Journal of Steroid Biochemistry & Molecular Biology 160 (2016) 78–87

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Neurosteroids and potential therapeutics: Focus on pregnenolone

Monique Vallée[a,b,*]

[a] INSERM U862, Neurocentre Magendie, Pathophysiology of Addiction, Bordeaux F33077, France
[b] Université de Bordeaux, Bordeaux F33077, France

ARTICLE INFO

Article history:
Received 28 May 2015
Received in revised form 18 September 2015
Accepted 21 September 2015
Available online 1 October 2015

Keywords:
Pregnenolone
Neurosteroids
Cognition
Stress
Depression
Cannabinoids
Schizophrenia
Therapy
Pregnenolone-like molecules

ABSTRACT

Considerable evidence from preclinical and clinical studies shows that steroids and in particular neurosteroids are important endogenous modulators of several brain-related functions. In this context, it remains to be elucidated whether neurosteroids may serve as biomarkers in the diagnosis of disorders and might have therapeutic potential for the treatment of these disorders.

Pregnenolone (PREG) is the main steroid synthesized from cholesterol in mammals and invertebrates. PREG has three main sources of synthesis, the gonads, adrenal glands and brain and is submitted to various metabolizing pathways which are modulated depending on various factors including species, steroidogenic tissues and steroidogenic enzymes.

Looking at the whole picture of steroids, PREG is often known as the precursor to other steroids and not as an active steroid *per se*. Actually, physiological and brain functions have been studied mainly for steroids that are very active either binding to specific intracellular receptors, or modulating with high affinity the abundant membrane receptors, GABAA or NMDA receptors.

However, when high sensitive and specific methodological approaches were available to analyze low concentrations of steroids and then match endogenous levels of different steroid metabolomes, several studies have reported more significant alterations in PREG than in other steroids in extraphysiological or pathological conditions, suggesting that PREG could play a functional role as well. Additionally, several molecular targets of PREG were revealed in the mammalian brain and beneficial effects of PREG have been demonstrated in preclinical and clinical studies.

On this basis, this review will be divided into three parts. The first provides a brief overview of the molecular targets of PREG and the pharmacological effects observed in animal and human studies. The second will focus on the possible functional role of PREG with an outline of the modulation of PREG levels in animal and in human research. Finally, the review will highlight the possible therapeutic uses of PREG that point towards the development of pregnenolone-like molecules.

Contents

Abbreviations: PREG, pregnenolone; PREG-S, pregnenolone sulfate; PROG, progesterone; 17OH-PREG, dihydropregnenolone; 5α-DHPROG, dihydroprogesterone; DOC, deoxycorticosterone; 17OH-PROG, dihydroprogesterone; 5α-DHDOC, dihydrodeoxycorticosterone; 3α,5α-THDOC, tetrahydrodeoxycorticosterone; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; GABA, acide γ-aminobutyrique; NMDA, *N*-methyl-D-aspartate; CB1, cannabinoid type 1 receptor; GPCR, G protein coupled receptor; MAP, microtubule-associated protein; AMG, aminogluthetimine; CSF, cerebrospinal fluid.

* Corresponding author at: INSERM U862, Neurocentre Magendie, 146 rue Léo Saignat, Bordeaux 33077, France. Fax: +3 355 757 3669.
E-mail address: monique.vallee@inserm.fr (M. Vallée).

http://dx.doi.org/10.1016/j.jsbmb.2015.09.030
0960-0760/

1. The synthesis and metabolism of pregnenolone

The conversion of cholesterol into pregnenolone (PREG) is the first step in the synthesis of steroids that is consistent from amphibians to mammalians [68,96] and is common to the different sites of steroidogenesis. Classical steroidogenic tissues, such as the gonads, adrenal glands and placenta, synthesize steroid hormones *de novo* from cholesterol [113]. Moreover, the brain is equipped with all the enzyme machinery of steroidogenesis and is thus capable of synthesizing steroids, named neurosteroids that act locally to modify brain functions. Neurosteroids are synthesized in the central and the peripheral nervous system, in glial cells, and also in neurons from cholesterol or steroidal precursors imported from peripheral sources [1,11,25,92,136].

The initiation steps of *de novo* steroid biosynthesis which are common to all steroidogenic tissues include the mobilization of cholesterol into the inner mitochondrial membrane and the subsequent cholesterol side-chain cleavage by the mitochondrial enzyme CYP11A1 (cytochrome P450scc) into PREG [113]. Conversion of cholesterol to PREG by mitochondrial P450scc is the first, rate-limiting step in the synthesis of all steroid hormones. P450scc is involved in three chemical reactions, the 22-hydroxylation of cholesterol, 20-hydroxylation of 22(R)-hydroxycholesterol, and oxidative scission of the C20-22 bond of 20(R), 22(R)-dihydroxycholesterol (the side-chain cleavage event). The presence of P450scc is necessary to render a cell steroidogenic and capable of making steroids *de novo*, as opposed to modifying steroids produced elsewhere, which occurs in many types of cells. Indeed, a spontaneous deletion of the rabbit CYP11A1 gene for P450scc [161], its knockout in the mouse [58], and rare patients with P450scc mutations [64] results in the loss of all steroidogenesis, indicating that steroidogenesis is initiated by this one enzyme.

Regulatory mechanisms of steroidogenesis mainly affect the transport of cholesterol from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM), triggered by the steroidogenic acute regulatory protein (StAR), the conversion of cholesterol to PREG on the IMM involving P450scc, and then the synthesis of downstream steroids. Hence, acute steroidogenic responses are mediated primarily by the availability of the substrate and thus involve the action of StAR, while the chronic regulation of steroidogenesis is quantitatively (how much) determined by P450scc gene expression and qualitatively (which steroids) determined by the expression of downstream enzymes [100].

It is noteworthy that PREG is submitted to various metabolic pathways that differ slightly depending on several factors, including steroidogenic tissues, species, and steroidogenic enzymes. For example, the steroid metabolic pathways in animal and human endocrine glands are different [74]. In rodents, PREG is mainly converted into its sulfated derivative (PREG-S) and progesterone (PROG), while in humans PREG is also converted into 17-hydroxy pregnenolone (17-OH PREG) [74]. The bi-directional pathway of sulfation and sulfate conjugate hydrolysis plays an important role in metabolism and is catalyzed by sulfotransferase and sulfatase enzymes, respectively. Sulfation is generally a deactivation pathway but the sulfate conjugate may be more reactive that the active parent. For instance, PREG-S which is predominantly a way of metabolism for elimination is however an active steroid within the brain binding γ-aminobutyric acid ($GABA_A$) and *N*-methyl-D-aspartate (NMDA) type of glutamate receptors with high affinity and mediating the regulation of many brain functions. It is unlikely that there is significant transfer of steroid sulfate across the blood-brain barrier, suggesting that PREG-S can be synthesized in the brain *de novo*. Nevertheless, no conversion of PREG to PREG-S occurred in brains of rodents, as evidenced in the low or even absent PREG-S found in brain rodents evaluated using very highly sensitive methods [46,54,55,71,72,73,101,132]; while in humans sulfated forms of steroids are present [156].

PROG can be metabolized in endocrine tissue in (1) dihydroxyprogesterone (DHPROG) with its main active metabolite, tetrahydroprogesterone (3α,5α-THPROG or allopregnanolone) or its isomer 3β,5α-THPROG (epiallopregnanolone), (2) in deoxycorticosterone and in corticosterone in rodents and then in aldosterone, or (3) in 17-OH PROG and in cortisol in humans and then in androgens and estrogens. 17-OH PREG is converted into dehydroepiandrosterone (DHEA) that can be sulfated in DHEA-S, which is the major circulating steroid in humans [106] or converted into androgens and then estrogens (Fig. 1).

In addition to the multitude of metabolic pathways, the complexity and specificity in given species of the steroid metabolic profile also depend on the tissue and /or cell-specific expression of steroidogenic enzymes. Moreover, the presence of one isoform rather than another disrupts the relative synthesis/metabolic balance in favor of one steroidogenesis pathway and allows the modulation of active steroid levels in a local manner.

Finally, *de novo* steroidogenic activity of other tissues, such as adipose and intestine tissues is of recent acquisition [20,70]. It has been suggested that adipose and intestine tissues may contain the steroidogenic machinery necessary for the initiation of steroid biosynthesis *de novo* from cholesterol. For instance, the presence of the mitochondrial cholesterol transport and metabolism machinery, including steroidogenic acute regulatory protein (STAR) and CYP11A1, suggests that adipose and intestine tissues may have the ability to synthesize PREG *de novo* [70,75]. Moreover, almost all the steroid-converting enzymes downstream of PREG have been discovered in mouse or human adipose and intestine tissue [12,20,137,142].

Hence, the site-specific production of steroids that contribute to the functional action of steroids within the brain must be taken into consideration. As a result, further investigations are needed for comparison studies of steroid levels, including PREG, within the

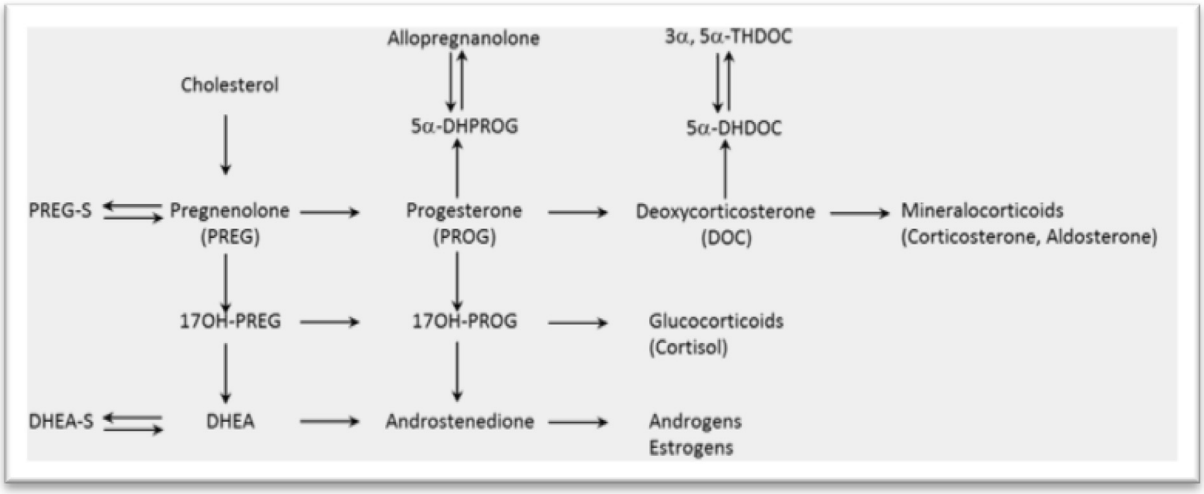

Fig. 1. Synthesis and main metabolic pathways of pregnenolone (PREG) in humans and rodents into pregnenolone sulfate (PREG-S), progesterone (PROG), or into dihydropregnenolone (17OH-PREG). PROG is metabolized in dihydroprogesterone (5α-DHPROG) with its main metabolite allopregnanolone (tetrahydroprogesterone), and in deoxycorticosterone (DOC), or dihydroprogesterone (17OH-PROG). DOC is then metabolized in dihydrodeoxycorticosterone (5α-DHDOC) and in tetrahydrodeoxycorticosterone (3α,5α-THDOC), or in mineralocorticoids including cortisosterone and aldosterone. 17-OH PREG can be metabolized in dehydroepiandrosterone (DHEA) or in 17OH-PROG. DHEA is metabolized into its sulfated form (DHEA-S) or in androstenedione. 17-OH PROG is metabolized in glucocorticoids, mainly cortisol, or in androstenedione, which is then metabolized in androgens and estrogens. The predomincance of metabolic pathways is influenced by the species and the steroidogenic tissues (see text).

different steroidogenic tissues in physiological and pathological conditions.

2. Pharmacological effects of pregnenolone

Neurosteroids have typical features including their rapid activity capability. Neurosteroids are synthesized on site "within the brain for the brain" [8] and act on membrane receptors that trigger signaling cascades resulting in rapid autocrine and paracrine effects independent of their relatively long genomic effects [89,112,129]. In this context, the main targets of neurosteroids involved the inhibitory and excitatory aminoacid receptors, $GABA_A$ and NMDA receptors, respectively [28,66,67,133]. The activation or inhibition of the activity of these neurotransmitter receptors by neurosteroids is dependent to the chemical structures of steroids; moreover the sub-unit composition of the receptor is a determinant key for the nature of modulation [13,27,60,108]. For example, the 3α derivative of PROG, allopregnanolone acts as an inhibitory steroid while the sulfated forms of PREG (PREG-S) and DHEA (DHEA-S) act as excitatory steroids. Moreover, PREG-S and DHEA-S inhibit and increase GABA current and NMDA-mediated current, respectively, in neuronal cultures of the hippocampus of rats [76,77,160], resulting then in a global activation. However, unlike the other neurosteroids, PREG expresses a poor affinity for these two receptors [108,155] but other targets have been described for PREG.

2.1. Molecular targets of pregnenolone

The most frequently described molecular targets for PREG include Sigma1 receptors and cytoplasmic microtubules and more recently a new target was discovered with the type-1 cannabinoid (CB1) receptor.

2.1.1. Sigma 1 receptor

Dr T. Maurice's group and its collaborators have worked intensively on the effects of steroids that involve Sigma 1 receptor activation. This receptor has been cloned in several species and is widely expressed both in the brain (in neurons and oligodendrocytes) and the periphery [85]. Sigma 1 receptor is an atypical protein, first identified as an opiate receptor, then as the phencyclidine binding site associated with the NMDA receptor and finally as a membrane-bound receptor distinct from other receptors. Numerous high-affinity and selective sigma1 ligands have been described, amongst them steroids, the more potent being PROG and PREG-S that act as an antagonist and as an agonist respectively while PREG is a less potent agonist [85,139]. Pleiotropic behavioral effects of sigma1 ligands have been described, including learning and memory functions, stress-anxiety- and depression-related functions, addiction and psychosis as well [14,86,87].

2.1.2. Microtubules

Prof E-E. Baulieu and collaborators have found a particular intracellular target for PREG, the microtubule-associated protein (MAP). The MAPs are involved in neuronal shape and control the balance between rigidity and plasticity in neuronal processes [84,146]. PREG binds specifically and with high affinity, by acting as an agonist, to microtubule-associated protein 2 (MAP2) [39,103] a protein family mainly expressed in neuronal cell bodies, dendrites, and dendritic spines where it modulates the assembly and stabilization of microtubules [26]. PREG dose-relatedly accelerated microtubule polymerization and increased the amount of microtubules formed in rat brain. Moreover, PREG is required for zebrafish embryonic cell movement and microtubule stability [57] and promotes cell migration and microtubule polymerization by binding a microtubule plus end-tracking protein, cytoplasmic linker protein 1, in cultured mouse adrenocortical Y1 cells and zebrafish embryos [157]. Thus, the actions of PREG on microtubules suggest a potential role in brain development, plasticity, aging and depression as well, since it has been suggested that hippocampal MAP-2 expression may be involved in the pathogenesis and pharmacology of depression [17,18].

2.1.3. CB1 receptor

Recently, our group discovered that PREG was capable of reducing central cannabinoid effects, which are mediated by the type-1 cannabinoid (CB1) receptor, the most abundant G protein

coupled receptor (GPCR). CB1 receptor is expressed widely within the brain [140] and is the main target of the major psychoactive component of *Cannabis sativa*, delta-9 tetrahydrocannabinol (THC) [114]. We discovered that the inhibitory action of PREG on THC-mediated effects involved CB1 receptors. Indeed, PREG was able to act as an endogenous allosteric negative modulator on the CB1 receptor by reducing downstream intracellular pathways including more specifically mitogen-activated protein (MAP) kinases without modulating the binding of the common CB1 ligands [152]. Interestingly, in collaboration with Dr. P. Reggio, we demonstrated a specific binding site, topographically distinct from orthosteric ligand sites, for PREG on CB1 receptor using an *in vitro* CB1 model simulating ligand–protein binding [24,152].

Thus, the action of PREG on CB1 receptor suggests that PREG could modulate the CB1-related processes which are involved in many brain functions, such as cognition, memory, anxiety, control of appetite, motor behavior, and reward-related behavior [40,43,52]. Moreover, the modulation of CB1 GPCR by PREG as an allosteric ligand may present a new opportunity in the field of drug development in relation to cannabinoid toxicity and addiction. Thus, it is believed that allosteric modulators may offer certain advantages for drug development compared to orthosteric ligands, which bind to the same site as the endogenous ligands and then modify the intrinsic activity of the receptor. However, allosteric ligands do not modify the activity of the receptors *per se*, and thus induce fewer side effects than orthosteric compounds [88].

Overall, the discovery of specific endogenous targets of PREG strongly suggests a biological action for endogenous PREG that could parallel its pharmacological effects.

2.2. Preclinical and clinical effects of pregnenolone

Given that PREG displays poor activity on the classical targets of neurosteroids, few studies exist on the biological effects of PREG, *per se*; however, some studies in animals suggest that PREG has effects on anxiety, cognition and memory [34,162]. Moreover, PREG displays neuroprotective actions and can play a beneficial role in the relief of chronic pain. Additionally, our group has demonstrated that PREG can counteract the classical cannabinoid-related effects in rodents [152].

In humans, PREG was first used in clinical practice as an anti-inflammatory agent in the 1940s [116], and placebo-controlled human trials with PREG demonstrated significant improvements in mood, general well-being, psychomotor performance and learning in normal controls tested under stressful conditions, with minimal side effects. More recently, PREG treatments have shown beneficial effects in depression- and psychosis-related disorders, such as shizophrenia.

2.2.1. Anxiety and depression-related functions

Preclinical findings suggest that PREG may play a role in anxiety and depression-regulatory mechanisms [93,123]. PREG displays antidepressant-like effects [123] and anxiogenic- or anxiolytic-like effects (depending on dose) in the elevated plus maze test in mice [93].

Although the first clinical investigations evaluating the therapeutic effects of PREG in healthy volunteers revealed no improvement in mood after 4 weeks of treatment [90] a general tendency for PREG to reduce subjective depression ratings could be detected [90]. Then, in a subgroup of subjects treated with PREG the sedative effects of a single dose of diazepam were significantly reduced suggesting a putative therapeutic benefit of PREG for the treatment of certain psychiatric conditions such as reversing the undesired sedative-hypnotic actions of benzodiazepines [90].

Furthermore, both preclinical and human data suggest that PREG may be a promising treatment for bipolar depression [23,80,126]. Accordingly, in a recent clinical study beneficial outcome was reported with PREG (titrated to 500 mg/day) therapy for 12 weeks [21] suggesting that PREG may improve depressive symptoms in patients with bipolar disorder.

2.2.2. Memory and cognitive-related functions

Cognitive improvement with PREG has been observed in animal research in several types of memory paradigms. For instance, promnesic effects have been reported in mice in avoidance paradigms [38], in a food search task [61] and in a working memory paradigm [94]. Moreover, beneficial effects of PREG have been shown by reducing the memory deficits induced by the CB1 agonist THC in an object recognition test in mice [152]. The last effect was not related to an increase in the downstream steroids of PREG in brain and plasma of the animals.

Furthermore, PREG significantly improved the synaptic plasticity of memory-related brain areas of aged rats, significantly increased brain cholinergic activity and thus helps to improve learning and memory in aged rats [22].

2.2.3. Neuroprotection-related functions

It was shown that PREG administration decreased the formation of gliotic tissue following a penetrating lesion in adult rat cerebral cortex and hippocampus [41] and displayed neuroprotective effects against glutamate and amyloid beta protein neurotoxicity in mouse hippocampal cells line [50]. Moreover, production of steroid hormones and their roles in the regulation of myelin synthesis and repair in both the central and peripheral nervous systems have been reported [69,91,111,131]. In this context, it was shown that Schwann cells were a major producer of steroid hormones and pregnenolone production by P450scc was an important regulatory step during myelination [163]. Additionally, following spinal cord injury, PREG reduced consecutive histopathological changes *in vivo*, saved the nervous tissue from secondary lesions and improved the recovery of motor functions [51]. This neuroprotective effect could result from direct action by PREG on spinal cord neurons since PREG may modulate the neuronal cytoskeleton dynamics by binding to MAP2 [103].

2.2.4. Chronic pain

It has been well documented that steroids play a key role in the regulation of neurobiological processes involved in the control of pain [98,143]. Endogenous neurosteroids which may be produced in the spinal cord dorsal horn, a pivotal structure involved in nociceptive transmission and pain modulation [99], may control the integration of somatosensory messages, including nociceptive signals and modulate their transmission toward the brain [97]. Indeed, a key action of PREG has been evidenced in a combinative therapy that promotes recovery after spinal cord injury in rats [51] suggesting that the endogenous neurosteroid PREG may play an important role in the modulation of spinal functions.

2.2.5. Drug addiction-related functions

PREG has been reported to dose dependently reduce ethanol self-administration without producing sedation in alcohol-preferring P rats, a prominent genetic model of high alcohol intake; suggesting that PREG may have potential as a novel therapy for reducing chronic alcohol drinking in individuals that abuse alcohol [15].

Recently, we discovered that PREG was able to counteract some cannabinoid-related effects mediated by CB1 pathways [152]. For instance, inhibition of the conversion of cholesterol into PREG by the P450scc inhibitor aminogluthetimine (AMG) amplified the tetrad effects, such as hypothermia, hypoactivity, catalepsy, and analgesia observed following high dose of the CB1 agonist, THC in mice. PREG was then able to relieve the effects of AMG and decrease THC-induced tetrad effects as well. Moreover, PREG was

able to dramatically reduce hyperphagia induced by a low dose of THC in food-restricted mice model and in *ad libitum* fed rats. Since PREG treatment did not increase levels of downstream steroids in the experimental conditions used, these data indicate that PREG can reduce acute THC effects in a high range of doses in rodents.

Furthermore, PREG has the ability to antagonize the addiction-related effects of cannabinoids [152]. A well-known consequence of the intake of *Cannabis sativa* and its derivatives is a positive reinforcing effect that can lead to regular use and ultimately to addiction [42]. First, we demonstrated that acute PREG decreased midbrain DA system activation that is involved in mediating addiction to most drugs of abuse, including cannabinoids [30,43,141]. PREG blunted the THC-induced increase in the extracellular levels of dopamine in the nucleus accumbens and the firing activity of neurons in the ventral tegmental area, which contains the cell bodies of the dopaminergic neurons projecting to the nucleus accumbens. We then confirmed that PREG can counteract the reinforcing effects of cannabinoid drugs by using an intravenous self-administration mouse model of the synthetic CB1 agonist, WIN 55212-2 [95]. After chronic self-administration of the CB1 agonist, acute PREG administration was able to decrease the intake of the drug and also reduce the motivation for taking the CB1 drug; revealing an inhibiting effect of PREG in chronic CB1 activation in an addiction animal model.

2.2.6. Psychosis-related functions

In addition to antipsychotics, there have been recent advances in the treatment of schizophrenia using alternative pharmacological agents [102] such as neurosteroids [78,164]. Evidence shows that the classical antipsychotic clozapine markedly increases PREG levels in rodent serum and hippocampus and it has been hypothesized that this neurosteroid induction participates in the greater beneficial therapeutic effects of clozapine observed in clinical studies [81].

Furthermore, administration of PREG has been shown to relieve certain schizophrenia symptoms in proof-of-concept, randomized controlled clinical trials [79,80,126]. For example, PREG treatment improved functional capacity in participants with schizophrenia, but did not improve cognitive symptoms over an 8-week treatment period (100 mg for 2 weeks, 300 mg for 2 weeks and 500 mg for 4 weeks) [79]. Moreover, PREG treatment (50 mg/day for 8 weeks) induced significant amelioration of the visual attention deficit in recent-onset schizophrenia [65]. Accordingly, it has been shown that PREG can markedly reduce schizophrenia-like behavior in dopamine transporter knockout (DAT-KO) mice [159]. For instance, PREG could reduce prepulse inhibition (PPI) deficits, which mimic schizophrenia-related symptoms in humans.

3. Modulation of pregnenolone levels

The availability of highly-specific and highly-selective analytical methods for the quantification of low levels of endogenous steroids offers the opportunity to evaluate the steroid metabolome and thus to compare the levels of several steroids in the same tissue sample in an individual subject in given physiological and/or pathological conditions. For instance, altered levels can be detected in tissues from animals or humans and can be correlated with a specific biological or behavioral outcome, which can highlight one specific steroid or several steroids as biomarkers for the diagnosis of disorders. In this way, quantification studies have revealed that PREG might be a crucial factor involved in several brain functions, such as cognitive-, stress-, depression-, addiction-, pain- and psychosis- related functions.

3.1. Cognitive functions

Decreased PREG-S levels in the hippocampus have been reported in cognitively impaired aged rats compared to unimpaired age-matched rats using a radio-immunoassay method [148,149,150]; however, recent studies using highly sensitive methods for quantification of PREG-S and highly-selective PREG-S extraction method as well, have revealed no PREG-S within the rodent brain [46,54,55,72,73,101] suggesting that a lipoidal form of PREG instead of a sulfated form may be related to cognitive deficits [71]. Moreover, neurosteroid alteration, including PREG increase, has been reported in the temporal cortex of patients that display cognitive impairments [104]. These data suggest that PREG may be relevant to the neurobiology and therapeutics of age-related cognitive disorders.

3.2. Stress- and depression- related functions

On the basis of the modulation of neurosteroid levels extensively described in stress-related functions, neurosteroids have been designated as major factors involved in mediating physiological and pathophysiological stress-related coping. Although abundant studies have reported modulations of the positive modulators of the $GABA_A$ receptors, allopregnanolone and 3α,5α-THDOC in response to acute [7,56,121] and chronic stress [31,48,62,134]; PREG can also be altered [121,151]. For example, following a 10-min swim stress the level of PREG was increased much more that other steroids in the frontal cortex while no change was observed in plasma [151]. Moreover, PREG levels are elevated in the plasma of monkeys by naloxone activation of hypothalamic–pituitary–adrenal (HPA) axis [117], which is activated during stress challenges.

Furthermore, the main alterations concern PREG levels in an unpredictable chronic stress animal model that achieved validity for depression-like patterns [45,153], suggesting that PREG may be a significant endogenous substrate involved in the depression phenotype [152]. Moreover, systemic treatment with the competitive 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibitor trilostane, which showed antidepressant-like properties in forced swim stress in mice [36] increased PREG levels in the hippocampus and frontal cortex of mice [35]. Accordingly, alterations in PREG levels in the hippocampus of animals receiving antidepressant treatments parallel to their antidepressant-like effects [82] have been demonstrated. Combination of the administration of olanzapine and fluoxetine, that is clinically effective in bipolar depression [145], produces elevations in hippocampal PREG levels in rats [82].

In line with these preclinical results, decreased cerebrospinal fluid (CSF) levels of PREG have been found in patients with anxiety-depressive disorder [44], and post-menstrual syndrome [154] suggesting a pathophysiological role of this neuroactive steroid in mood regulation. Furthermore, in hypercortisolemic depressed patients the beneficial effects of the steroid synthesis inhibitor ketoconazole have been accompanied by an increase in PREG levels [158] suggesting that changes in neuroactive steroid levels might contribute to its antidepressant effects [158].

3.3. Chronic pain

Chronic pain has profound physiological effects on the endocrine system. Steroid alterations can serve as biomarkers for the presence of severe pain and steroid homeostasis is then needed to achieve pain control [143]. For instance, sever pain initially activates the hypothalamic–pituitary–adrenal system which in humans results in elevated serum steroid levels such as cortisol, pregnenolone and DHEA. Following this stimulation phase a depletion phase takes place resulting in a drop of serum

steroid levels below normal levels [63]. Accordingly, patients suffering from severe and chronic pain for which standard treatment is ineffective have an abnormal serum hormonal profile [144]. Moreover, the local synthesis of neurosteroids near their sites of action in pain neural centers has been demonstrated. For instance, the rat spinal dorsal horn contains various key steroid-synthesizing enzymes and may therefore be an active center for producing neurosteroids [97,109,110]. The presence and activity of cytochrome P450scc in parallel with the production of PREG from cholesterol has been observed in rat pain pathways, including the dorsal root ganglia, spinal cord dorsal horn, nociceptive supra-spinal nuclei and somatosensory cortex [109]. Moreover, the relative amount of [3H]-neurosteroid newly synthesized in rat lumbar spinal cord after an 18-h incubation with [3H]-cholesterol resolved by HPLC-Flo/One characterization revealed a higher [3H]-PREG amount than other [3H]-neurosteroids (PROG, DHEA and ALLO), suggesting that endogenous neurosteroids, such as PREG may play an important role in the modulation of spinal functions [97].

3.4. Drug addiction-related functions

Animal studies have reported altered neurosteroid levels following acute treatment with drugs of abuse [2,47,105,118,152], however, we recently demonstrated that PREG was one of the main targets. Indeed, acute administration of the major classes of drugs of abuse, such as the psychostimulant cocaine, the opioid morphine, nicotine, alcohol and the cannabinoid THC, at doses corresponding to the ED50 for most of their unconditioned behavioral effects, widely enhanced brain levels of PREG without altering plasma levels [152]. A dramatic increase in PREG was observed for THC, while the other downstream neurosteroids, such as allopregnanolone, its 3β isomer epiallopregnanolone, 3α,5α-THDOC, DHEA or testosterone were almost unchanged. In addition, THC-induced increase in brain PREG was associated with an increase in the expression of the enzyme cytochrome P450scc, which synthesizes PREG from cholesterol.

3.5. Psychotic-related dysfunctions

PREG is one of the most altered neurosteroids observed in patients with schizophrenia [124,127]. For instance, in a recent study, serum PREG levels were significantly reduced in first-episode antipsychotic-naive participants with schizophrenia in both males and females [19]. A prior study also reported lower serum PREG levels in participants with schizophrenia [127]. In contrast, PREG levels in postmortem brain tissue samples (posterior cingulate and parietal cortex) were higher in schizophrenic subjects [83], potentially reflecting medication-induced PREG elevations. Specifically, clozapine [6,81] and olanzapine [82] significantly elevate PREG levels in multiple rodent brain regions, as do antidepressants such as fluoxetine [6,82,147].

Hence, the alteration of PREG levels in the disorders described here, strongly suggest that PREG could be an endogenous biomarker of these pathologies.

4. Potential therapeutic uses of pregnenolone

Overall, the above studies provide new insights into the role of PREG in mediating some physiological and pathophysiological functions and therefore identify PREG as a potential therapeutic candidate in brain-related disorders. For example, convergent evidence from animal studies and human clinical research involves PREG in neuroprotection; in stress-, anxiety, depression- and psychosis- related disorders, and in addiction processes with a great impact on cannabinoid-related dysfunctions (see Table 1). Moreover, low levels of PREG were associated with psychiatric disorders and PREG supplementation may result in improving the symptomatology (see Table 1). In parallel to these beneficial effects PREG therapy, even in high doses, was found to be well tolerated with a positive safety profile [21,79,80,107,125,126].

However, as regards medication, several factors have to be taken in account, such as the unwanted side effects, and the bioavailability of the compound after administration. Although the pharmacology and the toxicology have to be assessed, it is also crucial to know what the body does to the drug [119,120]. For instance, knowledge of the absorption, distribution, metabolism and excretion (ADME) properties of the drug and its metabolites in animals and humans should be assessed to understand the differences in effect among species and to optimize drug dosing [49] and drug metabolism and pharmacokinetic studies are needed to discover the duration of drug action [3,128]. Moreover, for orally administered drugs, adequate absorption and bioavailability must be achieved [119,120]. Absorption refers to the amount of total drug-derived material that crosses the gastrointestinal epithelium, whereas bioavailability addresses the amount of biologically active material that reaches the systemic circulation. Thus, oral medications because of first pass effects require high doses that may induce side effects. Other delivery forms have been proposed, for example recent evidence suggests that the delivery of intranasal steroids may be a more optimal way to deliver steroids with fewer peripheral side effects [5]. However, low bioavailability of around 23% has been observed following intranasal PREG administration [32].

Finally, when considering steroid therapy, their main disadvantages, such as their poor bioavailability and their short biological half-life caused by their rapid *in vivo* metabolism have to be taken in account. Thus, local metabolism may influence steroid activity by converting precursor steroid to differently active or inactive metabolites, which is a significant outcome for PREG since it is the

Table 1
Brief overview of animal and human research into the behavioral effects of PREG, the impact of extraphysiological and pathological-related processes on PREG levels suggesting that PREG may play a role as biomarker in the diagnosis of related disorders, and the ongoing trial therapy of PREG or derivatives. References: (1) [93,123]; (2) [90]; (3) [35,83,121,151]; (4) [44,158]; (5) [16,21]; (6) [22,38,61,94,152]; (7) [104]; (8) [10]; (9) [41,50,51,163]; (10) [152]; (11) [159]; (12) [127]; (13) [80]; (14) [79,125,126].

Preclinical targets of PREG	Clinical targets of PREG	PREG as biomarker of disorders in animals	PREG as biomarker in the diagnosis of human pathologies	Clinical trial therapy of PREG or derivatives
Anxiety and depression-like behaviors(1)	Depression(2)	Acute and Chronic stress coping; depression-like behavior(3)	Depression(4)	Depression(5)
Memory and cognition(6)			Cognitive phenotype in Alzheimer's disease(7)	Alzheimer's disease(8)
Neuroprotection(9)				
Cannabinoid-related effects(10)		Cannabinoid-related toxicity and addiction(10)		
Schizophrenia animal model(11)	Schizophrenia(12)		Schizophrenia(13)	Schizophrenia(14)

precursor of many steroids. For instance, high doses of PREG (50 mg/kg) administered intraperiteonally in ethanol-experienced rats induced a 3-fold increase in cortical allopregnanolone 45 min later [15]. Also in humans, PREG (400 mg) following oral administration is converted to multiple metabolites, among them allopregnanolone [138]. Moreover, after ingestion of 50 mg of PREG, major conversion to 5beta metabolites has been reported in urine [130].

5. Conclusions and future perspectives: development of pregnenolone-like molecules

For the above reasons, even if PREG has real potential for therapeutic use and can be safely administered, the development of synthetic steroids that display better bioavailability and efficacy might constitute promising novel strategies for the treatment of certain disorders. Recently, several synthetic analogs of PREG have been developed and some of them have successfully shown some benefits showing pregnenolone-like effects and are thus under investigation in preclinical or clinical trials.

Previously, different PREG derivatives were synthesized, and evaluated for various biological activities. The PREG analogs, hydroxylated at C-20, are known to affect calcium-dependent processes, and also affect the degree of depolarization of smooth muscles [53]. The hemisuccinate of pregnenolone-derivative significantly increases the perfusion pressure, and vascular resistance in isolated rat heart [37] and the nitrochlorambucil ester of PREG exhibited significant cytotoxic activity towards the brain posterior fossa, medullablastoma, and lung large cell carcinoma cell lines [135]. Moreover, 21-triazolyl derivatives and pyrazoline derivatives were identified as potential anticancer active compounds in human cancer cell lines [4,59] and pregnenolone derivatives with diamide side chains at C16 are able to act as antiviral agents against Herpes Simplex Virus Type 1 [29].

Also, knowing the strong interdependence between the chemical structure of the steroid and its biological activity, scientists were able to target specifics carbon positions on the steroid skeleton for the synthesis of analogs with specific activity. Indeed, the structure/activity studies initiated by the eminent chemist, Dr. R. Purdy [122,152], indicate that the 3α-hydroxyl configuration is required for binding and activity. Moreover, the enzymes involved in the synthesis/metabolism pathways of steroids mainly target the C3-position for progesterone and its hydroxyl metabolites and the C17-position for androgens and estrogens. Therefore, strategies have been developed to produce C3-analogs of PREG (for example 3-methoxy pregnenolone developed by Baulieu and collaborators and *MAPREG* (microtubule-associated protein/neurosteroidal pregnenolone) biotech [9,10,16]) and C3-17 analogs (C3,C17 Non Metabolized Pregnenolone Derivatives developed by INSERM and *Aelis Farma* Biotech [115]). For instance, therapeutic activity of the 3-methoxy analog of PREG (MAP4343) on spinal cord injuries has been demonstrated [33] and preclinical studies have shown that MAP4343 has antidepressant efficacy in rats [16]. Consequently, a patent has been proposed for the use of the 3-methoxy analog of PREG for treating depressive disorders and long-term neurological diseases [9]. Moreover, the use of C3-C17 analogs is being investigated in CB1-related disorders with cannabis abuse as a primarily therapeutic target, and other therapeutic applications, including psychosis, mental retardation, metabolic disorders and skin diseases (www.aelisfarma.com).

In conclusion, attempts at discovering therapeutic outcomes for pregnenolone-like molecules should reveal promising applications and future medications.

References

[1] R.C. Agís-Balboa, G. Pinna, A. Zhubi, E. Maloku, M. Veldic, E. Costa, A. Guidotti, Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 14602–14607.

[2] A.A. Alomary, M. Vallée, L.E. O'Dell, G.F. Koob, R.H. Purdy, R.L. Fitzgerald, Acutely administered ethanol participates in testosterone synthesis and increases testosterone in rat brain, Alcohol Clin. Exp. Res. 27 (2003) 38–43.

[3] T.A. Baillie, M.N. Cayen, H. Fouda, R.J. Gerson, J.D. Green, S.J. Grossman, L.J. Klunk, B. LeBlanc, D.G. Perkins, L.A. Shipley, Drug metabolites in safety testing, Toxicol. Appl. Pharmacol. 182 (2002) 188–196.

[4] A.H. Banday, S.A. Shameem, B.D. Gupta, H.M. Kumar, D-ring substituted 1,2,3-triazolyl 20-keto pregnenanes as potential anticancer agents: synthesis and biological evaluation, Steroids 75 (2010) 801–804.

[5] W.A. Banks, J.E. Morley, M.L. Niehoff, C. Mattern, Delivery of testosterone to the brain by intranasal administration: comparison to intravenous testosterone, J. Drug Target 17 (2009) 759–765.

[6] M.L. Barbaccia, D. Affricano, R.H. Purdy, E. Maciocco, F. Spiga, G. Biggio, Clozapine but not haloperidol, increases brain concentrations of neuroactive steroids in the rat, Neuropsychopharmacology 25 (2001) 489–497.

[7] M.L. Barbaccia, M. Serra, R.H. Purdy, G. Biggio, Stress and neuroactive steroid, Int. Rev. Neurobiol. 46 (2001) 243–272.

[8] E.E. Baulieu, Neurosteroids: of the nervous system by the nervous system, for the nervous system, Recent Prog. Horm. Res. 52 (1997) 1–32.

[9] E.E. Baulieu, E. Fellous, P. Robel, M. Bianchi, Use of 3-methoxy-pregnenolone for the preparation of a drug for treating depressive disorders and long-term neurological diseases, US 8334278 B2 (2012)

[10] E.E. Baulieu, P. Robel, A. Fellous, Y. Duchossoy, V. Fontaine-Lenoir, S. David, MAPREG: toward a novel approach of neuroprotection and treatment of Alzheimer's disease, J. Mol. Neurosci. 24 (2004) 63–65.

[11] E.E. Baulieu, P. Robel, M. Schumacher, Neurosteroids: beginning of the story, Int. Rev. Neurobiol. 46 (2001) 1–32.

[12] C. Bélanger, V. Luu-The, P. Dupont, A. Tchernof, Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity, Horm. Metab. Res. 34 (2002) 737–745.

[13] D. Belelli, J.J. Lambert, Neurosteroids: endogenous regulators of the GABAA receptor, Nat. Rev. Neurosci. 6 (2005) 565–575.

[14] J.E. Bermack, G. Debonnel, The role of sigma receptors in depression, J. Pharmacol. Sci. 97 (2005) 317–336.

[15] J. Besheer, T.G. Lindsay, T.K. O'Buckley, C.W. Hodge, A.L. Morrow, Pregnenolone and ganaxolone reduce operant ethanol self-administration in alcohol-preferring P rats, Alcohol Clin. Exp. Res. 34 (2010) 2044–2052.

[16] M. Bianchi, E.E. Baulieu, 3β-Methoxy-pregnenolone (MAP4343) as an innovative therapeutic approach for depressive disorders, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 1713–1718.

[17] M. Bianchi, J.J. Hagan, C.A. Heidbreder, Neuronal plasticity, stress and depression: involvement of the cytoskeletal microtubular system? Curr. Drug Targets CNS Neurol. Disord. 4 (2005) 597–611.

[18] M. Bianchi, A.J. Shah, K.C. Fone, A.R. Atkins, L.A. Dawson, C.A. Heidbreder, M.E. Hows, J.J. Hagan, C.A. Marsden, Fluoxetine administration modulates the cytoskeletal microtubular system in the rat hippocampus, Synapse 63 (2009) 359–364.

[19] M. Bicikova, M. Hill, D. Ripova, P. Mohr, R. Hampl, Determination of steroid metabolome as a possible tool for laboratory diagnosis of schizophrenia, J. Steroid Biochem. Mol. Biol. 133 (2013) 77–83.

[20] G. Bouguen, L. Dubuquoy, P. Desreumaux, T. Brunner, B. Bertin, Intestinal steroidogenesis Steroids (2015) 00315-00318 S0039-128X(14) 00315-8

[21] E.S. Brown, J. Park, C.E. Marx, L.S. Hynan, C. Gardner, D. Davila, A. Nakamura, P. Sunderajan, A. Lo, T. Holmes, A randomized double-blind, placebo-controlled trial of pregnenolone for bipolar depression, Neuropsychopharmacology 39 (2014) 2867–2873.

[22] J. Bu, H. Zu, Effects of pregnenolone intervention on the cholinergic system and synaptic protein 1 in aged rats, Int. J. Neurosci. 124 (2014) 117–124.

[23] M.G. Carta, K.M. Bhat, A. Preti, GABAergic neuroactive steroids: a new frontier in bipolar disorders? Behav. Brain Funct. 8 (2012) 61.

[24] M. Clark, S. Meshkat, J.S. Wiseman, Grand canonical Monte Carlo simulation of ligand–protein binding, J. Chem. Inf. Model 46 (2006) 231–242.

[25] N.A. Compagnone, S.H. Mellon, Neurosteroids: biosynthesis and function of these novel neuromodulators, Front. Neuroendocrinol. 21 (2000) 1–56.

[26] C. Conde, A. Cáceres, Microtubule assembly, organization and dynamics in axons and dendrites, Nat. Rev. Neurosci. 10 (2009) 319–332.

[27] D.F. Covey, A.S. Evers, S. Mennerick, C.F. Zorumski, R.H. Purdy, Recent developments in structure-activity relationships for steroid modulators of GABA(A) receptors, Brain Res. Brain Res. Rev. 37 (2001) 91–97.

[28] A.I. Czlonkowska, P. Krzascik, H. Sienkiewicz-Jarosz, M.J. Siemiatkowski, A. SzyndlerBidzinski, A. Plaznik, The effects of neurosteroids on picrotoxin-, bicuculline- and NMDA-induced seizures, and a hypnotic effect of ethanol, Pharmacol. Biochem. Behav. 67 (2000) 345–353.

[29] M.E. Davola, G.I. Mazaira, M.D. Galigniana, L.E. Alche, J.A. Ramirez, A.A. Barquero, Synthetic pregnenolone derivatives as antiviral agents against acyclovir-resistant isolates of Herpes Simplex Virus Type 1, Antiviral Res. 122 (2015) 55–63.

[30] G. Di Chiara, A. Imperato, Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 5274–5278.
[31] E. Dong, K. Matsumoto, V. Uzunova, I. Sugaya, H. Takahata, H. Nomura, H. Watanabe, E. Costa, A. Guidotti, Brain 5alpha-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 2849–2854.
[32] N. Ducharme, W.A. Banks, J.E. Morley, S.M. Robinson, M.L. Niehoff, C. Mattern, S.A. Farr, Brain distribution and behavioral effects of progesterone and pregnenolone after intranasal or intravenous administration, Eur. J. Pharmacol. 641 (2010) 128–134.
[33] Y. Duchossoy, S. David, E.E. Baulieu, P. Robel, Treatment of experimental spinal cord injury with 3beta-methoxy-pregnenolone, Brain Res. 1403 (2011) 57–66.
[34] D. Eser, T.C. Baghai, C. Schule, C. Nothdurfter, R. Rupprecht, Neuroactive steroids as endogenous modulators of anxiety, Curr. Pharm. Des. 14 (2008) 3525–3533.
[35] J. Espallergues, T. Mamiyad, M. Vallée, T. Kosekid, T. Nabeshimad, J. Temsamanig, C. Laruelle, T. Maurice, The antidepressant-like effects of the 3b-hydroxysteroid dehydrogenase inhibitor trilostane in mice is related to changes in neuroactive steroid and monoamine levels, Neuropharmacology 62 (2012) 492–502.
[36] J. Espallergues, J. Temsamani, C. Laruelle, A. Urani, T. Maurice, The antidepressant-like effect of the 3b-hydroxysteroid dehydrogenase inhibitor trilostane involves a regulation of b-type estrogen receptors, Psychopharmacology (Berl.) 214 (2011) 455–463.
[37] L. Figueroa-Valverde, F. Díaz-Cedillo, E. Diaz-Ku, A. Camacho-Luis, Effect induced by hemisuccinate of pregnenolone on perfusion pressure and vascular resistance in isolated rat heart, Afr. J. Pharm. Pharmacol. 3 (2009) 234–241.
[38] J.F. Flood, J.E. Morley, E. Roberts, Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 1567–1571.
[39] V. Fontaine-Lenoir, B. Chambraud, A. Fellous, S. David, Y. Duchossoy, E.E. Baulieu, P. Robel, Microtubule-associated protein 2 (MAP2) is a neurosteroid receptor, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 4711–4716.
[40] M. Fujiwara, N. Egashira, New perspectives in the studies on endocannabinoid and cannabis: abnormal behaviors associate with CB1 cannabinoid receptor and development of therapeutic application, J. Pharmacol. Sci. 96 (2004) 362–366.
[41] J. García-Estrada, S. Luquín, A.M. Fernández, L.M. Garcia-Segura, Dehydroepiandrosterone, pregnenolone and sex steroids down-regulate reactive astroglia in the male rat brain after a penetrating brain injury, Int. J. Dev. Neurosci. 17 (1999) 145–151.
[42] E.L. Gardner, Addictive potential of cannabinoids: the underlying neurobiology, Chem. Phys. Lipids 121 (2002) 267–290.
[43] E.L. Gardner, Endocannabinoid signaling system and brain reward: emphasis on dopamine, Pharmacol. Biochem. Behav. 81 (2005) 263–284.
[44] M.S. George, A. Guidotti, D. Rubinow, B. Pan, K. Mikalauskas, R.M. Post, CSF neuroactive steroids in affective disorders: pregnenolone, progesterone, and DBI, Biol. Psychiatry 35 (1994) 775–780.
[45] A. Gregus, A.J. Wintink, A.C. Davis, L.E. Kalynchuk, Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats, Behav. Brain Res. 156 (2005) 105–114.
[46] W.J. Griffiths, S. Liu, Y. Yang, R.H. Purdy, J. Sjövall, Nano-electrospray tandem mass spectrometry for the analysis of neurosteroid sulphates, Rapid Commun. Mass Spectrom. 13 (1999) 1595–1610.
[47] A.C. Grobin, M.J. VanDoren, L.J. Porrino, A.L. Morrow, Cortical 3 alpha-hydroxy-5 alpha-pregnan-20-one levels after acute administration of delta 9-tetrahydrocannabinol, cocaine and morphine, Psychopharmacology (Berl.) 179 (2005) 544–550.
[48] A. Guidotti, E. Dong, K. Matsumoto, G. Pinna, A.M. Rasmusson, E. Costa, The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5alpha-dihydroprogesterone in psychiatric disorders, Brain Res. Brain Res. Rev. 37 (2001) 110–115.
[49] C. Gunaratna, Drug metabolism & pharmacokinetics in drug discovery: a primer for bioanalytical chemists, part I, Curr. Sep. 19 (2000) 17–23.
[50] E. Gursoy, A. Cardounel, M. Kalimi, Pregnenolone protects mouse hippocampal (HT-22) cells against glutamate and amyloid beta protein toxicity, Neurochem. Res. 26 (2001) 15–21.
[51] L. Guth, Z. Zhang, E. Roberts, Key role for pregnenolone in combination therapy promotes recovery after spinal cord injury, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12308–12312.
[52] Y. Hashimotodani, T. Ohno-Shosaku, M. Kano, Endocannabinoids and synaptic function in the CNS, Neuroscientist 13 (2007) 127–137.
[53] A. Hidalgo, R.C. Suzano, M.P. Revuelta, C. Sanchez-Diaz, A. Baamonde, B. Cantabrana, Calcium and depolarization-dependent effect of pregnenolone derivatives on uterine smooth muscle, Gen. Pharmacol. 27 (1996) 879–885.
[54] T. Higashi, Y. Daifu, T. Ikeshima, T. Yagi, K. Shimada, Studies on neurosteroids XV. Development of enzyme-linked immunosorbent assay for examining whether pregnenolone sulfate is a veritable neurosteroid, J. Pharm. Biomed. Anal. 30 (2003) 1907–1917.
[55] T. Higashi, H. Sugitani, T. Yagi, K. Shimada, Studies on neurosteroids XVI. Levels of pregnenolone sulfate in rat brains determined by enzyme-linked immunosorbent assay not requiring solvolysis, Biol. Pharm. Bull. 26 (2003) 709–711.
[56] T. Higashi, N. Takido, K. Shimada, Studies on neurosteroids XVII: analysis of stress-induced changes in neurosteroid levels in rat brains using liquid chromatography-electron capture atmospheric pressure chemical ionization-mass spectrometry, Steroids 70 (2005) 1–11.
[57] H.J. Hsu, M.R. Liang, C.T. Chen, B.C. Chung, Pregnenolone stabilizes microtubules and promotes zebrafish embryonic cell movement, Nature 439 (2006) 480–483.
[58] M.C. Hu, N.C. Hsu, N.B. El Hadj, C.I. Pai, H.P. Chu, C.K. Wang, B.C. Chung, Steroid deficiency syndromes in mice with targeted disruption of Cyp11a1, Mol. Endocrinol. 16 (2002) 1943–1950.
[59] M. Iqbal Choudhary, M. Shahab Alam, S. Yousuf, Y.C. Wu, A.S. Lin, F. Shaheen, Pregnenolone derivatives as potential anticancer agents, Steroids 76 (2011) 1554–1559.
[60] R.P. Irwin, S.Z. Lin, M.A. Rogawski, R.H. Purdy, S.M. Paul, Steroid potentiation and inhibition of *N*-methyl-D-aspartate receptor-mediated intracellular Ca++ responses: structure-activity studies, J. Pharmacol. Exp. Ther. 271 (1994) 677–682.
[61] R.L. Isaacson, P.E. Yoder, J. Varner, The effects of pregnenolone on acquisition and retention of a food search task, Behav. Neural. Biol. 61 (1994) 170–176.
[62] R.T. Khisti, C.T. Chopde, S.P. Jain, Antidepressant-like effect of the neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one in mice forced swim test, Pharmacol. Biochem. Behav. 67 (2000) 137–143.
[63] S. Khoromi, R. Muniyappa, L. Nackers, N. Gray, H. Baldwin, K.A. Wong, L.A. Matheny, B. Moquin, A. Rainer, S. Hill, A. Remaley, L.L. Johnson, M.B. Max, M.R. Blackman, Effects of chronic osteoarthritis pain on neuroendocrine function in men, J. Clin. Endocrinol. Metab. 91 (2006) 4313–4318.
[64] C.J. Kim, L. Lin, N. Huang, C.A. Quigley, T.W. AvRuskin, J.C. Achermann, W.L. Miller, Severe combined adrenal and gonadal deficiency caused by novel mutations in the cholesterol side chain cleavage enzyme, P450scc, J. Clin. Endocrinol. Metab. 93 (2008) 696–702.
[65] A. Kreinin, N. Bawakny, M.S. Ritsner, Adjunctive pregnenolone ameliorates the cognitive deficits in recent-onset schizophrenia, Clin. Schizophr. Relat. Psychoses (2014) 1–31.
[66] J.J. Lambert, D. Belelli, D.R. Peden, A.W. Vardy, J.A. Peters, Neurosteroid modulation of GABAA receptors, Prog. Neurobiol. 71 (2003) 67–80.
[67] J.J. Lambert, M.A. Cooper, R.D. Simmons, C.J. Weir, D. Belelli, Neurosteroids: endogenous allosteric modulators of GABA(A) receptors, Psychoneuroendocrinology 34 (2009) S48–58.
[68] C. Le Goascogne, P. Robel, M. Gouezou, N. Sananes, E.E. Baulieu, Neurosteroids: cytochrome P-450scc in rat brain, Science 237 (1987) 1212–1215.
[69] E. Leonelli, M. Ballabio, A. Consoli, I. Roglio, V. Magnaghi, R.C. Melcangi, Neuroactive steroids: a therapeutic approach to maintain peripheral nerve integrity during neurodegenerative events, J. Mol. Neurosci. 28 (2006) 65–76.
[70] J. Li, E. Daly, E. Campioli, M. Wabitsch, V. Papadopoulos, De novo synthesis of steroids and oxysterols in adipocytes, J. Biol. Chem. 289 (2014) 747–764.
[71] P. Liere, A. Pianos, B. Eychenne, A. Cambourg, S. Liu, W. Griffiths, M. Schumacher, J. Sjövall, E.E. Baulieu, Novel lipoidal derivatives of pregnenolone and dehydroepiandrosterone and absence of their sulfated counterparts in rodent brain, J. Lipid Res. 45 (2004) 2287–22302.
[72] S. Liu, W.J. Griffiths, J. Sjövall, Capillary liquid chromatography/electrospray mass spectrometry for analysis of steroid sulfates in biological samples, Anal. Chem. 75 (2003) 791–797.
[73] S. Liu, J. Sjövall, W.J. Griffiths, Neurosteroids in rat brain: extraction, isolation, and analysis by nanoscale liquid chromatography–electrospray mass spectrometry, Anal. Chem. 75 (2003) 5835–5846.
[74] V. Luu-The, Assessment of steroidogenesis and steroidogenic enzyme functions, J. Steroid Biochem. Mol. Biol. 137 (2013) 176–182.
[75] S.M. MacKenzie, S.S. Huda, N. Sattar, R. Fraser, J.M. Connell, E. Davies, Depot specific steroidogenic gene transcription in human adipose tissue, Clin. Endocrinol. (Oxf.) 69 (2008) 848–854.
[76] M.D. Majewska, S. Demirgören, E.D. London, Binding of pregnenolone sulfate to rat brain membranes suggests multiple sites of steroid action at the GABAA receptor, Eur. J. Pharmacol. 189 (1990) 307–315.
[77] M.D. Majewska, S. Demirgören, C.E. Spivak, E.D. London, The neurosteroid dehydroepiandrosterone sulfate is an allosteric antagonist of the GABAA receptor, Brain Res. 526 (1990) 143–146.
[78] C.E. Marx, D.W. Bradford, R.M. Hamer, J.C. Naylor, T.B. Allen, J.A. Lieberman, J. L. Strauss, J.D. Kilts, Pregnenolone as a novel therapeutic candidate in schizophrenia: emerging preclinical and clinical evidence, Neuroscience 191 (2011) 78–90.
[79] C.E. Marx, J. Lee, M. Subramaniam, A. Rapisarda, D.C.T. Bautista, E. Chan, J.D. Kilts, R.W. Buchanan, E. Pui Wai, S. Verma, K. Sim, J. Hariram, R. Jacob, R.S.E. Keefe, S. Ann Chong, Proof-of-concept randomized controlled trial of pregnenolone in schizophrenia, Psychopharmacology (Berl.) 231 (2014) 3647–3662.
[80] C.E. Marx, R.S. Keefe, R.W. Buchanan, R.M. Hamer, J.D. Kilts, D.W. Bradford, J.L. Strauss, J.C. Naylor, V.M. Payne, J.A. Lieberman, A.J. Savitz, L.A. Leimone, L. Dunn, P. Porcu, A.L. Morrow, L.J. Shampine, Proof-of concept trial with the neurosteroid pregnenolone targeting cognitive and negative symptoms in schizophrenia, Neuropsychopharmacology 34 (2009) 1885–1903.
[81] C.E. Marx, L.J. Shampine, G.E. Duncan, M.J. VanDoren, A.C. Grobin, M.W. Massing, R.D. Madison, D.W. Bradford, M.I. Butterfield, J.A. Lieberman, A.L. Morrow, Clozapine markedly elevates pregnenolone in rat hippocampus, cerebral cortex, and serum: candidate mechanism for superior efficacy? Pharmacol. Biochem. Behav. 84 (2006) 598–608.

[82] C.E. Marx, L.J. Shampine, R.T. Khisti, W.T. Trost, D.W. Bradford, A.C. Grobin, M. W. Massing, R.D. Madison, M.I. Butterfield, J.A. Lieberman, A.L. Morrow, Olanzapine and fluoxetine administration and coadministration increase rat hippocampal pregnenolone, allopregnanolone and peripheral deoxycorticosterone: implications for therapeutic actions, Pharmacol. Biochem. Behav. 84 (2006) 609–617.
[83] C.E. Marx, R.D. Stevens, L.J. Shampine, V. Uzunova, W.T. Trost, M.I. Butterfield, M.W. Massing, R.M. Hamer, A.L. Morrow, J.A. Lieberman, Neuroactive steroids are altered in schizophrenia and bipolar disorder: relevance to pathophysiology and therapeutics, Neuropsychopharmacology 31 (2006) 1249–1263.
[84] A. Matus, Microtubule-associated proteins: their potential role in determining neuronal morphology, Annu. Rev. Neurosci. 11 (1988) 29–44.
[85] T. Maurice, C. Grégoire, J. Espallergues, Neuro(active) steroids actions at the neuromodulatory sigma1 (s1) receptor: biochemical and physiological evidences consequences in neuroprotection, Pharmacol. Biochem. Behav. 84 (2006) 581–597.
[86] T. Maurice, P. Romieu, Involvement of the sigma1 receptor in the appetitive effects of cocaine, Pharmacopsychiatry 37 (2004) S198–207.
[87] T. Maurice, A. Urani, V.L. Phan, P. Romieu, The interaction between neuroactive steroids and the sigma1 receptor function: behavioral consequences and therapeutic opportunities, Brain Res. Brain Res. Rev. 37 (2001) 116–132.
[88] L.T. May, V.A. Avlani, P.M. Sexton, A. Christopoulos, Allosteric modulation of G protein-coupled receptors, Curr. Pharm. Des. 10 (2004) 2003–2013.
[89] B.S. McEwen, Non-genomic and genomic effects of steroids on neural activity, Trends Pharmacol. Sci. 12 (1991) 141–147.
[90] S.E. Meieran, V.I. Reus, R. Webster, R. Shafton, O.M. Wolkowitz, Chronic pregnenolone effects in normal humans: attenuation of benzodiazepine induced sedation, Psychoneuroendocrinology 29 (2004) 486–500.
[91] R.C. Melcangi, L.M. Garcia-Segura, A.G. Mensah-Nyagan, Neuroactive steroids: state of the art and new perspectives, Cell. Mol. Life Sci. 65 (2008) 777–797.
[92] R.C. Melcangi, A.G. Mensah-Nyagan, Neurosteroids: measurement and pathophysiologic relevance, Neurochem. Int. 52 (2008) 503–505.
[93] C.L. Melchior, R.F. Ritzmann, Pregnenolone and pregnenolone sulfate alone and with ethanol, in mice on plus-maze, Pharmacol. Biochem. Behav. 48 (1994) 893–897.
[94] C.L. Melchior, R.F. Ritzmann, Neurosteroids block the memory-impairing effects of ethanol in mice, Pharmacol. Biochem. Behav. 53 (1996) 51–56.
[95] V. Mendizábal, A. Zimmer, R. Maldonado, Involvement of kappa/dynorphin system in WIN 55, 212-2 self-administration in mice, Neuropsychopharmacology 31 (2006) 1957–1966.
[96] A.G. Mensah-Nyagan, D. Beaujean, V. Luu-The, G. Pelletier, H. Vaudry, Anatomical and biochemical evidence for the synthesis of unconjugated and sulfated neurosteroids in amphibians, Brain Res. Brain Res. Rev. 37 (2001) 13–24.
[97] A.G. Mensah-Nyagan, C. Kibaly, V. Schaeffer, C. Venard, L. Meyer, C. Patte-Mensah, Endogenous steroid production in the spinal cord and potential involvement in neuropathic pain modulation, J. Steroid Biochem. Mol. Biol. 109 (2008) 286–293.
[98] A.G. Mensah-Nyagan, L. Meyer, V. Schaeffer, C. Kibaly, C. Patte-Mensah, Evidence for a key role of steroids in the modulation of pain, Psychoneuroendocrinology 34 (2009) S169–S177.
[99] M.J. Millan, Descending control of pain, Prog. Neurobiol. 66 (2002) 355–474.
[100] W.L. Miller, H.S. Bose, Early steps in steroidogenesis: intracellular cholesterol trafficking, J. Lipid Res. 52 (2011) 2111–2135.
[101] K. Mitamura, M. Yatera, K. Shimada, Quantitative determination of pregnenolone 3-sulfate in rat brains using liquid chromatography/electrospray ionization-mass spectrometry, Anal. Sci. 15 (1999) 951–955.
[102] S. Miyamoto, L.F. Jarskog, W.W. Fleischhacker, Alternative pharmacologic targets for the treatment of schizophrenia: results from phase I and II trials, Curr. Opin. Psychiatry 26 (2013) 158–165.
[103] K. Murakami, A. Fellous, E.E. Baulieu, P. Robel, Pregnenolone binds to microtubule-associated protein 2 and stimulates microtubule assembly, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3579–3784.
[104] J.C. Naylor, J.D. Kilts, C.M. Hulette, D.C. Steffens, D.G. Blazer, J.F. Ervin, J.L. Strauss, T.B. Allen, M.W. Massing, V.M. Payne, N.A. Youssef, L.J. Shampine, C.E. Marx, Allopregnanolone levels are reduced in temporal cortex in patients with Alzheimer's disease compared to cognitively intact control subjects, Biochim. Biophys. Acta 1801 (2010) 951–959.
[105] L.E. O'Dell, A.A. Alomary, M. Vallée, G.F. Koob, R.L. Fitzgerald, R.H. Purdy, Ethanol-induced increases in neuroactive steroids in the rat brain and plasma are absent in adrenalectomized and gonadectomized rats, Eur. J. Pharmacol. 484 (2004) 241–247.
[106] L. Orienteich, J.L. Brind, R.L. Rizer, J.H. Vogelman, Age changes and sex differences in serum DHEAS concentration throughout adulthood, J. Clin. Endocrinol. Metab. 59 (1984) 551–555.
[107] I.J. Osuji, E. Vera-Bolaños, T.J. Carmody, E.S. Brown, Pregnenolone for cognition and mood in dual diagnosis patients, Psychiatry Res. 178 (2010) 309–312.
[108] M. Park-Chung, A. Malayev, R.H. Purdy, T.T. Gibbs, D.H. Farb, Sulfated and unsulfated steroids modulate gamma-aminobutyric acid A receptor function through distinct sites, Brain Res. 830 (1999) 72–87.
[109] C. Patte-Mensah, V. Kappes, M.J. Freund-Mercier, K. Tsutsui, A.G. Mensah-Nyagan, Cellular distribution and bioactivity of the key steroidogenic enzyme cytochrome P450 side chain cleavage, in sensory neural pathways, J. Neurochem. 86 (2003) 1233–1246.
[110] C. Patte-Mensah, S. Li, A.G. Mensah-Nyagan, Impact of neuropathic pain on the gene expression and activity of cytochrome P450 side-chain-cleavage in sensory neural networks, Cell Mol. Life Sci. 61 (2004) 2274–2284.
[111] C. Patte-Mensah, A.G. Mensah-Nyagan, Peripheral neuropathy and neurosteroid formation in the central nervous system, Brain Res. Rev. 57 (2008) 454–459.
[112] S.M. Paul, R.H. Purdy, Neuroactive steroids, FASEB J. 6 (1992) 2311–2322.
[113] A.H. Payne, D.B. Hales, Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones, Endocr. Rev. 25 (2004) 947–970.
[114] R.G. Pertwee, The central pharmacology of psychotropic cannabinoids, Pharmacol. Ther. 36 (1988) 189–261.
[115] P.V. Piazza, M. Vallée, F.X., Felpin, J.M., Revest, S.. Fabre, 3-(4′-substituted)-benzyl-ether derivatives of pregnenolone, WO2014083068 A1 (2014)
[116] G. Pincus, H. Hoagland, Effects on industrial production of the administration of Delta5 pregnenolone to factory workers, Psychosom. Med. 7 (1945) 342–352.
[117] P. Porcu, L.S. Rogers, A.L. Morrow, K.A. Grant, Plasma pregnenolone levels in cynomolgus monkeys following pharmacological challenges of the hypothalamic-pituitary-adrenal axis, Pharmacol. Biochem. Behav. 84 (2006) 618–627.
[118] P. Porcu, C. Sogliano, M. Cinus, R.H. Purdy, G. Biggio, A. Concas, Nicotine-induced changes in cerebrocortical neuroactive steroids and plasma corticosterone concentrations in the rat, Pharmacol. Biochem. Behav. 74 (2003) 683–690.
[119] J.F. Pritchard, S.R. Anderson, C. Breuckner, N.D. Premkumar, J.W. Polli, Conference report: a hitchhiker's guide to outsourcing ADME studies: the inside of outsourcing, Bioanalysis 5 (2013) 403–405.
[120] J.F. Pritchard, M. Jurima-Romet, M.L. Reimer, E. Mortimer, B. Rolfe, M.N. Cayen, Making better drugs: decision gates in non-clinical drug development, Nat. Rev. Drug Discov. 2 (2003) 542–553.
[121] R.H. Purdy, A.L. Morrow, P.H. Moore, S.M. Paul, Stress-induced elevations of γ-aminobutyric acic type A receptor-active steroids in the rat brain, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 4553–4557.
[122] R.H. Purdy, A.L. Morrow, J.R. Blinn, S.M. Paul, 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 (1990) 1572–1581.
[123] D.S. Reddy, S.K. Kulkarni, Proconvulsant effects of neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate in mice, Eur. J. Pharmacol. 345 (1998) 55–59.
[124] M.S. Ritsner, The clinical and therapeutic potentials of dehydroepiandrosterone and pregnenolone in schizophrenia, Neuroscience 191 (2011) 91–100.
[125] M.S. Ritsner, H. Bawakny, A. Kreinin, Pregnenolone treatment reduces severity of negative symptoms in recent-onset schizophrenia: an 8-week double-blind, randomized add-on two-center trial, Psychiatry Clin. Neurosci. 68 (2014) 432–440.
[126] M.S. Ritsner, A. Gibel, T. Shleifer, I. Boguslavsky, A. Zayed, R. Maayan, A. Weizman, V. Lerner, Pregnenolone and dehydroepiandrosterone as an adjunctive treatment in schizophrenia and schizoaffective disorder: an 8-week double-blind, randomized, controlled, 2-center, parallel-group trial, J. Clin. Psychiatry 71 (2010) 1351–1362.
[127] M.S. Ritsner, R. Maayan, A. Gibel, A. Weizman, Differences in blood pregnenolone and dehydroepiandrosterone levels between schizophrenia patients and healthy subjects, Eur. Neuropsychopharmacol. 17 (2007) 358–365.
[128] S.A. Roberts, Drug metabolism and pharmacokinetics in drug discovery, Curr. Opin. Drug Discov. Dev. 6 (2003) 66–80.
[129] R. Rupprecht, Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties, Psychoneuroendocrinology 28 (2003) 139–168.
[130] C. Saudan, N. Baume, P. Mangin, M. Saugy, Urinary analysis of 16(5a)-androsten-3a-ol by gas chromatography/combustion/isotope ratio mass spectrometry: implications in anti-doping analysis, J. Chromatogr. B 810 (2004) 157–164.
[131] M. Schumacher, R. Guennoun, D.G. Stein, A.F. De Nicola, Progesterone: therapeutic opportunities for neuroprotection and myelin repair, Pharmacol. Ther. 116 (2007) 77–106.
[132] M. Schumacher, P. Liere, Y. Akwa, K. Rajkowski, W. Griffiths, K. Bodin, J. Sjövall, E.E. Baulieu, Pregnenolone sulfate in the brain: a controversial neurosteroid, Neurochem. Int. 52 (2008) 522–540.
[133] M. Sedlacek, M. Korinek, M. Petrovic, O. Cais, E. Adamusova, H. Chodounska, L. Vyklicky Jr, Neurosteroid modulation of ionotropic glutamate receptors and excitatory synaptic transmission, Physiol. Res. 57 (2008) S49–S57.
[134] M. Serra, E. Sanna, M.C. Mostallino, G. Biggio, Social isolation stress and neuroactive steroids, Eur. Neuropsychopharmacol. 17 (2007) 1–11.
[135] L.A. Shervington, N. Smith, E. Norman, T. Ward, R. Phillips, A. Shervington, To determine the cytotoxicity of chlorambucil and one of its nitro-derivatives conjugated to prasterone and pregnenolone, towards eight human cancer cell-lines, Eur. J. Org. Chem. 44 (2009) 2944–2951.
[136] K. Shibuya, N. Takata, Y. Hojo, A. Furukawa, N. Yasumatsu, T. Kimoto, T. Enami, K. Suzuki, N. Tanabe, H. Ishii, H. Mukai, T. Takahashi, T.A. Hattori, S. Kawato, Hippocampal cytochrome P450s synthesize brain neurosteroids which are

paracrine neuromodulators of synaptic signal transduction, Biochem. Biophys. Acta 1619 (2003) 301–316.
[137] D. Sidler, P. Renzulli, C. Schnoz, B. Berger, S. Schneider-Jakob, C. Flück, D. Inderbitzin, N. Corazza, D. Candinas, T. Brunner, Colon cancer cells produce immunoregulatory glucocorticoids, Oncogene 30 (2011) 2411–2419.
[138] R.K. Sripada, C.E. Marx, A.P. King, J.C. Rampton, S.S. Ho, I. Liberzon, Allopregnanolone elevations following pregnenolone administration are associated with enhanced activation of emotion regulation neurocircuits, Biol. Psychiatry 73 (2013) 1045–1053.
[139] T.P. Su, E.D. London, J.H. Jaffe, Steroid binding at σ receptors suggests a link between endocrine, nervous, and immune systems, Science 204 (1988) 219–221.
[140] I. Svízenská, P. Dubový, A. Sulcová, Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures: a short review, Pharmacol. Biochem. Behav. 90 (2008) 501–511.
[141] G. Tanda, F.E. Pontieri, G. Di Chiara, Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism, Science 276 (1997) 2048–2050.
[142] A. Tchernof, M.F. Mansour, M. Pelletier, M.M. Boulet, M. Nadeau, V. Luu-The, Updated survey of the steroid-converting enzymes in human adipose tissues, J. Steroid Biochem. Mol. Biol. 147 (2015) 56–69.
[143] F. Tennant, The physiologic effects of pain on the endocrine system, Pain Ther. 2 (2013) 75–86.
[144] F. Tennant, Hormone abnormalities in patients with severe and chronic pain who fail standard treatments, Postgrad. Med. 127 (2015) 1–4.
[145] M. Tohen, E. Vieta, J. Calabrese, T.A. Ketter, G. Sachs, C. Bowden, P.B. Mitchell, F. Centorrino, R. Risser, R.W. Baker, A.R. Evans, K. Beymer, S. Dube, G.D. Tollefson, A. Breier, Efficacy of olanzapine and olanzapine–fluoxetine combination in the treatment of bipolar I depression, Arch. Gen. Psychiatry 60 (2003) 1079–1088.
[146] R.P. Tucker, The roles of microtubule-associated proteins in brain morphogenesis: a review, Brain Res. Rev. 15 (1990) 101–120.
[147] D.P. Uzunov, T.B. Cooper, E. Costa, A. Guidotti, Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 12599–12604.
[148] M. Vallée, W. Mayo, M. Darnaudéry, C. Corpéchot, J. Young, M. Koehl, M. Le Moal, E.E. Baulieu, P. Robel, H. Simon, Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 14865–14870.
[149] M. Vallée, W. Mayo, G.F. Koob, M. Le Moal, Neurosteroids in learning and memory processes, Int. Rev. Neurobiol. 46 (2001) 273–320.
[150] M. Vallée, R.H. Purdy, W. Mayo, G.F. Koob, M. Le Moal, Neuroactive steroids: new biomarkers of cognitive aging, J. Steroid Biochem. Mol. Biol. 86 (2003) 329–335.
[151] M. Vallée, J.D. Rivera, G.F. Koob, R.H. Purdy, R.L. Fitzgerald, Quantification of neurosteroids in rat plasma and brain following swim stress and allopregnanolone administration using negative chemical ionization gas chromatography/mass spectrometry, Anal. Biochem. 287 (2000) 153–166.
[152] M. Vallée, S. Vitiello, L. Bellocchio, E. Hébert-Chatelain, S. Monlezun, E. Martin-Garcia, F. Kasanetz, G.L. Baillie, F. Panin, A. Cathala, V. Roullot-Lacarrière, S. Fabre, D.P. Hurst, D.L. Lynch, D.M. Shore, V. Deroche-Gamonet, U. Spampinato, J.M. Revest, R. Maldonado, P.H. Reggio, R.A. Ross, G. Marsicano, P.V. Piazza, Pregnenolone can protect the brain from cannabis intoxication, Science 343 (2014) 94–98.
[153] B. Vollmayr, F.A. Henn, Stress models of depression, Clin. Neurosci. Res. 3 (2003) 245–251.
[154] M. Wang, L. Seippel, R.H. Purdy, T. Backstrom, Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, pregnenolone sulfate, 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one, J. Clin. Endocrinol. Metab. 81 (1996) 1076–1082.
[155] C.E. Weaver, M.B. Land, R.H. Purdy, K.G. Richards, T.T. Gibbs, D.H. Farb, Geometry and charge determine pharmacological effects of steroids on *N*-methyl-D-aspartate receptor-induced Ca(2+) accumulation and cell death, J. Pharmacol. Exp. Ther. 293 (2000) 747–754.
[156] S. Weill-Engerer, J.P. David, V. Sazdovitch, P. Liere, B. Eychenne, A. Pianos, et al., Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients, J. Clin. Endocrinol. Metab. 87 (2002) 5138–5143.
[157] J.H. Weng, M.R. Liang, C.H. Chen, S.K. Tong, T.C. Huang, S.P. Lee, Y.R. Chen, C.T. Chen, B.C. Chung, Pregnenolone activates CLIP-170 to promote microtubule growth and cell migration, Nat. Chem. Biol. 9 (2013) 636–642.
[158] O.M. Wolkowitz, V.I. Reus, T. Chan, F. Manfredi, W. Raum, R. Johnson, J. Canick, Antiglucocorticoid treatment of depression: doubleblind ketoconazole, Biol. Psychiatry 45 (1999) 1070–1074.
[159] P. Wong, C.C. Chang, C.E. Marx, M.G. Caron, W.C. Wetsel, X. Zhang, Pregnenolone rescues schizophrenia-like behavior in dopamine transporter knockout mice, PLoS One 7 (2012) e51455.
[160] F.S. Wu, T.T. Gibbs, D.H. Farb, Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor, Mol. Pharmacol. 40 (1991) 333–336.
[161] X. Yang, K. Iwamoto, M. Wang, J. Artwohl, J.I. Mason, S. Pang, Inherited congenital adrenal hyperplasia in the rabbit is caused by a deletion in the gene encoding cytochrome P450 cholesterol side-chain cleavage enzyme, Endocrinology 132 (1993) 1977–1982.
[162] P. Zheng, Neuroactive steroid regulation of neurotransmitter release in the CNS: action, mechanism and possible significance, Prog. Neurobiol. 89 (2009) 134–152.
[163] T.S. Zhu, M. Glaser, Regulatory role of cytochrome P450scc and pregnenolone in myelination by rat Schwann cells, Mol. Cell. Biochem. 313 (2008) 79–89.
[164] C.F. Zorumski, S. Mennerick, Neurosteroids as therapeutic leads in psychiatry, JAMA Psychiatry 70 (2013) 659–660.