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Effects of neurosteroids on neuronal survival: Molecular basis and clinical perspectives

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Review

Abstract. Neurosteroids have long been known to act as important modulators of central nervous system functions. The concept of their mechanism of action, however, have essentially undergone an evolution. Previously, these compounds were postulated to regulate neuronal function mainly via allosteric regulation of some membrane-bound receptors, such as $GABA_A$ and NMDA receptors, in a non-genomic way. Recent studies have provided evidence for intracellular targets for neurosteroids, e.g., transcription factors (NFκ-B, progesterone receptors), protein kinases (phosphatidylinositol 3-kinase, protein kinase C), or microtubule-associated proteins, i.e. factors essential in regulation of neuronal survival and apoptosis. This paper reviews *in vitro* and *in vivo* data on neurosteroid involvement in the regulation of neurodegenerative processes with emphasis on new intracellular and genomic mechanisms of their action. Potential utility of neurosteroids in the treatment of some neurodegenerative disorders has been also discussed.

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INTRODUCTION

Neurosteroids are precursors (pregnenolone, dehydroepiandrosterone; PREG, DHEA) and metabolites (allopregnanolone, allotetrahydrodeoxycorticosterone; THP, THDOC) of steroid hormones which influence the excitability of neurons predominantly by non-genomic mechanisms. In contrast to steroid hormones that regulate gene transcription through interactions with intracellular receptors, neurosteroids can alter the function of membrane-bound receptors in the central nervous system. Recent studies have shown that neurosteroids can be synthesized in the brain, are strong modulators of some membrane receptors for neurotransmitters, influence neurodevelopmental processes and modulate bioelectric activity of neurons.

SYNTHESIS OF NEUROSTEROIDS IN THE CNS

Neurosteroids are synthesized *de novo* from cholesterol or peripherally derived sources, in mitochondria of glial cells, such as oligodendrocytes and astrocytes, and neurons (Mellon et al. 2001). Cholesterol is transported into the mitochondrion by a peripheral type of benzodiazepine receptor and then is converted into pregnenolone, the main precursor of all steroids, by the cytochrome P450 side-chain cleavage (P450scc) enzyme (Compagnone and Mellon 2000). Pregnenolone is then converted into progesterone by a 3β-hydroxysteroid dehydrogenase or to DHEA by a 17α-hydroxylase and 17, 20-liase. Oligodendrocytes are the main source of pregnenolone whereas conversion of pregnenolone to progesterone and DHEA to androstendione occurs in astrocytes (Zwain and Yen 1999).

Dehydroepiandrosterone and pregnenolone are found at high concentration in the central nervous system both as free compounds and sulfate esters. High activity of 5 α-reductase and 3 α-hydroxysteroid oxidoreductase, which process peripheral progesterone and desoxycorticosterone to allopregnanolone and THDOC, respectively, has been detected in the brain. The latter neurosteroids are ring A-reduced derivatives of steroid hormones and possess hydroxyl group in 3α position (Fig. 1).

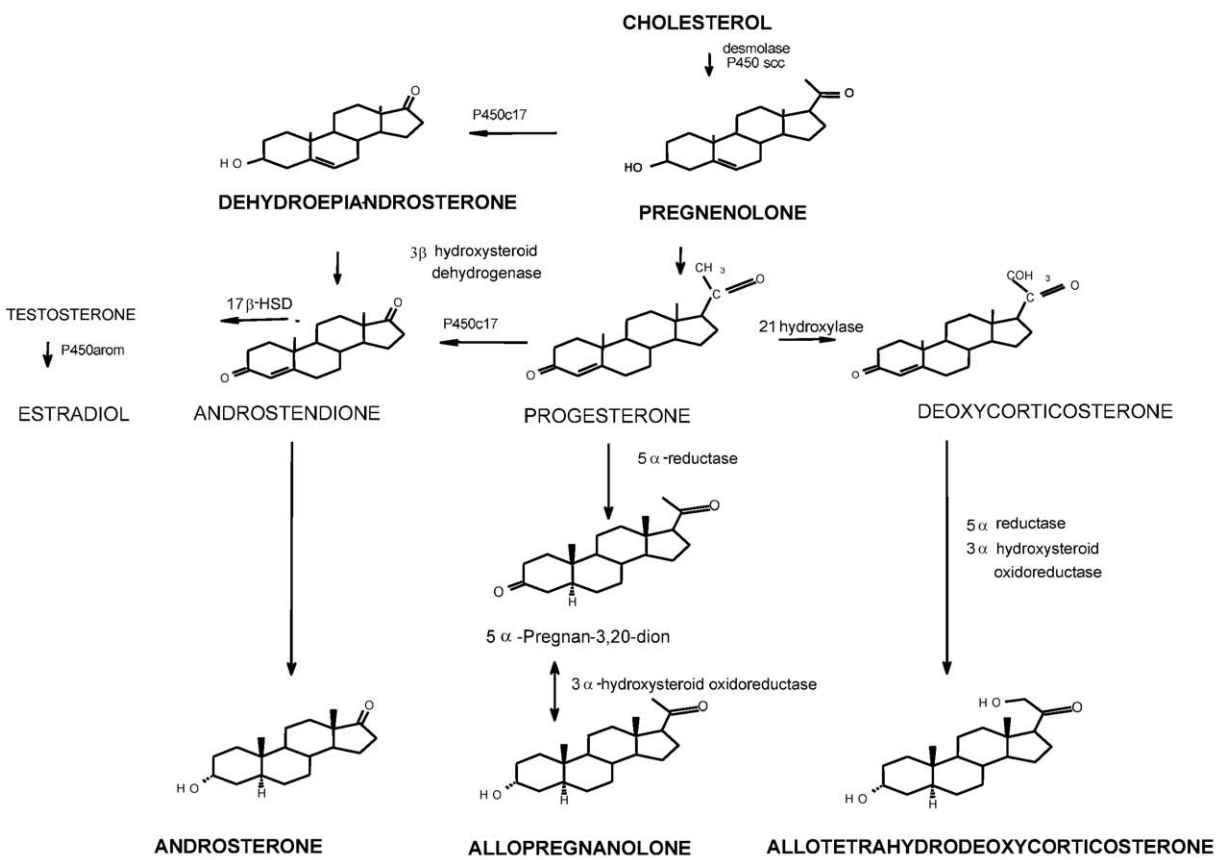

Fig. 1 Biosynthesis and metabolism of steroid hormones in CNS

MECHANISM OF ACTION

Neurosteroids influence the excitability of the central nervous system by an allosteric modulation of $GABA_A$ receptors, excitatory amino acid receptors (NMDA, AMPA, kainate) and σ receptors (Majewska et al. 1986, Maurice et al. 1999, Wu et al. 1991).

$GABA_A$ receptors are molecular targets for a variety of sedative-hypnotic drugs including benzodiazepines and barbiturates. Neurosteroids which possess reduced ring A and hydroxyl group in the position 3 α (THP and THDOC) show agonistic activity at $GABA_A$ receptor (inhibitory neurosteroids). This receptor is an oligomeric gated chloride channel that, when activated by GABA or agonists, initiates chloride entry into neurons thereby inducing membrane hyperpolarization and reducing neuronal excitability. THP and THDOC at nanomolar concentrations augment GABA action, potentiate binding of agonists: muscimol and benzodiazepines and decrease binding of an antagonist t-butylbicyclophosphorothionate (TBPS), whereas at high micromolar concentrations neurosteroids open chloride channels (Majewska et al. 1986). The affinity of inhibitory neurosteroids for $GABA_A$ receptors is responsible for their anxiolytic, antiepileptic and sleep-inducing properties (Budziszewska et al. 1998, Crawley et al. 1986, Gąsior et al. 1999, Mendelson et al. 1987, Reddy 2004). Among neurosteroids, allopregnanolone is the most potent agonist of $GABA_A$ receptor. It inhibits seizures evoked by $GABA_A$ receptor antagonists, e.g., bicuculline, picrotoxine, pentetrazole (Belelli et al. 1990, Kokate et al. 1994), and those induced by excitatory amino acids such as NMDA or kainate (Budziszewska et al. 1998, Leśkiewicz et al. 1997). Activation of $GABA_A$ receptors by allopregnanolone also leads to the inhibition hypothalamic-pituitary-adrenal (HPA) axis activity.

On the other hand, PREG(S) and DHEA(S) have been reported to antagonize the $GABA_A$ receptor by interacting with other sites then inhibitory neurosteroids (Park-Chung et al. 1999). Neurosteroids may also activate receptors of other neurotransmitter systems, first of all the ionotropic NMDA receptor. PREGS and DHEAS positively modulate several NMDA-receptor-mediated responses, and thus they appear to be excitatory neurosteroids. At nanomolar concentration, PREGS specifically enhanced the NMDA-gated currents in spinal cord neurons and cultured rat hippocampal neurons (Irwin et al. 1992, Wu et al. 1991). DHEAS potentiated the intracellular Ca^{2+} fluxes mediated through NMDA-receptor channels in mouse neocortical neuronal cultures (Compagnone and Mellon 1998). Some neurosteroids, isomers of allopregnanolone (5β-pregnan-3α-ol-20-one, 5β-pregnan-3β-ol-20-one) behave like negative modulators and inhibit NMDA-induced conductance. Neurosteroids also interact with the sigma (σ) receptors. PREGS acts as a σ_1 receptor inverse agonist, DHEAS appears to be a σ_1 receptor agonist, whereas progesterone acts as a σ_1 receptor antagonist (Monnet et al. 1995). The agonistic effect of PREG and DHEA on σ_1 receptors enhances acetylcholine release. Besides acetylcholine, neurosteroids modulate release of other neurotransmitters, such as noradrenaline, dopamine and excitatory amino acids (Budziszewska et al. 1998, Dazzi et al. 1996, Jaworska-Feil et al. 1998, Leśkiewicz et al. 2002, Monnet et al. 1995).

Although it is commonly accepted that the modulation of membrane neurotransmitter receptors is the main mechanism of neurosteroid action, recent data point also to the involvement of intracellular nuclear receptors in effects of some reduced derivatives of progesterone or deoxycorticosterone. The latter compounds themselves are devoid of affinity for nuclear receptors; however, they are oxidized in vivo to dihydroxy derivatives, which can activate progesterone receptors. For example, it has been demonstrated that allopregnanolone, after conversion to 5α-dihydroprogesterone, stimulates progesterone receptor-mediated gene transcription (Rupprecht 1997). Regarding excitatory neurosteroids, it has been found that DHEA enhances androgen receptor-mediated gene expression (Mo et al. 2004).

No neurosteroid itself has been found to activate the glucocorticoid receptor (Rupprecht et al. 1996), but they might modulate glucocorticoid action *via* their interaction with various protein kinases, e.g., protein kinase A (PKA), protein kinase C (PKC), Ca^{2+}/calmodulin-dependent protein kinase (CaMK), mitogen-activated protein kinase (MAPK) involved in the regulation of corticosterone-induced gene transcription (Maroder et al. 1993, Moyer et al. 1993, Ning and Sanchez 1995, Nordeen et al. 1994). In fact, inhibitory action of allopregnanolone on glucocorticoid-induced gene transcription in mouse fibroblast cells was shown to involve protein kinase C and extracellular signal-regulated kinase (ERK)-MAPK (Basta-Kaim et al. 2007). On the other hand, DHEAS which has been

shown to possess antiglucocorticoid activity *in vivo*, had no effect on GR-mediated gene transcription (Basta-Kaim et al. 2007, Di Santo et al. 1996b).

Apart from membrane and intracellular receptors, a new target for neurosteroid action has been recently proposed by Plassart-Schiess and Baulieu (2001). These investigators found that PREG and PREGS bind to neural microtubule-associated protein type 2 and accelerate microtubule polymerization in this way regulating neuronal plasticity (Plassart-Schiess and Baulieu 2001).

EFFECTS OF NEUROSTEROIDS ON NEURONAL DEGENERATION

A growing body of evidence indicates that both positive and negative steroid modulators of $GABA_A$ receptors are involved in the regulation of excitotoxic and apoptotic processes (Wojtal et al. 2006). *In vivo* studies demonstrated protective effects of allopregnanolone in models of traumatic brain injury (Djebaili et al. 2004, He et al. 2004) and in focal cerebral ischemia (Sayeed et al. 2006). Repeated peripheral administration of allopregnanolone in rats subjected to contusion of the prefrontal cortex inhibited both apoptotic (caspase-3 activity, DNA fragmentation and Bax level) and astrocytic (glial fibrillary acidic protein) markers in the lesion area and attenuated cognitive deficits in these animals (Djebaili et al. 2004, 2005). In the rat model of bilateral medial prefrontal cortex contusions, allopregnanolone, when administered for five days post-injury, enhanced behavioral recovery as estimated in the Morris water maze test and decreased neuronal loss in the mediodorsal nucleus of the thalamus and the nucleus basalis magnocellularis (He et al. 2004). Further study conducted by these investigators shed some light on the mechanism of neuroprotective action of allopregnanolone showing that this compound decreased the level of interleukin-1β and tumor necrosis factor-α, cytokines which play the major role in neuronal loss and cognitive deficits in traumatic brain injury. In the model of focal cerebral ischemia in rats, morphometric analysis revealed that allopregnanolone significantly reduced cortical and hemispheric infarct volumes (Sayeed et al. 2006). The above *in vivo* data indicate that neuroprotective effects of allopregnanolone are stereospecific (epiallopregnanolone was inactive), require repeated administration and are usually stronger than those exerted by progesterone. Intraperitoneal injection of allopregnanolone at non-sedative doses also attenuates status-epilepticus-related hippocampal cell damage: however, this effect may be connected with its anticonvulsant activity (Leśkiewicz et al. 1997).

In vitro data also point to neuroprotective effects of allopregnanolone in models of neuronal injury induced mainly by excitatory amino acids. Lockhart and coauthors (2002) showed that allopregnanolone inhibited NMDA-induced lactate dehydrogenase (LDH) release, reduced the percentage of TUNEL-positive cells and prevented a decrease in mitochondrial membrane potential in human NT2 cell line. This neurosteroid showed also ability to inhibit the NMDA-induced apoptosis in P19-derived neurons, preserving cytochrome c release to the cytoplasm and Bax translocation to the mitochondria (Xilouri and Papazafiri 2006). Regarding the mechanism of allopregnanolone action, it has been suggested that it interacts with mitochondrial pathway of apoptosis (Lockhart et al. 2002). Besides attenuating the NMDA toxicity, allopregnanolone was reported to prevent the kainate-induced LDH release in the primary culture of rat cerebral cortical cells and in differentiated PC-12 pheochromocytoma cells (Kajta et al. 1999, Yu et al. 2002). Moreover, this neurosteroid protected PC12 rat cells from serum deprivation-induced apoptosis, and induced the expression of the anti-apoptotic Bcl-2 proteins (Charalampopoulos et al. 2004). It should be mentioned here that there are large differences in concentrations of allopregnanolone used in various *in vitro* studies. Indeed, under the basal physiological condition, the level of allopregnanolone in brain tissue is low (nanomolar range), whereas in some *in vitro* studies it exerted neuroprotective effects when used at micromolar concentrations. However, this steroid can reach locally high concentrations because its synthesis in brain tissue can be enhanced under some pathological conditions, such a stress, seizures, or trauma.

Excitatory neurosteroids are an interesting group of potential neuroprotective agents. They are negative and positive modulators of $GABA_A$ and NMDA receptors, respectively, and interact with σ1 receptors and modulate acetylcholine release in the hippocampus. These features may play a role in positive effects of PREG(S) and DHEA(S) on cognitive and memory processes (Flood et al. 1988, Mayo et al. 1993). Besides the stimulatory effect of pregnenolone on memory, this steroid also modulates neurotoxic phe-

nomena. It protects spinal cord neurons against mechanical injury *in vivo* and hippocampal cells against glutamate- or β-amyloid-induced damage (Gursoy et al. 2001, Guth et al. 1994). On the other hand, pregnenolone sulfate enhances NMDA receptor activity, aggravates NMDA-induced seizures and calcium ion influx into the cells. In contrast to pregnenolone, pregnenolone sulfate potentiates NMDA-induced neuronal damage. Indeed, it has been reported that pregnenolone sulfate at a concentration of 50 μM induced apoptosis of retinal cells (Cascio et al. 2002).

DHEA is another stimulatory neurosteroid which is produced at high concentrations in the human embryo, enhancing neuronal development. Its concentration in blood decreases with age, and it has been postulated that its deficit contributes to some disturbances of the central nervous system function in the elderly. In contrast to DHEA, the level of cortisol increases with age, and there is evidence that high concentrations of this glucocorticoid enhance hippocampal cell loss. DHEA is regarded as a functional antagonist of glucocorticosteroids. In fact, cortisol and DHEA act oppositely on immune system activity, glucose uptake and neurogenesis in the hippocampus. Neuroprotective effects of DHEA have been demonstrated *in vitro* and *in vivo*. DHEA attenuated NMDA-induced CA1 and CA2 hippocampal cell damage (Kimonides et al. 1998) and inhibited lipid peroxidation evoked by hyperglycemia (Aragno et al. 1997). *In vitro* studies have shown that DHEA (10–100 nM) prevents neuronal damage evoked by NMDA, AMPA, kainate and corticosterone (Kimonides et al. 1998, 1999). Furthermore, DHEA ameliorated hippocampal cell injury induced by H_2O_2, sodium nitroprusside and anoxia (Bastianetto et al. 1999, Marx et al. 2000). Anti-apoptotic effects of DHEA and pro-apoptotic effects of DHEAS have been shown in two models of apoptosis, i.e., serum and FGF deprivation (Zhang et al. 2002). In contrast, Kaasik and others (2001) showed anti-apoptotic effects of DHEAS in cells deprived of oxygen and glucose. It is hypothesized that the activation of phosphatidylinositol 3 kinase (PI3-K)/Akt pathway may be important to neuroprotective effects of neurosteroids. Activation of this pathway inhibits effects of several pro-apoptotic agents, such as Bad, GSK-3β, Forkhead and stimulates glycolysis enhancing ATP level and membrane potential. It is known that DHEA activates the above-mentioned pathway in adipocytes; however, its effect on PI3-K/Akt in neuronal cells has been only partially elucidated (Perrini et al. 2004). Our recent results (unpublished), as well as data of Zhang and others (2002), indicate an involvement of PI3-K/Akt pathway in DHEA anti-apoptotic action.

Other intracellular mechanisms of DHEA neuroprotective effects may involve the activation of NFκ-B, inhibition of tumor necrosis factor synthesis in microglia and astrocytes, activation of superoxide dismutase, elevation of reduced glutathione level, and inhibition of glucose-6 phosphate dehydrogenase (Di Santo 1996a, Mao and Barger, 1998, Oberbeck et al. 2001). Moreover, DHEA inhibits corticosterone-induced translocation of JNK-3 to the nucleus (Kimonides et al. 1999). That is an important event, since phosphorylation and activation of MAP kinases (JNK, p38, ERK) is regarded as one of the first hallmarks of cell damage.

Neurosteroids may also affect cytoarchitectonic changes in the central nervous system, stimulating neurogenesis. DHEAS and PGLS have been found to enhance neurogenesis in dentate gyrus of rat hippocampus and prevent age-dependent cognitive impairments (Karishma and Herbert 2002, Mayo et al. 2005). It should be stressed that neurosteroids are implicated in protecting not only brain and spinal cord but also peripheral neurons. Indeed, studies on the peripheral nervous system provide evidence that progesterone, dihydroprogesterone and allopregnanolone increase expression of two myelin proteins (glycoprotein Po and peripheral myelin protein 22) in the sciatic nerve of aged male rats (Melcangi et al. 2003), thus preventing age-related demyelinization.

A POSSIBLE ROLE OF NEUROSTEROIDS IN NEURODEGENERATIVE DISEASES

Both inhibitory and stimulatory neurosteroids may have beneficial effects in some neurodegenerative diseases. Some damaging factors (stress, seizures, traumatic injury in the brain and spinal cord) are known to increase local neurosteroid production, which can be considered as a neuroprotective reaction (di Michelle et al. 2000). A lowered brain concentration of PREGS and DHEAS has been detected in Alzheimer's disease (AD) patients. Moreover, a negative correlation between β-amyloid peptide and PREGS level and between phosphorylated tau proteins and DHEAS brain concentration has been demonstrated (Weill-Engerer et al. 2002).

In contrast, to the level of DHEAS, DHEA level, at least in the cerebrospinal fluid, of patients with AD is higher than in age-matched controls. The enhanced level of DHEA may result from β-amyloid-induced stimulation of its synthesis, which has been postulated as an adaptive neuroprotective response of the organism to β-amyloid toxicity (Brown et al. 2000). Recently, it has been hypothesized that the activity of sufotransferase, 7α-, 7β-hydroxylase and 16α-hydroxylase may be lower in AD. This suggests that the elevated level of DHEA may be a consequence of deficient DHEA sulfatation and hydroxylation. In line with this assumption, approximately 50% decline in cytochrome P45-CYP7B mRNA (7α-hydroxylase, an enzyme which metabolizes DHEA to 7α-hydroxy-DHEA) has been detected in dentate gyrus neurons from AD patients (Yau et al. 2003). Since sulfate and 7-hydroxy-derivatives of DHEA are generally more potent neuroprotectants than DHEA itself, the decrease in DHEA metabolism may lead to weaker action of this neurosteroid in AD patients.

In contrast to AD, less is known about neurosteroid participation in the mechanism of other neurodegenerative disorders. In an experimental model of irreversible fatal Niemann-Pick type C disease associated with deficiency of intracellular cholesterol and/or ganglioside trafficking, a decrease in allopregnanolone level in the cerebral cortex has been reported. Moreover, neonatal administration of this steroid delays occurrence of neurological symptoms, suppresses astrocytic reaction, reduces microglial activation and increases myelination, thus elongating life-span in the model of Npc1 (-/) mice (Ahmad et al. 2005, Mellon et al. 2004). Only experimental evidence suggests a possible participation of DHEA in preventing Parkinson's disease as it has been shown that DHEA protects mouse striatal dopamine neurons against 1-methyl, 4-phenyl,-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic cell loss (D'Astous et al. 2003).

Recent data suggest that neurodegenerative processes may play a role in pathogenesis of some psychiatric diseases, such as major depression and schizophrenia. Therefore, it seems justified to mention that some depressed patients show lower CSF and plasma levels of allopregnanolone, which can be normalized upon antidepressants administration, especially by the selective serotonin reuptake inhibitors (Van Broekhoven and Verkes 2003). Furthermore, in animal models, allopregnanolone showed antidepressant activity. Interestingly, a decrease in DHEA/cortisol ratio in plasma was also observed in depressed patients, and in a few clinical studies the administration of DHEA for 4–6 weeks ameliorated depressive symptoms (Van Broekhoven and Verkes 2003, Wolkowitz et al. 1999). Such long-term treatment with DHEA is known to increase insulin-like growth factor-1 (IGF-1) plasma concentration. Assuming that similar changes occur in the central nervous system, DHEA might activate, *via* IGF-1, the PI3-K/Akt neuroprotective pathway. With regard to the involvement of neurosteroids in pathogenesis of schizophrenia, the data are controversial. Harris and colleagues (2001) found an inverse correlation between schizophrenia symptoms and the DHEA and DHEAS plasma concentration. Furthermore, in one study chronic treatment with DHEA attenuated negative symptoms of this disease (Strous 2005). However, these data have not been confirmed in other studies on psychotic patients (Harris et al. 2001).

CONCLUSIONS

On the basis of preclinical studies, neurosteroids appear to be an important group of endogenous neuroprotectants which affect neuronal survival engaging multiple extra- and intracellular mechanisms. Although traditionally action of neurosteroids was connected mainly with the modulation of $GABA_A$ receptors, new facts suggest that intracellular mechanisms, such as activation of PI3-K/Akt pathways, NFκ-B transcription factor and enhanced microtubule polymerization, might play a more essential role in their neuroprotective effects. It has also been more often postulated that genomic mechanisms involving activation of progesterone and indirect inhibition of glucocorticoid receptors can participate in attenuation of neuronal damage by neurosteroids. In fact, action of excitatory neurosteroids resembles that exerted by some neurotrophic factors. These data indicate that neurosteroids hold promise as potential neuroprotective drugs in the treatment of some neurodegenerative disorders; however, available clinical data are insufficient to support definitive conclusions.

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