Neurosteroids, GABA\(_A\) receptors and neurosteroid based drugs: are we witnessing the dawn of the new psychiatric drugs?

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**Abstract**

In broad biological terms, neurosteroids can be defined as a class of endogenous steroids synthesized in the brain or in peripheral steroidogenic tissues having potent and relatively selective activity on brain gamma-aminobutyric acid A (GABA\(_A\)) receptors. In this regard, the most important neurosteroids are allopregnanolone and allotetrahydrodeoxycorticosterone (allo THDOC). These \(\alpha\)-reduced derivatives of pregnenolone and progesterone act as positive allosteric modulators of GABA\(_A\) receptors. As such, they potentiate the inhibitory action of GABA on GABA\(_A\) receptors and produce a wide spectrum of behavioral actions ranging from anxiolytic, anticonvulsive, sedative, hypnotic, amnestic (loss of memory), myorelaxant, and anesthetic effects. Sulfated derivatives of pregnenolone and dehydroepiandrosterone, pregnenolone sulfate (PS) and dehydroepiandrosterone sulfate (DHEAS), are also very important neurosteroids. In contrast to allopregnanolone and allo THDOC, PS and DHEAS induce excitatory effect on neurons because they facilitate the block of GABA\(_A\) receptors. The spectrum of behavioral effects of PS and DHEAS consists of analeptic, anxiogenic, proconvulsive, and anamnestic (cognitive enhancing). The purpose of this review paper is to analyze recent research in the field of neurosteroids and neurosteroid-based drugs with emphasis on interaction of neurosteroids with brain GABA\(_A\) receptors. This article also provides an overview of innovative therapeutic approaches. GABA\(_A\) receptor modulating steroids (GAMS), GABA\(_A\) receptor modulating steroid antagonists (GAMSA), and translocator protein (TSPO) activators are examples of innovative therapeutic approaches in treating clinically important neurological and psychiatric diseases. Consequently, the therapeutic potential of GAMS, GAMSA, and TSPO activators will be briefly evaluated.

**Key words:** Neurosteroids; GABA\(_A\) receptors; translocator protein; drugs
1. Introduction

By definition, neurosteroids are steroid molecules synthesized within the brain under physiologic conditions, and released in a paracrine or autocrine way to induce fast (nongenomic) or long-lasting (genomic) modulation of nerve cell function [1]. The term neuroactive steroid is usually reserved for steroids that act on the central or peripheral nervous system, wherever they come from: by exogenous application (drugs), from steroidogenic tissues, by endocrine secretion (hormones), or from local nerve tissues (neurotransmitters and neuromodulators). For simplicity, in this review, the term neurosteroids and neuroactive steroids will be used as interchangeable terms. In this review, we will focus on neurosteroids, neurosteroid-based drugs, and the interaction of neurosteroids with GABA_A receptors to produce rapid, nongenomic modulatory effects on brain cells. Genomic effects of (neuro)steroids mediated by classical hormonal receptors, as well as nongenomic, rapid effects, other than the one mediated by GABA_A receptors are reviewed in details elsewhere.

2. Biosynthesis of neurosteroids and sites of drug action

The biosynthesis of neurosteroids in the brain tissue is very similar to the synthesis in any other steroidogenic organ - ovary, testis, placenta, or adrenal gland. Neurosteroidogenesis is detected in both, neurons [2] as well as in glial cells [3]. The first phase of neurosteroid synthesis takes place inside the mitochondria. The mitochondrial phase of steroidogenesis yields pregnenolone as a final metabolic product. Pregnenolone arises from cholesterol (Figure 1.) by the action of cytochrome P450 enzyme 11A1 (CYP11A1), also known as CYPscc (2). Lipophilic cholesterol is not able to freely pass through the aqueous environment in order to reach the inner mitochondrial membranes. Thus, cholesterol must be translocated through the hydrophilic media by a process that involves a specific transport system. By the coordinated action of translocator protein (TSPO; for details see text bellow) and steroidogenic acute regulatory protein (STARD1), cholesterol is transported from the outer to the inner mitochondrial membrane [4, 5]. Enzyme CYP11A1, which is responsible for pregnenolone synthesis, is located close to the inner mitochondrial membrane. This enzyme removes the side chain from the 27-carbon ring of cholesterol and produces the 21-carbon ring steroid molecule of pregnenolone. Although pregnenolone produces behavioral effects on its own [5], it is much more important as a precursor of the all other endogenous neurosteroids. Since the transport of cholesterol to the inner mitochondrial membrane is “bottle-neck” in the production of pregnenolone, and pregnenolone is the “mother” of all neurosteroids, translocation of cholesterol from the outer to inner mitochondrial membrane is considered to be a rate-limiting step in the synthesis of neurosteroids [6].

The neurosteroid precursor pregnenolone, is further metabolized in the endoplasmic reticulum by multiple, subsequent enzymatic reactions (microsomal phase of neurosteroid synthesis). As it is shown in Figure 1., these reactions are catalyzed with a variety of enzymes (CYP450 as well as non CYP450 types) and are able to produce functionally and structurally diverse neurosteroids [7-9].

For example, pregnenolone (Figure 1., white box) is conjugated by the enzyme sulfotransferase (SULT2A1) yielding pregnenolone sulfate (Figure 1., PREGN.S, red box), the neurosteroid with excitatory action on nerve cells [9, 10]. The biochemical reactions and pathways involved in neurosteroid synthesis are usually bidirectional. In the case of excitatory pregnenolone sulfate, a reverse reaction catalyzed by sulfatase, returns inhibitory acting pregnenolone [6]. Also, in two subsequent reactions catalyzed by CYP17A1, pregnenolone yields dehydroepiandrosterone (Figure 1., DHEA, red box), another active neurosteroid that seems to have a paradoxical, dual mode of action on neurons [11]. Namely, DHEA induces an excitatory response when it is used acutely. On the contrary, when DHEA is used chronically, inhibitory effects on neurons should be expected. DHEA is sulfated by sulfotransferase to produce dihydriopandrosterone sulfate (Figure 1., DHEAS, red box), a neurosteroid with pure excitatory action on neurons [12]. By removing sulfate from the DHEAS molecule with the enzyme sulfatase, DHEAS is metabolized back into DHEA.

From DHEA, by the action of 3α- hydroxysteroid dehydrogenase (3α-HSD) or 5-α and 5-β reductase [13], the endogenous neurosteroids 5α,3α-androsterone (Figure 1., ANDROSTERONE, blue box) and 5β,3α-androsterone (also known as ethiocolanolone) are produced. These metabolites of DHEA act as inhibitory neurosteroids [14, 15]. Also, the pheromone androstenol, could
be synthesized from DHEA in human and boar testicles [16]. This lipophilic and musk smelling substance, acts on GABA-A receptors located in the olfactory bulb to increase sexual receptive behavior [16]. As an interesting coincidence, it must be emphasized that two Nobel Prize laureates from Croatia, Vladimir Prelog and Lavoslav Ružička, were pioneers of androstenol isolation from boar testicles [17].

Progesterone (Figure 1, white box) is synthesized from pregnenolone in a reduction reaction, which is catalyzed by 3β-hydroxysteroid dehydrogenase or 3β-HSD [7]. From a historical perspective, progesterone had a fundamental role in the development of the neurosteroid concept. Namely, a report published by Hans Selye on the anesthetic effect of progesterone in rats and mice was the first experimental evidence for the rapid central nervous system (CNS) effect of steroid molecules [18]. Like pregnenolone, progesterone also serves as a precursor for the synthesis of important neurosteroids. In two subsequent metabolic steps catalyzed by 5-α reductase and 3α- or 3β-HSD, the following neurosteroids are derived from progesterone: 3α,5α-tetrahydroprogesterone (known as allopregnanolone; Figure 1, 3α5αTH-PROGEST., blue box), 3α,5β-tetrahydroprogesterone (known as pregnanolone.), 3β,5α-tetrahydroprogesterone (known as isoallopregnanolone), and 3β,5β-tetrahydroprogesterone (known as epiallopregnanolone). Both 3α-tetrahydroprogesterone molecules produce inhibitory actions on neurons. On the other hand, 3β-tetrahydroprogesterone molecules are natural excitatory neurosteroids with the ability to antagonize the anesthetic effect of inhibitory neurosteroids, including allopregnanolone [19-21]. Important groups of inhibitory acting neurosteroids produced from progesterone are derivatives of deoxycorticosterone. In a three step reaction catalyzed by CYP21, 5-α reductase, and 3α-HSD, with deoxycorticosterone and dihydrodeoxycorticosterone (Figure 1, 5αDH-DEOXYCORT., blue box) as intermediate products, progesterone yields 3α,5α-tetrahydrodeoxycorticosterone (alloTHDOC; Figure 1, 3α5α TH-DEOXYCORT., blue box) and 3α,5β-tetrahydrodeoxycorticosterone thDOC [6, 22]. Both reduced derivatives of deoxycorticosterone produce inhibitory effects on neuronal cells [23, 24].

It is interesting that protein or mRNA for the CYP21 enzyme, which is necessary for the production of deoxycorticosterone from progesterone, and for the production of deoxycortisol from 17OH-progesterone, have

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**Figure 1.** A schematic diagram of neurosteroidogenesis with selected enzymes and enzyme inhibiting drugs. Synthesis of neurosteroids is not complete, for the sake of visibility. Some neurosteroids that are missing here are mentioned in the text (ethiocolanolone and androstenedol, DHEA metabolites; pregnanolone and 3β-tetrahydroprogesterones, progesterone metabolites; THDOC, deoxycorticosterone metabolite). Neurosteroids with inhibitory actions on neurons are presented in blue boxes. Sulfated neurosteroids with excitatory actions on neurons are presented in red boxes. DHEA has dual mode of action, depending on the way of use. Acute effect of DHEA is excitation of neurons. Thus, DHEA is presented in form of red box.

Abbreviations: TSPO = translocation protein; STRD1 = steriodogenic acute regulatory protein; PREGN.S. = pregnenolone sulfate; SULT2A1 = sulfotransferase; 17OH-PREGN. = 17 hydroxy pregnenolone; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate; 3α-RED. = 3α-reductase; 3α5αTH-PROGEST. = 3α5α tetrahydroprogesterone, also known as alloprogesterone; 17OH-PROGEST. = 17 hydroxyprogesterone; ANDROSTENED. = androstenedione; 3α5αTH-DEOXYCORT. = 3α5α tetrahydro deoxycorticosterone; AROM. = aromatase, also known as CYP19A1 or P450arom; abirat. a. = abiraterone acetate; finast. = finasteride; MPa = medroxyprogesterone acetate
not yet been detected in brain tissue [6]. On the other hand, mRNA and proteins for CYP11B and CYP11AS, which are responsible for cortisol and aldosterone synthesis, respectively, were found in a significant amount in the cortex, hippocampus, basal ganglia, cerebellum (CYP11B), and hypothalamus (CYP11AS) [6]. Specific drugs could manipulate the synthesis of both excitatory and inhibitory neurosteroids. For example, the biosynthesis of inhibitory acting neurosteroids can be inhibited by 5α-reductase inhibitors (Figure 1., 5α-RED.) such as finasteride (13,25) or by inhibitors of 3α-HSD such as medroxyprogesterone acetate [26]. Inhibition of inhibitory neurosteroids with finasteride or medroxyprogesterone acetate (Figure 1., MPA) has been associated with depression, anxiety, irritability, and sexual dysfunction. These behavioral alternations are well known side effects of 5α-reductase inhibitors [27, 28] or MPA [29, 30].

Inhibitors of CYP11A (aminogluthethimide and ketoconazole), by blocking the production of pregnenolone (Figure 1), may inhibit the production of all neurosteroids [31, 32]. Inhibitors of CYP17A1, such as abiraterone acetate (Fig. 1; antiandrogen), may block the production of DHEA from pregnenolone [33] and, indirectly, the production of its derivatives DHEAS (excitatory neurosteroid) and androsterone (inhibitory neurosteroid). The CYP11B1 inhibitor metyrapone (anticortisol drug) may inhibit the synthesis of potential excitatory neurosteroids, which are reduced metabolites of cortisol [6, 34].

Trilostan inhibits 3β-HSD (Figure 1) and conversion of pregnenolone to progesterone [35]. Thus, trilostan inhibits the production of all classical steroid hormones – progesterone, estrogens, androgens, glucocorticoids, and mineralocorticoids. Likewise, trilostan inhibits the production of all brain neurosteroids, with the exception of pregnenolone and its derivatives PS, DHEA, DHEAS, and the DHEA derivative androsterone. Synthesis of these neurosteroids does not depend on the 3βHSD enzyme.

Aromatase (Figure 1., AROM) is the enzyme that catalyzes the conversion of androgens to estrogens [35]. In mice, letrozole (Fig. 1), an aromatase inhibitor that blocks the conversion of testosterone to 17β-estradiol, significantly decreased testosterone potentiation of pen-tylentetrazole-induced seizures. Thus, endogenously produced 17β-estradiol derived from testosterone might contribute to the observed pro-convulsive actions of testosterone in mice [37].

Also, specific drugs could activate steroidogenic enzymes. The term “selective steroidogenic brain stimulants” (SSBSs) has been proposed for drugs that activate steroidogenesis and potentiate the effects of endogenously synthesized neurosteroids [38]. Good examples of SSBSs are fluoxetine and norfluoxetine. Fluoxetine is a well-known antidepressant drug that belongs to a class of selective serotonin reuptake inhibitors (SSRIs), and norfluoxetine is an active metabolite of fluoxetine. Fluoxetine, norfluoxetine, several other SSRI (paroxetine, sertraline), and other non-SSRI antidepressant drugs (venlafaxine, mirtazapine) are potent inducers of the 3α-HSD enzyme [39]. These drugs have been found to increase levels of inhibitory neurosteroid alloprevagenalone and to potentiate its behavioral effects [40]. The TSPO activators, a new group of investigational drugs that have the potential to treat important neurological and psychiatric diseases [41] also increase brain levels of inhibitory neurosteroids and potentiate their behavioral action (for details about TSPO activators, see text below).

3. GABA A receptors as a target for the nongenomic action of neurosteroids

3.1. GABA A receptors – how many binding sites are there?

The GABA A receptor is a protein complex made up of five protein subunits and associated with the chloride channel (Figure 2). Each protein subunit (Figure 2., left) contains four transmembrane regions with a characteristic cysteine loop (cys-loop), and one intracellular loop and one extracellular COOH terminus [42]. Protein subunits of GABA A receptors are symmetrically arranged around the chloride channel, building the wall of this channel (Figure 2, right). The main inhibitory neurotransmitter, γ-aminobutyric acid (GABA), by acting on its binding site at the GABA A receptor, opens the chloride channel and increases the transmembrane inflow of chloride anions, inducing a rapid (in milliseconds), inhibitory, hyperpolarization potential on neurons. Due to its typical structure and function, the GABA A receptor belongs to a family of cys-loop ligand gated ion channel (LGIC) receptors [43].

Also, the GABA A receptor is a receptor complex, which
is composed of multiple binding sites. It is generally accepted that this receptor complex consists of the following binding sites: binding sites for the neurotransmitter GABA and competitive antagonists [44-47], modulatory binding sites for sedative/hypnotic and anxiolytic benzodiazepines [48-51], binding sites for sedative/hypnotic and anesthetic barbiturates [52-54], binding sites for convulsive toxins like picrotoxine and pyrethroid insecticides [55, 56], and neurosteroid modulatory sites [57-60] for neurosteroids. The existence of these “classical” binding sites is consistently and repeatedly confirmed by different research groups all over the world. In almost 70 years of research devoted to GABA neurotransmission [62-66], evidence on “classical” GABA\(_A\) receptor binding sites has been accumulated at different levels of body organization (organism, tissue, cellular, and subcellular level) and by using of various research methods, including behavioral (in vivo testing in models for anxiety, depression, sedation/hypnosis, epilepsy), biochemical (receptor binding studies and \(36\)chlorine uptake in synaptosomes), electrophysiological (“patch clamp” and similar techniques), and molecular biology (receptor subunit transfection, gene knockout, gene knockdown, site directed mutagenesis) methods [64-66].

Despite this, more than a dozen binding sites, other than the “classical” ones mentioned above, were postulated to exist within GABA\(_A\) receptors. Just to mention few: binding sites for general anesthetics and ethanol [67-70], binding sites for furosemid and amiloride diuretics [71, 72], binding sites for Zn\(_2+\) [73], binding sites for fluoroquinolone antibiotics [74], binding sites for fenamate NSAIDs [74, 75], and binding sites for dehydrogenated ergot drugs [76-78]. The 2015 Nobel Prize in Physiology or Medicine was awarded with one half jointly to W.C. Campbell and S. Ōmura for their discovery of broad spectrum antihelmintic drugs, avermectine, and ivermectin (79). A long time ago, Wang and Pong proposed the mechanism of action of those two drugs which involved the interaction of the antihelmintic drug with the specific binding site at the GABA\(_A\) receptor complex in invertebrate muscles [80]. Only recently, Estrada-Mondragon and Lynch published results that confirm the direct interaction between ivermectin and vertebrate GABA\(_A\) receptors [81].

3.2. GABA\(_A\) receptor assembly and GABA\(_A\) receptor subtype heterogeneity

So far, eight GABA\(_A\) receptor subunits (named by the Greek alphabet - alpha, beta, gamma, delta, eta, omega, pi, and rho) and their 19 isoforms (\(\alpha1-6\), \(\beta1-3\), \(\gamma1-3\), \(\delta\), \(\epsilon\), \(\theta\), \(\pi\), \(\rho1-3\)) have been identified [82]. From these, theoretically \(\approx 800\) different hetero or homo pentamer subunit combinations and structurally different GABA\(_A\) receptors are possible to build. However, only 11 subunit combinations were positively identified as native GABA\(_A\) receptors in mammalian brain. With 9 GABA\(_A\) receptor subunit combinations in the category “highly probable” and another 6 in the category “tentative”, that makes the final list of 26 GABA\(_A\) receptors. The most abundant native GABA\(_A\) receptor combination is the \(\alpha1\beta2\gamma2\) (60% of all). GABA\(_A\) receptors which are assembled of \(\alpha2\beta3\gamma2\) and \(\alpha3\beta2\gamma2\) subunit combinations represent 15–20% and 10–15% of the total native receptors, respectively [83].

3.3. Modulation of GABA\(_A\) receptors by neurosteroids

Excitatory neurosteroids (PS, DHEAS, DHEA if acutely applied, isoalphopregnanolone, and epialphopregnanolone) act as negative allosteric modulators of GABA\(_A\) receptors and facilitate closing of GABA\(_A\) receptor-associated Cl\(^-\) ionophore [5, 10, 12, 84]. In contrast, inhibitory neurosteroids (allopregnanolone, pregnanolone, THDOC, alloTHDOC, androsterone, DHEA if chronically applied, ethiocholanolone, and androstenol) act as positive allosteric modulators of GABA\(_A\) receptors and
potentiate opening of GABA<sub>λ</sub> receptor associated Cl-channels by GABA [14, 15, 19-21, 23, 85, 86]. GABA<sub>λ</sub> receptor binds two GABA molecules, at the interface between the α and β subunit. This means that the cooperative action of two GABA molecules is needed for the GABA gate chloride channel. Binding of benzodiazepines (i.e. diazepam) at the interface of α and γ subunits induces positive allosteric modulation of GABA<sub>λ</sub> receptors and facilitates opening of chloride channels by GABA. In the model of neurosteroid binding proposed by Hosie [87], four molecules of neurosteroids interact with the GABA<sub>λ</sub> receptor. Two neurosteroid molecules interact with GABA binding sites to potentiate the opening of Cl channels with GABA. The other two molecules interact with the hydrophobic M region to directly open GABA<sub>λ</sub> receptor associated chloride channels. It seems quite possible that neurosteroid binding sites consists of multiple, overlapping binding sites, one potentiating binding site, one activating binding site, and one binding site for excitatory (sulfated) neurosteroids [58, 88].

GABA receptors consisting of α1/β subunits or ρ subunits are relatively insensitive to neurosteroids [89]. In contrast, GABA<sub>λ</sub> receptors containing δ subunit, particularly in combination with α4 or α6 subunits are highly sensitive to low, nanomolar concentrations of neurosteroids [90, 91].

4. Behavioral effects of neurosteroids

Inhibitory neuroactive steroids induce dose dependent anxiolytic, anticonvulsive, sedative/hypnotic, amnestic (high dose), and anesthetic effects [92]. The spectrum of activity of inhibitory neuroactive steroids is, therefore, very similar to those of classical benzodiazepines, which are full agonists at benzodiazepine receptors. However, in comparison to full agonist benzodiazepines, inhibitory neuroactive steroids seem to have a decreased potential for tolerance and abuse, but only to locomotor effects. Also, when compared to benzodiazepines, inhibitory neurosteroids exhibit less effect on locomotor coordination in combination with ethanol. These data suggest that inhibitory neurosteroids might have a better safety profile than classical sedative hypnotic drugs, like benzodiazepines or barbiturates. Inhibitory neuroactive steroids also induce anti-aggressive behavior.

The inhibitory neuroactive steroids seem to decrease depressive mood. The antidepressant effect of systemically applied allopregnenolone and other α reduced neurosteroids have been repeatedly reported in different animal models of depression – forced swimming test, olfactory bulbectomy, and social isolation test [93]. Moreover, results of recent preclinical and clinical studies, connected pregnane neurosteroids (allopregnanolone and alloTHDOC) and pregnenolone with the pathophysiology of bipolar disease [94] and schizophrenia [95].

Inhibitory neuroactive steroids also modulate the acute and chronic stress reaction [96]. Brain levels of inhibitory neuroactive steroids rise during acute stress, which is followed by a decrease in HPA activity, suggesting a protective role of inhibitory neurosteroids in acute stress. On the contrary, brain levels of inhibitory neuroactive steroids decrease during chronic stress, which is followed by increased HPA activity.

Excitatory neuroactive steroids induce dose dependent spectra of activity, which includes analeptic, anxiogenic (high dose), and proconvulsive actions [92]. These neurosteroids also have an amnestic (cognitive enhancing) effects [97]. The profile of behavioral activities induced by excitatory neurosteroids could be compared with that of partial inverse agonists to the benzodiazepine modulatory site. Excitatory neuroactive steroids (PS, DHEAS and DHEA) decrease depressive mood and clearly induce an antidepressant action in animals and humans [92]. They also induce aggressive behavior, impulsivity, and stimulate “acute stress like” behaviors (anxiety and excitation). In some countries, DHEA supplementation in elderly people has been advertised as an anti-aging medication [98].

Neurosteroids play an important role in alcohol tolerance and withdrawal [92, 11, 99]. In addition to the behavioral effects mentioned above, neurosteroids are also able to induce analgesic [100], anti-inflammatory [101], and neuroprotective [102, 103] effects.

5. Therapeutic potentials of synthetic GABA<sub>λ</sub> modulatory steroids (GAMS) and GABA<sub>λ</sub> modulatory steroid antagonists (GAMSA)

Synthetic GAMS may represent a rational alternative approach to therapy with endogenous neurosteroids. One problem with natural neurosteroids is low oral
bioavailability, due to rapid hepatic oxidation and inactivation. Another problem is their potential for unwanted, genomic effects. Namely, natural neurosteroids could be converted to precursor steroids (progesterone, testosterone), which have a high affinity for classical hormonal receptors and low affinity for neurosteroid binding sites at GABA<sub>α</sub> receptors.

The neurosteroid ganaxolon is an example of a synthetic steroid, which seems to be able to overcome the mentioned problems above. Ganaxolon is a 3β- methylated synthetic analog of the endogenous neuroactive steroid allopregnanolone. It is well known that 3β- substitution enhances the bioavailability of pregnane steroids, without altering their primary pharmacodynamic properties. Ganaxolon is a positive allosteric modulator at GABA<sub>α</sub> receptors with high potency for receptors and high efficacy against chemically induced convulsions (bicuculline, pentylenetetrazole, aminophylline), maximal electroshock and amigdala kindling seizures in animals [104].


Also, it is under clinical investigation for the treatment of PTSD (https://ClinicalTrials.gov/show/identifier:NCT01339689), fragile X syndrome (https://ClinicalTrials.gov/show/identifier:NCT01725152) and smoking cessation (https://ClinicalTrials.gov/show/identifier:NCT01857531).

GR3027 and UC1010 are investigational GABAA receptor modulating steroid antagonists (GAMSA). Both are 3β-hydroxy steroids and, as such, they are able to antagonize the enhancement of GABA<sub>α</sub> receptor activation caused by 3α-hydroxy neurosteroids. Increasing signaling through the GABA<sub>α</sub> receptor is a key driver for the neurological symptoms associated with hepatic encephalopathy (HE) as well as for CNS symptoms (depression, anxiety) associated with the premenstrual dysphoric disorder (PMDD). GR3027 and UC1010 are promising candidate drugs intended to treat the symptoms of patients with HE or PMDD, respectively [106, 107]. Also, investigational GAMSA drugs are 17PA (3α5α-17-phenylandrost-16-en-3-ol) and HBAO (20R-17β-(1-hydroxy-2,3-butadienyl)-5α-androstene-3α-ol). Both, 17PA and HBAO are synthetic derivatives and antagonists of allopregnenolone [107, 108], with relative selectivity for a6β2–3δ GABA<sub>α</sub> receptors (HBAO) or for α1β2γ2 GABA<sub>α</sub> receptors (17PA). Basic search of the web site ClinicalTrials.gov for “GR3027”, or “UC1010” or “HBAO” made on the 12th of January 2016 yielded only 1 heat. The study is in phase I/II, and its aim is to evaluate UC1010 (https://ClinicalTrials.gov/show/identifier:NCT01875718) treatment in premenstrual dysphoric disorder (PMDD).

6. Therapeutic potential of TSPO activators

By definition, an “ideal” anxiolytic drug is anxioselective anxiolytic. It induces rapid and selective anxiolytic effect, without significant sedative, hypnotic, amnestic, or myorelaxant effect. Also, anxioselective anxiolytics should not have significant abuse potential or the ability to induce tolerance during long-term therapy. In the last two decades of the 20th century, a substantial amount of money was invested in the pharmaceutical industry for research and development of anxioselective drugs. But, there is no result of this investment [109]. With time, the idea of anxioselective anxiolytics became the “holy grail” of anxiolytic research and therapy [110].

The pharmacological properties of nonsteroid TSPO activators emapunil (XBD173 or AC-5216) and etifoxine seem to match the above-mentioned definition of an “ideal” anxioselective drugs. Both, emapunil (N-benzyl-N-ethyl-2-(7-methyl-8-oxo-2-phenylpurin-9-yl)acetamide) and etifoxine (2-ethylamino-6-chloro-4-methyl-4-phenyl-4H-3,1-benzoxazine hydrochloride) are synthetic, nonsteroidal TSPO agonists. As such, emapunil as well as etifoxine, stimulate the synthesis of neurosteroids. Emapunil exhibited high affinity for TSPO (previously known as peripheral benzodiazepine receptors) in the crude mitochondrial fraction prepared from the whole brain of rats (Ki = 0.297 nM), but only negligible affinity for central benzodiazepine receptors [111]. In work reported by Kita and Furukawa, the anxiolytic-like
effects of emapunil were inhibited by trilostane (Figure 1., 3β-HSD inhibitor), finasteride (Fig. 1, 5-α reductase inhibitor), and picrotoxin (blocker of GABA$_A$ receptor associated chloride channel), while those of diazepam (full agonist at benzodiazepine binding site) were inhibited by picrotoxin only [112]. These results clearly demonstrate that the anxiolytic-like effects of emapunil are due to newly synthesized inhibitory neurosteroids that enhance GABA$_A$ receptor function. Emapunil produces anti-anxiety and antidepressant-like effects that are mediated by TSPO and increased synthesis of inhibitory neurosteroids [111]. However, it does not cause myorelaxant, sedative/hypnotic effect, or addiction with repeated dosing [113], the side effects usually associated with conventional benzodiazepines. Like emapunil, etifoxine activates TSPO and rapidly increases the production of neurosteroids [114], especially allopregnanolone [115]. The modulatory action of etifoxine on GABA$_A$ receptor is mediated by the β subunit [116].

In addition to behavioral actions, TSPO activators have anxiolytic effects [117, 118], as well as neuroprotective, and anti-inflammatory actions in experimental models for neurodegenerative diseases [119-121] and neurotrauma [122].

Basic search of web site ClinicalTrials.gov for “XBD173”, or “emapunil” or “AC5216” made on the 12th of January 2016 yielded 4 hits. There is only one ongoing clinical trial (phase II) with the nonsteroid TSPO agonist emapunil for the treatment of generalized anxiety disorder (GAD). Three clinical trials with emapunil are aimed at investigating emapunil as a ligand for positron emission tomography (PET). In one of these studies, emapunil is intended for use in brain imaging of neurodegenerative diseases. Search of the same web site with the term “Etifoxine” gave another two hits. Both are Phase III studies. One study recruits volunteers aged between 65 and 75 years old (https://ClinicalTrials.gov/show/identifier:NCT02143895) and another recruits healthy volunteers (https://ClinicalTrials.gov/show/identifier:NCT02147548). The studies are evaluating the effects of 100 mg of Etifoxine and 2 mg of Lorazepam on vigilance and cognitive function in the elderly.

7. Conclusions

Neurosteroids are important endogenous modulators of neurons and glial cell activity. They are implicated in the pathophysiology of clinically important diseases, like anxiety, unipolar depression, postmenstrual dysphoria syndrome, bipolar depression, schizophrenia, hepatic encephalopathy, epilepsy, painful states, and brain injury. In search for etiopharmacological approaches in treating these diseases, neurosteroids should be seriously taken into account.

Neurosteroid-based drugs produce a full spectrum of pharmacological actions that overlap with those of other positive allosteric modulators of the GABA-A receptor but, at the same time, exhibit important quantitative and qualitative differences. Preclinical evaluations of GAMS, GAMSA, or TSPO activators have predicted the efficacy of these drugs in the treatment of several important CNS disorders. It seems that with these compounds, new and exciting therapeutic avenues might be opened.

Author contributions

Both authors contributed in manuscript drafting, writing and revising it critically for its scientific content. Both authors have approved the final version of article.

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