cholesterol or by *in situ* metabolism of blood-borne precursors, and that accumulate in the nervous system independently of classical steroidogenic gland secretion rates [2]. The term 'neuroactive steroids' refers to steroid hormones that exert their effects on neural tissue. Neuroactive steroids may be synthesized both in the nervous system and in endocrine glands [23].

Neurosteroids, including pregnenolone (PREG), dehydroepiandrosterone (DHEA), progesterone (PROG) and their derivatives, are synthesized in the presence of the steroidogenic enzymes [4, 18, 24, 28]. The first step in the biosynthesis of neurosteroids is the conversion of cholesterol to PREG. This reaction is catalyzed by the cytochrome P450 cholesterol side-chain cleavage (P450SCC) in three successive chemical reactions: 20 α -hydroxylation, 22-hydroxylation and scission of the C₂₀-C₂₂ carbon bond of cholesterol. The products of this reaction are PREG and isocaproic acid. PREG can be converted to DHEA, *via* cytochrome P450c17. Both PREG and DHEA are 3 β -hydroxy- Δ 5-steroids which are present in neural tissue in the free forms and their sulfate ester forms [18, 24]. 17 β -hydroxysteroid dehydrogenase (17 β -HSD) and 3 β -hydroxysteroid dehydrogenase-isomerase (3 β -HSD) mediate conversion of DHEA into androgens. Aromatase converts testosterone to estradiol, while 5 α -reductase converts testosterone to dihydrotestosterone [18].

PREG can be oxidized to active 3-oxo- Δ 4-steroids such as PROG by the 3 β -HSD. PROG is a substrate for 5 α / β -reductase enzymes and is converted into 5 α / β -dihydroprogesterone (5 α / β -DH PROG). Further reduction of 5 α / β -DH PROG at the C₃ position, by the 3 α -hydroxysteroid oxidoreductase (3 α -HSOR), yields 3 α -hydroxy-5 α / β -pregnan-20-one (3 α ,5 α / β -tetrahydroprogesterone; 3 α ,5 α / β -TH PROG; allo-pregnanolone) [24].

Neurosteroids act in the nervous system in an auto-/paracrine configuration [24]. They may regulate gene expression by binding to nuclear receptors or affect neurotransmission through action at membrane ion-gated and other neurotransmitter receptors [18, 24]. PROG, produced by Schwann cells in response to their stimulation by adjacent neurons, regulates myelin protein synthesis *via* a nuclear PROG receptor [24]. The 3 α -reduced metabolites of PROG (3 α ,5 α / β -TH PROG; allopregnanolone) are potent positive allosteric modulators of γ -amino-butyric acid

(GABA_A) receptors. Activation of GABA_A receptor increases the duration and frequency of ligand-gated chloride channel opening, which leads to chloride entry into neurons and thus membrane hyperpolarization and reduced neuronal excitability [15]. Behavioral effects associated with GABA_A receptor activation are as follows: decreased anxiety, sedation and decrease in seizure activity [18]. 3 α ,5 α -TH PROG affects neuronal growth, survival and differentiation, causes regression of neuritic extensions before they have established contact with other neurons or glia, and protects neurons from death induced by picrotoxin [9]. In contrast to 3 α -reduced pregnane steroids, PREGS and DHEAS display GABA-antagonistic properties [15]. Inhibition of GABA_A receptor function produces effects ranging from anxiety and excitability to seizure susceptibility [29].

PREGS and DHEA, but not DHEAS, potentiate the effects of N-methyl-D-aspartate (NMDA) in increasing intracellular calcium level. This mechanism may be responsible for growth of axons induced by DHEA. DHEAS stimulates dendrites to grow, but underlying mechanism remains unknown [18, 24].

DHEAS acts as sigma-1 receptor agonist, while PREGS appears to be a sigma-1 receptor inverse agonist. PROG acts as sigma-1 receptor antagonist [19]. Selective sigma-1 receptor ligands exert a potent neuromodulating effect on excitatory neurotransmitter systems, including the glutamatergic and cholinergic systems. Modulation of NMDA-mediated glutamatergic neurotransmission by selective sigma-1 receptor ligands plays a vital role in major neuroadaptational phenomena, such as long-term potentiation, learning and memory, seizures, acute neuronal death and neurodegeneration [17, 19].

Modulatory role of neurosteroids includes also their influence on nicotinic, muscarinic, serotonin, kainate and glycine receptor functions [5, 17, 28].

Neuroprotective properties of neurosteroids

The pathophysiology of AD is thought to be attributable to the effects of β -amyloid, a peptide that accumulates in the brain causing neurotoxicity and degeneration [30]. Weil-Engerer et al. [32] have found decreased levels of neurosteroids in certain brain

Tab. 1. Neuroprotective properties of neurosteroids

Neurosteroid	Neuroprotective properties	References
DHEA	– protection of mouse striatal DA neurons against MPTP	[6]
	– protection of rat hippocampal hilar neurons against KA-induced death	[31]
7- α -hydroxy-DHEA	– antiglucocorticoid-mediated neuroprotection*	[20]
7- β -hydroxy-DHEA	– antiglucocorticoid-mediated neuroprotection*	[20]
DHEAS	– protection of hippocampal neurons against glutamate-induced neurotoxicity*	[16]
	– partial neuroprotection against 1-methyl-4-phenylpyridinium-, colchicine-, glutamate- and NMDA-induced neurotoxicity*	[11]
	– protection of rat cultured cerebellar granule cells against oxygen and glucose deprivation*	[11]
	– neuroprotection in an animal model of reversible spinal cord ischemia (in rabbits)	[13]
7- α -hydroxy-EPIA	– protection against hypoxia-induced neuronal damage*	[25]
7- β -hydroxy-EPIA	– protection against hypoxia-induced neuronal damage*	[25]
	– neuroprotection in rat model of global forebrain ischemia	[25]
	– neuroprotection in rat model of focal cerebral ischemia	[25]
PROG	– prevention of striatal depletion in C57B/6 mice evoked by MPTP	[4]
	– protection of rat hippocampal hilar neurons against KA-induced death	[31]
	– neuroprotection against glutamate-induced neurotoxicity*	[22]
	– neuroprotection in Wobbler mouse mutants presenting severe motoneuron degeneration and astrogliosis of the spinal cord	[7, 8]
	– neuroprotection after experimental central and peripheral nervous system injury in rats	[12]
19-norprogesterone	– neuroprotection against glutamate-induced neurotoxicity*	[22]
allopregnanolone	– neuroprotection in NP-C mouse	[9]

* – *in vitro* study. DA – dopaminergic, DHEA – dehydroepiandrosterone, DHEAS – dehydroepiandrosterone sulfate, EPIA – epiandrosterone, MPTP – 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, KA – kainic acid, NMDA – N-methyl-D-aspartate, NP-C – Niemann-Pick type C disease, PROG – progesterone

regions of AD patients compared with aged patients without dementia. Moreover, the higher concentrations of proteins implicated in the formation of plaques and neurofibrillary tangles (β -amyloid peptides and phosphorylated tau proteins), the lower the levels of pregnenolone sulfate (PREGS) in the striatum and cerebellum and dehydroepiandrosterone sulfate (DHEAS) in the hypothalamus have been observed [32].

Manifestation of PD are the result of reduced dopaminergic input into the striatum, caused by neuronal degeneration in the pars compacta of the substantia nigra. In some families PD is inherited as an autosomal dominant trait. In experimental animals, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication exerts similar disorders to those observed in patients who suffered from PD [30]. DHEA proved to provide protection of dopaminergic (DA) neurons in the striatum of mice against MPTP [6].

Studies by Mao and Barger [16] have shown that DHEAS protects hippocampal neurons against glutamate-induced neurotoxicity, while little protection is obtained with equivalent doses of DHEA itself. The authors conclude that the neuroprotective properties of DHEAS may be connected with its ability to enhance a kappa B-dependent transcription factor activity, which has also been confirmed by the fact that suppression of kappa B binding to DNA inhibits the neuroprotective activity of DHEAS [16]. Neuroprotective action of DHEAS has been also documented in an *in vitro* model of ischemia (oxygen and glucose deprivation in rat cultured cerebellar granule cells). DHEAS offered almost full neuroprotection and eliminated the apoptotic features of the oxygen-glucose deprivation-evoked neuronal death. These effects were inhibited by both a GABA_A receptor-linked chloride channel agonist (pentobarbital) and

antagonist (picrotoxin) [11]. DHEAS has been noted to afford partial neuroprotection against 1-methyl-4-phenylpyridinium-, colchicine-, glutamate- and NMDA-induced neurotoxicity [11]. In the study carried out by Lapchak et al. [13], DHEAS proved to have neuroprotective properties in an animal model of reversible spinal cord ischemia. If administered 5, but not 30 minutes, after the start of occlusion of the infrarenal aorta, it allowed to prolong aorta clamping without the risk of permanent paraplegia in 50% animals. This effect was abolished by bicuculline, the GABA_A antagonist, which suggests possible involvement of GABA_A-mediated events in the neuroprotective action of DHEAS [13].

Current data indicate that 7-hydroxylation may play an important role in the neuroprotection afforded by neurosteroids. Jellinck et al. [10] have found that 7- α - and 7- β -hydroxy-DHEA are the main metabolites formed by the rat hippocampal astrocytes in response to excitatory amino acid-induced toxicity. In the experiment carried out by Pringle et al. [25], the steroids: estradiol, DHEA and epiandrosterone (EPIA) exhibited no protection against *in vitro* hypoxia-induced neuronal damage, while 7- α - and 7- β -hydroxy metabolites of EPIA significantly reduced neurotoxicity in this model of ischemia. Moreover, 7- β -hydroxy-EPIA turned out to be effective in two *in vivo* rat models of cerebral ischemia (global forebrain ischemia and focal ischemia) [25]. In another study, 7- α - and 7- β -hydroxy-DHEA revealed its neuroprotective potency mediated by antiglycorticoid activity, which once again confirmed the hypothesis that the 7-hydroxylation process may be relevant to neuroprotection offered by neurosteroids [20].

Veiga et al. [31] reckon that neuroprotective effects of PREG and DHEA may be due to their conversion to testosterone and the consecutive conversion of testosterone to estradiol, whose neuroprotective properties have been fairly well documented in current literature. PROG and DHEA, in a dose-dependent manner, protected hilar neurons in the rat hippocampus against kainic acid-induced neuronal death. However, these hormones showed no effect in the presence of fadrozole, an aromatase inhibitor, which blocked estradiol formation [31].

Several studies have demonstrated that PROG exhibits neuroprotective effects in experimental central and peripheral nervous system injury. Intensive PROG treatment of spinal-cord-transected rats restored choline acetyltransferase (CHAT) immunore-

activity and the expression of the regulatory subunits of neuronal Na⁺-K⁺-ATP-ase reduced by the injury, and also up-regulated GAP-43 mRNA in the ventral horn neurons, which may be responsible for repair processes after injury [12]. PROG therapy has also been proved effective in the Wobbler mouse mutants presenting severe motoneuron degeneration and astrogliosis of the spinal cord. According to Gonzalez Deniselle et al. [7, 8], PROG diminishes symptoms of neurodegeneration in Wobbler mice and prolongs their lives. The hormone, however, remains ineffective in preventing astrocytosis. The neuroprotective properties of PROG may be connected with its ability to normalize Na⁺-K⁺-ATP-ase mRNA levels [7, 8]. PROG and 19-norprogesterone, unlike to medroxyprogesterone acetate (MPA), protected the hippocampal neuron cultures against glutamate toxicity. In this case, neuroprotective effects of PROG and 19-norprogesterone probably resulted from their ability to increase the expression of the antiapoptotic factor bcl-2, as MPA is devoided of this potency [22]. In the study carried out by Callier et al. [4], PROG prevented striatal dopamine depletion in C57B1/6 mice caused by MPTP.

Considering the fact that PROG is also a gonadal hormone, it should be mentioned that the susceptibility to neurological injury differs depending on gender and the day of ovarian cycle in females. Recent studies have demonstrated that sexually mature females are less susceptible to central nervous system injury than males and ovariectomized females. Furthermore, during estrous phase of the cycle female animals are more protected than during proestrus [1, 3, 27]. These data indicate that circulating ovarian hormones, including neuroactive PROG, afford neuroprotection.

Nevertheless, not all results are so promising. PROG by itself did not prevent neuronal loss induced by kainic acid in ovariectomized rats, although when combined with estradiol proved to be efficacious [1]. In the ovariectomized female rats, after middle cerebral artery occlusion (MCAO), exogenous PROG therapy not only turned out to be ineffective in terms of neuroprotection, but even, when administered chronically, exacerbated striatal stroke injury [21].

NP-C is an autosomal recessive neurodegenerative disease caused by mutations in the lysosomal NPC1 protein. The lack of NPC1 protein in cells results in the accumulation of unesterified cholesterol and glycosphingolipids in lysosomes contributing to the NP-C neurological phenotype (ataxia and tremor) and

to the neuronal and glial loss [30]. Griffin et al. [9] have shown a decreased level of neurosteroids, especially allopregnanolone, and also a decrease in steroidogenic enzymes (3β -HSD and 5α -reductase) activity in the cortex and cerebellum of NP-C mice. Treatment of NP-C mice with allopregnanolone alleviated some of the neurodegenerative features of NP-C, which suggests that disrupted synthesis of neurosteroids may be a factor leading to neurodegeneration and supplementation of allopregnanolone deficits may protect neurons from death in NP-C. Neuroprotective effects of neurosteroids are listed in Tab. 1.

Conclusions

In the light of the experimental data concerning neuroprotection it should be emphasized that neurosteroids do exhibit neuroprotective properties. The mechanism by which these agents provide neuroprotection is probably multifactorial and both, genomic and nongenomic mechanisms may be involved. However, in some studies neurosteroids were shown to be neutral or even, in one study [21], had adverse effect on the central nervous system (chronic treatment with PROG in the ovariectomized rats potentiated striatal stroke injury after MCAO), which, of course, requires further research.

Most studies on neuroprotective action of neurosteroids have been conducted on animals and its results cannot be fully extrapolated to humans, therefore, there is also a need for clinical trials confirming neuroprotective effects of neurosteroids in humans. Another problem may be associated with common metabolic pathways of neurosteroids. In this context, administration of a given neuroactive steroid would affect concentrations of the other ones.

Although the existing knowledge on the neuroprotective properties of neurosteroids is still incomplete, this group of steroids seems to hold promise in the management of neurodegenerative conditions such as age-dependent dementia, cerebrovascular diseases, traumatic cerebral injuries, epilepsy, AD or Parkinson's disease. It is very likely that the GABA-mimetic effects of neurosteroids would be of particular importance in this regard – to be true, a synthetic neurosteroid, ganaxolone with an apparent GABAergic activity, is at present an effective drug for the treatment of

catamenial epilepsy [26]. However, other mechanisms cannot remain underestimated – Leśkiewicz et al. [14] are of opinion that a negative modulation of excitatory amino acid release may be important as well.

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