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INVITED REVIEW

Synthetic neurosteroids on brain protection

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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, anesthetics 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 3α-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*

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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 5α-pregnane-3α-ol-20-one (allopregnanolone) and its isomer 5α-pregnane-3β-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 3α-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 3β-methylated synthetic analog of allopregnanolone, ganaxolone (3α-hydroxy-3β-methyl-5α-prengan-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; 3α-hydroxy-5α-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 (3α-5α-androsten-16-one and 3α-5α-4methoxy-androsten-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 astrogliosis (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 antiglucorticoids, 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 5α-androst-3β,5,6β-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-3α,17β-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 17β-estradiol (Covey, 2009).

Neurosteroids synthesis: steroid effects on 3β-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 (CYP450scc; Iwahashi et al., 1990). Then, the pregnenolone is oxidized to progesterone by the 3β-hydroxysteroid dehydrogenase enzyme (3β-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 5α-reductase (5α-R) and the 3α-hydroxysteroid dehydrogenase enzymes (3α-HSD; Mellon et al., 2001). The rate-limiting step in neurosteroidogenesis is the unidirectional reduction of progesterone to the 5α-dihydroprogesterone (5α-DHP) by the 5α-R. Subsequently, the 3α-HSD catalyzes conversion of 5α-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 3β-HSD enzyme has been demonstrated in several tissues like adrenal glands, gonads and CNS (Rheaume 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 3β-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 3β-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 3β-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 3β-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, anesthetics 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 3α-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 traduced in SS anxiolytic properties. This type of effects was observed with the synthetic allopregnanolone analogue Co 2-6749 (GMA-839; WAY-141839; 3α,21-dihydroxy-3β-trifluoromethyl-19-nor-5β-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 5α-androst-3β,5,6β-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-3α,17β-diol and ent-steroid of 17β-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 3β-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.

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References

Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A (2006) Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proc Natl Acad Sci U S A 103:14602-14607.

Akk G, Covey DF, Evers AS, Steinbach JH, Zorumski CF, Mennerick S (2007) Mechanisms of neurosteroid interactions with $GABA_A$ receptors. Pharmacol Ther 116:35-37.

Akk G, Covey DF, Evers AS, Steinbach JH, Zorumski CF, Mennerick S (2009) The influence of the membrane on neurosteroid actions at GABA(A) receptors. Psychoneuroendocrinology 34:S59-S66.

Albertson BD, Hill RB, Sprague KA, Wood KE, Nieman LK, Loriaux DL (1994) Effect of the antiglucocorticoid RU486 on adrenal steroidogenic enzyme activity and steroidogenesis. Eur J Endocrinol 130:195-200.

Ardeshiri A, Kelley MH, Korner IP, Hurn PD, Herson PS (2006) Mechanism of progesterone neuroprotection of rat cerebellar Purkinje cells following oxygen-glucose deprivation. Eur J Neurosci 24:2567-2574.

Baulieu EE, Robel P (1990) Neurosteroids: a new brain function? J Steroid Biochem Mol Biol 37:395-403.

Behl C, Trapp T, Skutella T, Holsboer F (1997) Protection against oxidative stress-induced neuronal cell death-a novel role for RU486. Eur J Neurosci 9:912-920.

Bernardi F, Salvestroni C, Casarosa E, Nappi RE, Lanzone A, Luisi S, Purdy RH, Petraglia F, Genazzani AR (1998) Aging is associated with changes in allopregnanolone concentrations in brain, endocrine glands and serum in male rats. Eur J Endocrinol 138:316-321.

Bialer M, Johannessen SI, Levy RH, Perucca E, Tomson T, White HS (2010) Progress report on new antiepileptic drugs: a summary of the Tenth Eilat Conference (EILAT X). Epilepsy Res 92:89-124.

Brinto RD, Wang JM (2006) Preclinical analyses of the therapeutic potential of allopregnanolone to promote neurogenesis in vitro and in vivo in transgenic mouse model of Alzheimer's disease. Curr Alzheimer Res 3:11-17.

Carter RB, Wood PL, Wieland S, Hawkinson JE, Belelli D, Lambert JJ, White HS, Wolf HH, Mirsadeghi S, Tahir SH, Bolger MB, Lan NC, Gee KW (1997) Characterization of the anti convulsant properties of ganaxolone (CCD 1042; 3alpha-hydroxy-3beta-methyl-5alpha-pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma- aminobutyricacid (A) receptor. J Pharmacol Exp Ther 280:1284-1295.

Chen J, Leng T, Chen W, Yan M, Yin W, Huang Y, Lin S, Duan D, Lin J, Wu G, Zhang J, Yan G (2013) A synthetic steroid 5α-androst-3β,5,6β-triol blocks hypoxia/reoxygenation-induced neuronal injuries via protection of mitochondrial function. Steroids 78:996-1002.

Coirini H, Gouézou M, Delespierre B, Liere P, Pianos A, Eychenne B, Schumacher M, Guennoun R (2003) Characterization and regulation of the 3β-hydroxysteroid dehydrogenase isomerase enzyme in the rat sciatic nerve. J Neurochem 84:119-126.

Cordey M, Gundimeda U, Gopalakrishna R, Pike CJ (2005) The synthetic estrogen 4-stren-3 alpha,17 beta-diol (estren) induces estrogen-like neuroprotection. Neurobiol Dis 19:331-339.

Covey DF (2009) ent-Steroids: novel tools for studies of signaling pathways. Steroids 74:577-585.

Djebaili M, Hoffman SW, Stein DG (2004) Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. Neuroscience 123:349-359.

Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, Tonon MC, Pelletier G, Vaudry H (2009) Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. Front Neuroendocrinol 30:259-301.

Gasior M, Carter RB, Goldberg SR, Witkin JM (1997) Anticonvulsant and behavioral effects of neuroactive steroids alone and in conjunction with diazepam. J Pharmacol Exp Ther 282:543-553.

Gasior M, Carter RB, Witkin JM (1999) Neuroactive steroids: potential therapeutic use in neurological and psychiatric disorders. Trends Pharmacol Sci 20:107-111.

Genazzani AR, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, Tonetti A, Nappi RE, Luisi S, Palumbo M, Purdy RH, Luisi M (1998) Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. J Clin Endocrinol Metab 83:2099-2103.

Ghoumari AM, Dusart I, El-Etr M, Tronche F, Sotelo C, Schumacher M, Baulieu EE (2003) Mifepristone (RU486) protects Purkinje cells from cell death in organotypic slice cultures of postnatal rat and mouse cerebellum. Proc Natl Acad Sci U S A 100:7953-7958.

Guennoun R, Fiddes RJ, Gouézou M, Lombès M, Baulieu EE (1995) A key enzyme in the biosynthesis of neurosteroids; 3β-hydroxysteroid dehydrogenase/Δ5–Δ4-isomerase (3β-HSD) is expressed in rat brain. Brain Res Mol Brain Res 30:287-300.

Harrison NL, Simmonds MA (1984) Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res 323:287-292.

Hawkinson JE, Kimbrough CL, Belelli D, Lambert JJ, Prurdy RH, Lan NC (1994) Correlation of neuroactive steroid modulation of [35S] t-butylbicyclophosphorothionate and [3H]-flunitrazepam binding and gamma-aminobutyric acid receptor function. Mol Pharmacol 46:977-985.

He J, Evans O, Hoffman SW, Oyesiku NM, Stein DG (2004a) Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. Exp Neurol 189:404-412.

He J, Hoffman SW, Stein DG (2004b) Allopregnanolone, a progesterone metabolite, enhances behavioral recovery and decreases neuronal loss after traumatic brain injury. Restor Neurol Neurosci 22:19-31.

Herzog AG (1999) Progesterone therapy in women with epilepsy, a 3-year follow-up. Neurology 52:1917-1918.

Hirst JJ, Yawno T, Nguyen P, Walker DW (2006) Stress in pregnancy activates neurosteroid production in the fetal brain. Neuroendocrinology 84:264-274.

Ibanez C, Shields SA, El-Etr M, Baulieu EE, Schumacher M, Franklin RJ (2004) Systemic progesterone administration results in a partial reversal of the age-associated decline in CNS remyelination following toxin-induced demyelination in male rats. Neuropathol Appl Neurobiol 30:80-89.

Irwin RW, Brinton RD (2014) Allopregnanolone as regenerative therapeutic for Alzheimer's disease: Translational development and clinical promise. Prog Neurobiol 113:40-55.

Iwahashi K, Ozaki H S, Tsubaki M, Ohniski J, Taheuchi Y, Ichikawa Y (1990) Studies of the immunohistochemical and biochemical localization of the cytochrome P-450-scclinked monooxygenase system in the adult rat brain. Biochem Biophys Acta 1035:182-189.

Kousteni S, Chen JR, Bellido T, Han L, Ali AA, O'Brien CA, Plotkin L, Fu Q, Mancino AT, Wen Y, Vertino AM, Powers CC, Stewart SA, Ebert R, Parfitt AM, Weinstein RS, Jilka RL, Manolagas SC (2002) Reversal of bone loss in mice by non genotropic signaling of sex steroids. Science 298:843-846.

Kruse MS, Rey M, Barutta J, Coirini H (2009) Allopregnanolone effects on astrogliosis induced by hypoxia in organotypic cultures of striatum, hippocampus and neocortex. Brain Res 1303:1-7.

Kruse MS, Rey M, Veleiro AS, Burton G, Coirini H (2010) Hypoxia impairs the morphology of neurons in cortex and hippocampus organotypic cultures. Biocell 34:A97.

Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA (2003) Neurosteroid modulation of $GABA_A$ receptors. Prog Neurobiol 71:67-80.

Laurie DJ, Wisden W, Seeburg PH (1992) The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J Neurosci 11:4151-4172.

Majewska MD (1992) Neurosteroids: endogenous bimodal modulators of the $GABA_A$ receptor. Mechanism of action and physiological significance. Prog Neurobiol 38:379-395.

Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM (1986) Steroid hormone metabolites are barbiturate-like modulators of the $GABA_A$ receptor. Science 232:1004-1007.

McCullers DL, Sullivan PG, Scheff SW, Herman JP (2002) Mifepristone protects CA1 hippocampal neurons following traumatic brain injury in rat. Neuroscience 109:219-230.

Melcangi RC, Froelichsthal P, Martini L, Vescovi AL (1996) Steroid metabolizing enzymes in pluripotential progenitor central nervous system cells: effect of differentiation and maturation. Neuroscience 72:467-475.

Melcangi RC, Garcia-Segura LM, Mensah-Nyagan AG (2008) Neuroactive steroids: state of the art and new perspectives. Cell Mol Life Sci 65:777-797.

Mellon SH, Griffin LD, Compagnone NA (2001) Biosynthesis and action of neurosteroids. Brain Res Brain Res Rev 37:3-12.

Mensah-Nyagan AG, Do-Rego JL, Beaujean D, Luu-The V, Pelletier G, Vaudry H (1999) Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. Pharmacol Rev 51:63-81.

Morrow AL (2007) Recent developments in the significance and therapeutic relevance of neuroactive steroids-Introduction to the special issue. Pharmacol Ther 116:1-6.

Nguyen PN, Yan EB, Castillo-Melendez M, Walker DW, Hirst JJ (2004) Increased allopregnanolone levels in the fetal sheep brain following umbilical cord occlusion. J Physiol 560:593-602.

Patchev VK, Montkowski A, Rouskova D, Koranyi L, Holsboer F, Almeida OF (1997) Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavioral and neuroendocrine consequences of adverse early life events. J Clin Invest 99: 962-966.

Poisbeau P, Keller AF, Aouad M, Kamoun N, Groyer G, Schumacher M (2014) Analgesic strategies aimed at stimulating the endogenous production of allopregnanolone. Front Cell Neurosci 8:174.

Purdy RH, Morrow AL, Blinn JR, Paul SM (1990) Synthesis, metabolism, and pharmacological activity of 3α-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. J Med Chem 33:1572-1581.

Qian M, Krishnan K, Kudova E, Li P, Manion BD, Taylor A, Elias G, Akk G, Evers AS, Zorumski CF, Mennerick S, Covey DF (2013) Neurosteroid analogues. 18. Structure-activity studies of ent-steroid potentiators of γ-aminobutyric acid type A receptors and comparison of their activities with those of alphaxalone and allopregnanolone. J Med Chem 57:171-190.

Rakotomamonjy J, Levenes C, Baulieu EE, Schumacher M, Ghoumari AM (2011) Novel protective effect of mifepristone on detrimental $GABA_A$ receptor activity to immature Purkinje neurons. FASEB J 25:3999-4010.

Ramanjaneyulu R, Ticku MK (1984) Binding characteristics and interactions of depressant drugs with [35S]t-butylbicyclophosphorothionate; a ligand that binds to the picrotoxin site. J Neurochem 42:221-229.

Reddy DS (2003) Is there a physiological role for the neurosteroid THDOC in stress-sensitive conditions? Trends Pharmacol Sci 24:103-106.

Reddy DS (2010) Neurosteroids: endogenous role in the human brain and therapeutic potentials. Prog Brain Res 186:113-137.

Reddy DS, Kulkarni SK (1997) Differential anxiolytic effects of neurosteroids in the mirrored chamber behavior test in mice. Brain Res 752:61-71.

Reddy DS, Kulkarni SK (2000) Development of neurosteroid-base novel psychotropic drugs. Prog Med Chem 37:135-175.

Reddy DS, Woodward R (2004) Ganaxolone, a prospective overview. Drugs Future 29:227-242.

Rey M, Kruse MS, Alvarez LD, Ghini AA, Veleiro AS, Burton G, Coirini H (2013) Neuroprotective action of synthetic steroids with oxygen bridge. Activity on $GABA_A$ receptor. Exp Neurol 249:49-58.

Rheaume E, Lachance Y, Zhao HF, Breton N, Dumont M, de Launoit Y, Trudel C, Luu-The V, Simard J, Labrie F (1991) Structure and expression of a new complementary DNA encoding the almost exclusive 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase in human adrenals and gonads. Mol Endocrinol 5:1147-1157.

Sanchez PE, Ryan MA, Kridelka F, Gielen I, Ren SG, Albertson B, Malozowski S, Nieman L, Cassorla F (1989) RU-486 inhibits rat gonadal steroidogenesis. Horm Metab Res 21:369-371.

Schüle C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R (2011) Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? Neuroscience 191:55-77.

Schumacher M, Guennoun R, Robert F, Carelli C, Gago N, Ghoumari A, Gonzalez Deniselle MC, Gonzalez SL, Ibanez C, Labombarda F, Coirini H, Baulieu EE, De Nicola AF (2004) Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. Growth Horm IGF Res 14:S18-S33.

Vanover KE, Rosenzweig-Lipson S, Hawkinson JE, Lan NC, Belluzzi JD, Stein L, Barrett JE, Wood PL, Carter RB (2000) Characterization of the anxiolytic properties of a novel neuroactive steroid, Co 2-6749 (GMA-839; WAY-141839; 3alpha, 21-dihydroxy-3beta-trifluoromethyl-19-nor-5beta-pregnan-20-one), a selective modulator of gamma-aminobutyric acid(A) receptors. J Pharmacol Exp Ther 295:337-345.

Veleiro AS, Burton G (2009) Structure-activity relationships of neuroactive steroids acting on the $GABA_A$ receptor. Curr Med Chem 16:1-18.

Wang JM, Johnston PB, Ball BG, Brinton RD (2005) The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. J Neurosci 25:4706-4718.

Wang JM, Liu L, Irwin RW, Chen S, Brinton SD (2008) Regenerative potential of allopregnanolone. Brain Res Rev 57:398-409.

Weill-Engerer S, David JP, Sazdovitch V, Liere P, Eychenne B, Pianos A, Schumacher M, Delacourte A, Baulieu EE, Akwa Y (2002) Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. J Clin Endocrinol Metab 87:5138-5143.

Weinfeld J, Siegel RA, Chowers I (1980) In vitro conversion of pregnenolone to progesterone by discrete brain areas of the male rat. J Steroid Biochem 13:961-963.

Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 3:1040-1062.

Zwain IH, Yen SS (1999) Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. Endocrinology 140:3843-3852.