



● INVITED REVIEW

Synthetic neurosteroids on brain protection

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doi:10.4103/1673-5374.150640

<http://www.nrronline.org/>

Accepted: 2014-12-17

Abstract

Neurosteroids, like allopregnanolone and pregnanolone, are endogenous regulators of neuronal excitability. Inside the brain, they are highly selective and potent modulators of GABA_A receptor activity. Their anticonvulsant, anesthetic and anxiolytic properties are useful for the treatments of several neurological and psychiatric disorders *via* reducing the risks of side effects obtained with the commercial drugs. The principal disadvantages of endogenous neurosteroids administration are their rapid metabolism and their low oral bioavailability. Synthetic steroids analogues with major stability or endogenous neurosteroids stimulation synthesis might constitute promising novel strategies for the treatment of several disorders. Numerous studies indicate that the 3 α -hydroxyl configuration is the key for binding and activity, but modifications in the steroid nucleus may emphasize different pharmacophores. So far, several synthetic steroids have been developed with successful neurosteroid-like effects. In this work, we summarize the properties of various synthetic steroids probed in trials throughout the analysis of several neurosteroids-like actions.

Key Words: *allopregnanolone; synthetic steroids; GABA_A receptor; neuroprotection; cerebral cortex; hippocampus*

Funding: *This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT, PICT-2006-727) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP-860).*

Rey M, Coirini H. Synthetic neurosteroids on brain protection. Neural Regen Res. 2015;10(1):17-21.

Natural neurosteroids and synthetic steroids

Neurosteroids (NS), a term proposed by the physiologists Baulieu and Robel (1990), is widely used to refer to the steroids synthesized in the brain. Through their interaction with neuronal membrane receptors and ion channels, they are capable to modify the brain excitability (Lambert et al., 2003; Akk et al., 2009). Depending on its chemical structure, the steroids interactions with the GABA_A receptor may produce positive or negative modulations (Majewska, 1992; Reddy, 2003). Among the positive modulators of this receptor are two progesterone's metabolites: the 5 α -pregnane-3 α -ol-20-one (allopregnanolone) and its isomer 5 α -pregnane-3 β -ol-20-one (pregnanolone; Gasior et al., 1999). The interest on these steroids arises from their potential activity as anticonvulsants, anesthetics, anxiolytic or sedative-hypnotic agents (Akk et al., 2007) useful for the treatment of several neurological and psychiatric disorders (Gasior et al., 1999). Also, various physiological and pathophysiological conditions have been associated with changes in allopregnanolone and pregnanolone levels (Akk et al., 2007).

Although the natural NS can be used in epileptic patients (Herzog, 1999), certain properties, like their short biological half-life, avoid their clinical use. For that reason, synthetic

steroids (SS), that exhibit better bioavailability and efficacy, have an important therapeutic potential in brain disorders, becoming an alternative for this kind of pathologies (Reddy and Kulkarni, 2000; Morrow, 2007).

Therefore, there is a considerable interest around NS physiology and synthetic analogues development. The medicinal chemistry of neuroactive steroids (NAS) has been focused in the development of SS analogues preserving the absolute configuration of naturally occurring steroids. Structure/activity studies indicate that the 3 α -hydroxyl configuration is required for binding and activity (Purdy et al., 1990). However, modifications of the steroid nucleus may emphasize different pharmacophores. For example, the 3 β -methylated synthetic analog of allopregnanolone, ganaxolone (3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one) is capable to overcome these limitations, showing effective anticonvulsant properties (Carter et al., 1997; Reddy and Woodward, 2004). In fact, until now, it is the only SS that has been proved in human clinical trials for epilepsy (Nohria et al., 2010).

Neurosteroids and GABA_A receptor function

GABA binding to its receptor gates an intrinsic anion-selective channel. According to the reversal potential of the

permeate ions, the postsynaptic GABA response can be excitatory or inhibitory (Akk et al., 2007). The binding of the convulsant t-butyl-bicyclophosphorothionate (TBPS) to the GABA_A receptor can be allosterically modulated by allopregnanolone and pregnanolone (Ramanjaneyulu and Ticku, 1984). When GABA is present, these metabolites have a significantly increased binding affinity, and under this condition, it is possible to reflect the functional state of this receptor (Majewska, 1992; Hawkinson et al., 1994). Similarly, NAS can also stimulate the binding of flunitrazepam or muscimol to the receptor (Majewska et al., 1986; Hawkinson et al., 1994). The NS exposure enhances the opening probability of the chloride channel, so that the mean time open is increased, resulting in a reduction of neuronal excitability.

Harrison and Simmons (1984) demonstrated that alphaxalone (ALPX; 3 α -hydroxy-5 α -pregnane-11,20-dione), another allopregnanolone synthetic analogue, was able to enhance the GABA-evoked responses. Also, a positive allosteric modulation of GABA_A receptor was found with the SS ganaxolone (Carter et al., 1997; Gasior et al., 1997). Since then, several SS with different features have been developed. It has been described that at least two ent-16-ketosteroid synthetic analogues (3 α -5 α -androst-16-one and 3 α -5 α -4methoxyandrost-16-one; with an absolute opposite configuration to NAS), produced a more potent inhibition of the TBPS binding than ALPX (Qian et al., 2013). Moreover, we showed a decrease in TBPS binding and an increase in flunitrazepam and muscimol binding by the administration of SS epoxies (analogues to allopregnanolone and pregnanolone) with an intramolecular oxygen bridge that keeps the A/B angle of the steroid nucleus in a controlled way (Veleiro and Burton, 2009; Rey et al., 2013).

NAS and SS neuroprotective role

Cumulative evidence indicates the existence of neuroprotective properties of NAS in a variety of experimental paradigms (Schumacher et al., 2004). They have a major influence on the central nervous system (CNS) activity and are essential for growth and survival of neurons and glial cells (Wang et al., 2005; Melcangi et al., 2008). Studies in adult animals after brain injury indicate that NAS have an important role in repairing processes, enhancing myelination and reducing apoptotic processes (Ibanez et al., 2004). During pregnancy, stressful events which lead to transient hypoxia/ischemia, stimulate NAS production in the brain providing further protection (Nguyen et al., 2004). This supports the importance of NAS in brain development and suggests that the exposure to normal NAS levels is critical. In traumatic brain injury, progesterone has the most important repair-promoting actions (He et al., 2004a) and it acts through its reduced metabolites like allopregnanolone (Djebaili et al., 2004; He et al., 2004b; Ardeshiri et al., 2006). The neuroprotective actions of allopregnanolone have been shown in hypoxia-induced brain injury models, where its levels increase in response to acute hypoxic stress, as a protective mechanism to reduce excitotoxicity (Hirst et al., 2006). In fact, we have described a protective effect of allopregnanolone on as-

troglisis (Kruse et al., 2009) and neuronal damage (Kruse et al., 2010) caused by hypoxia in perinatal cultures of cerebral cortex and hippocampus of the rat. Studies with the SS mifepristone (RU486), reported that it acts as a neuroprotective agent against excitotoxicity and traumatic brain injury (Behl et al., 1997; McCullers et al., 2002) and protects Purkinje cells from cell death in postnatal rat and mouse cerebellum organotypic slice cultures (Ghoumari et al., 2003), through the reversion of chloride efflux in the GABA_A receptor elicited by GABA (Rakotomamonjy et al., 2011). Other properties like antiprogestagen and antiglucocorticoids, were observed with their administration. We have also demonstrated that two SS epoxies, (analogues of allopregnanolone and pregnanolone,) were capable to prevent the glial and neuronal damages in the perinatal cultures of cerebral cortex and hippocampus (Rey et al., 2013).

In adults, the brain ischemic stroke is also considered a hypoxic event that compromises the brain functionality. During ischemia, the loss of energy supply by the mitochondrial dysfunction and posterior increased oxidative stress contributed to the neuronal injury. Therefore, a trend has been set in the development of steroid drugs that reduce the excitotoxicity and the oxidative stress, for treatments of acute brain injuries or chronic neurodegenerative diseases. Because the current therapies are still limited the promotion of novel neuroprotectants is essential for the ischemic stroke treatment. One example is the SS 5 α -androst-3 β ,5,6 β -triol showed a robust neuroprotective effects when it was tested *in vitro* (Chen et al., 2013).

The Alzheimer's disease (AD) produces a brain degenerative process, with neuronal losses and decreased synapses. Present therapies are focused on stopping the progression of the disease, but the major challenge remains, in restore cognitive function through the regeneration of lost neurons and neural circuitry. In aged and AD brains, the pool of neural stem cells, their proliferative potential and the allopregnanolone content are markedly diminished (Bernardi et al., 1998; Genazzani et al., 1998; Weill-Engerer et al., 2002). Studies using transgenic AD mice showed that allopregnanolone has neurogenic properties (Wang et al., 2008). These *in vitro* and *in vivo* neurogenic features, coupled to low molecular weight, easy blood brain barrier penetration and lack of toxicity, are the key elements required to consider the use of allopregnanolone as a neurogenic/regenerative therapy for neurons restoration in AD patients (Brinton and Wang, 2006; Irwin and Brinton, 2014). Estrogen has also showed neuroprotective properties, preventing the development of neurodegenerative disorders like AD. Hormonal therapy at menopause (to restore normal levels) appears to reduce the risks, but this kind of treatment has been associated with detrimental effects. Therefore, the development of SS with a selective agonist action is promising. Moreover, estrogen like neuroprotection effects were observed with the SS 4-estren-3 α ,17 β -diol that differs structurally from estrogens only on the A ring (Kousteni et al., 2002; Cordey et al., 2005). In addition, similar neuroprotective actions have been described with the SS ent-steroid of 17 β -estradiol (Covey, 2009).

Neurosteroids synthesis: steroid effects on 3 β -HSD activity

Another important issue is the influence of the SS on the local natural NS synthesis. NAS are present in the nervous system and in other steroidogenic tissues, like gonads and adrenal glands. In the CNS, NS synthesis occurs in glial and neuronal cells. Within the mitochondrial matrix, the cholesterol is converted to pregnenolone by the cytochrome P450 side-chain cleavage enzyme (CYP450sc; Iwahashi et al., 1990). Then, the pregnenolone is oxidized to progesterone by the 3 β -hydroxysteroid dehydrogenase enzyme (3 β -HSD; Zwain and Yen, 1999) being this conversion an essential step in the biosynthesis of all steroid hormones. Allopregnanolone is synthesized from progesterone, by the sequential enzymatic steps of the type I 5 α -reductase (5 α -R) and the 3 α -hydroxysteroid dehydrogenase enzymes (3 α -HSD; Mellon et al., 2001). The rate-limiting step in neurosteroidogenesis is the unidirectional reduction of progesterone to the 5 α -dihydroprogesterone (5 α -DHP) by the 5 α -R. Subsequently, the 3 α -HSD catalyzes conversion of 5 α -DHP into allopregnanolone. Functionally expression of these enzymes has been described in pluripotent progenitor cells (Melcangi et al., 1996).

On the other hand, the expression of 3 β -HSD enzyme has been demonstrated in several tissues like adrenal glands, gonads and CNS (Rheume et al., 1991; Guennoun et al., 1995; Coirini et al., 2003). Moreover, pregnenolone conversion into progesterone has been demonstrated in rat homogenates from septum and amygdala (Weinfeld et al., 1980). The co-expression of 3 β -HSD and GABA_A receptor subunits in different brain regions (Laurie et al., 1992; Wisden et al., 1992) gives an anatomo-functional support for the *in situ* production of progesterone and the GABA_A receptor modulation (Guennoun et al., 1995). Although regulatory mechanisms underlying the NS biosynthesis inside the brain remain unclear, it is well known the capacity of steroids of negatively modulate the 3 β -HSD activity in different steroidogenic endocrine glands and in peripheral nervous system, like sciatic nerve (Guennoun et al., 1995; Coirini et al., 2003). Among SS, the RU486 caused an impact on the 3 β -HSD enzyme activity in rat adrenal gland (Albertson et al., 1994) but not in gonads (Sanchez et al., 1989). In our work, we described that SS epoxies caused a dose-dependent decrease on the 3 β -HSD activity. In fact, the analogues of pregnanolone produced less inhibition than those with the conformation allopregnanolone-like (Rey et al., 2013).

Conclusion

NS are endogenous regulators of neuronal excitability (Lambert et al., 2003; Akk et al., 2009). Within the brain, reduced steroids (like allopregnanolone and pregnanolone) are highly selective and potent modulators of the GABA_A receptor functions (Gasior et al., 1999). Thus, their anticonvulsant, anesthetic and anxiolytic properties are useful in the treatment of several neurological and psychiatric disorders (Schüle et al., 2011). Neuroprotective effects against adverse early life

events (Patchev et al., 1997) and neurogenic effects on neurodegenerative diseases, like AD (Brinton and Wang, 2006), have been observed with allopregnanolone administration. Steroids with similar activity like this progesterone metabolite provide big opportunities for therapeutic treatments reducing hormonal side effects (Morrow, 2007; Reddy, 2010). The principal disadvantage of endogenous NS administration is their poor bioavailability caused by their rapid *in vivo* metabolism. Thus, endogenous NS stimulation synthesis or synthetic steroids analogues (Poisbeau et al., 2014) might constitute promising novel strategies for several disorders treatments. The current medicinal chemistry around NAS is focused on the development of new SS analogues, having the absolute configuration of natural steroids. Several studies indicate that the 3 α -hydroxyl configuration is the key for binding and activity, but modifications in the steroid nucleus may emphasize different pharmacophores. Among the SS developed are ganaxolone and ALPX which have anesthetic and anticonvulsant properties. Until now, ganaxolone is the only one SS that has been used on human clinical trials for epilepsy (Nohria et al., 2010). On the other hand, the SS ent-neurosteroids produced more potent inhibition of TBPS binding from the GABAA receptor than ALPX (Qian et al., 2013). Moreover, we found that some SS epoxies reduce the TBPS binding and stimulate the flunitrazepam and muscimol binding in a dose-dependent manner (Rey et al., 2013). On the other hand, anxiolytic effects are mediated by GABAA receptors (Reddy and Kulkarni, 1997). Therefore NS modulation of this receptor can be translated in SS anxiolytic properties. This type of effects was observed with the synthetic allopregnanolone analogue Co 2-6749 (GMA-839; WAY-141839; 3 α ,21-dihydroxy-3 β -trifluoromethyl-19-nor-5 β -pregnan-20-one; Vanover et al., 2000). In fact, neurosteroidogenic agents, that lack benzodiazepine-like side effects, are promising for the treatment of anxiety and depression (Reddy, 2010).

Neuroprotective effects have been described with several SS in hypoxia-induced brain injury models. Among others, the SS RU486 was able to protect against excitotoxicity and traumatic brain injury (Behl et al., 1997; McCullers et al., 2002) and the 5 α -androst-3 β ,5,6 β -triol showed a neuroprotective action in an ischemic stroke model *in vitro* (Chen et al., 2013). Moreover, in perinatal brain tissues submitted to hypoxic conditions, restricted analogues from allopregnanolone or pregnanolone showed similar properties preventing the glial and neuronal damage (Rey et al., 2013). On the other hand, neurogenic properties on AD were observed with the 4-estren-3 α ,17 β -diol and ent-steroid of 17 β -estradiol administrations (Kousteni et al., 2002; Covey, 2009).

Another issue to take in consideration for the development of SS is related to the presence of all the enzymes necessary for NS synthesis in the brain (Mensah-Nyagan et al., 1999; Agis-Balboa et al., 2006; Do Rego et al., 2009). Although regulatory mechanisms around NS biosynthesis are still unclear, it is well known the capacity of steroids to negatively modulate the 3 β -HSD activity (in almost all steroidogenic tissues) and the importance of a minor effect on these activities by

the SS administration.

Specific enzymes and nuclear hormone receptors for endogenous steroids have structurally defined binding sites. It is important that the SS should be developed lacking the possibility to bind with high affinity to these proteins. Therefore the SS drugs might not strongly interfere with the natural steroids biosynthesis or their specific receptors. It would be also advantageous that the half-life of these new SS might be quite different and potentially longer, than those of steroid already used as anticonvulsants, anxiolytics, or another neuroactive-neurogenic agents. Thus, it is likely that the development of new SS for therapeutical use will continue requiring a great deal of effort with the attendant generation of new knowledge.

Author contributions: MR was responsible for writing the first draft of the manuscript and contributed to its editing and revision. HC was responsible for the review conception, and contributed to the design of the manuscript, writing and editing. Both authors approved the final version of this review.

Conflicts of interest: None declared.

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