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Neurosteroid Biosynthesis Upregulation: A Novel Promising Therapy for Anxiety Disorders and PTSD

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1. Introduction

Generalized anxiety, panic, and posttraumatic stress disorder (PTSD) are debilitating conditions, which have an incidence of one in ten persons in the general population and epidemiological studies also report that these disorders often occur with depression (1-3). Anxiolytic benzodiazepines, including diazepam and alprazolam, remain the best and most used treatments for these conditions (4-7). However, their therapeutic use is associated with side effects, which include sedation and rapid development of tolerance as well as dependence. This results in severe discontinuation symptoms and often to drug abuse (4-6, 8; 9).

In many patients, including patients with PTSD, the pharmacological effects of these drugs are very weak and there is a large number of non-responders (10-12). This has stimulated drug design that for many decades has focused in the development of new more effective therapies for anxiety disorders (13-15). Novel neuronal biomarkers for the pharmacological targets of the next generation of anxiolytic drugs have been discovered.

The downregulation of neurosteroid biosynthesis has been implicated in the pathophysiology of anxiety and depressive disorders (reviewed in 16). Decreases in cerebrospinal fluid (clinical studies) and brain content (preclinical studies) of the $GABA_A$ receptor-active progesterone derivative, allopregnanolone, have been associated with affective and mood disorders, which includes depression, anxiety spectrum disorders, PTSD, premenstrual dysphoric disorder, schizophrenia, and impulsivity (17-27). Thus, elevating or normalizing the downregulation of brain allopregnanolone levels could be a promising therapeutic strategy for these psychiatric disorders. This prompted investigations to develop new neurosteroidogenic agents to contrast allopregnanolone biosynthesis deficits in anxiety and depression (28-31).

We measured allopregnanolone levels in the cerebrospinal fluid (CSF) of PTSD patients assuming that allopregnanolone levels in the CSF reflect the levels of this neurosteroid in the brain (17). Also, in depressed patients, the concentration of allopregnanolone in the CSF was decreased by about 50-60% of the levels measured in non-psychiatric patients (26). The CSF allopregnanolone level decrease is likely induced by a downregulation of the expression of 5α-reductase type I mRNA in the prefrontal-cortex (area BA9) that we measured in

depressed patients and age- and sex-matched non-psychiatric subjects (32). The cortical level of 5α-reductase mRNA in depressed patients was dramatically decreased to about 50% of the levels measured in non-psychiatric comparison subjects, whereas the levels of 5α-reductase mRNA was unchanged in the cerebellum (32). In depressed patients, SSRI treatment with fluoxetine and fluvoxamine normalized the CSF allopregnanolone content (26) in a manner that correlated with the improvement in depressive symptoms. These results were confirmed in studies that determined allopregnanolone or levels of 5α-tetrahydrodeoxycorticosterone, another positive modulator of $GABA_A$ receptor function, in the plasma of depressed patients treated with SSRIs (33).

In premenopausal women with PTSD, the CSF allopregnanolone levels were decreased by about 60% and were inversely correlated with PTSD re-experiencing and comorbid depressive symptoms (17). Interestingly, CSF allopregnanolone levels were lowest in those patients with PTSD and comorbid depression. Also, the *ratio* of allopregnanolone to its steroid precursor, 5α-dihydroprogesterone (5α-DHP), was decreased among the PTSD patients, suggesting the presence of an impairment in the biosynthesis of allopregnanolone from its precursor 5α-DHP (17). These data suggest that the downregulation of brain allopregnanolone levels in PTSD and depressed patients may cause a GABAergic neurotransmission dysfunction, which in turn results in the behavioral symptoms seen in these patients.

Following the finding that fluoxetine and paroxetine and other SSRIs increase the content of allopregnanolone in several rodent brain structures (34), we hypothesized that normalization of brain allopregnanolone levels may underlie the pharmacological effects of the so called "selective serotonin reuptake inhibitors" or SSRIs in mood disorders. To test this hypothesis, we conducted experiments using the socially isolated mouse as an animal model of anxiety disorders and PTSD (16; 35-38). The socially isolated mouse expresses a robust decrease of corticolimbic allopregnanolone levels, which are associated with anxiety-like behaviors, fear, resistance to sedation, and heightened aggression (16; 35; 39). These behavioral deficits can be ameliorated by administration of fluoxetine and other SSRIs that upregulate allopregnanolone levels. Interestingly, fluoxetine's pharmacological effects resulted to be independent from the ability of this drug to inhibit serotonin reuptake (35; 36).

Our experiments support a selective and novel mechanism whereby SSRIs, acting as selective brain *steroidogenic* stimulants (SBSSs), increase brain corticolimbic allopregnanolone levels and improve PTSD, anxiety, and depression behavioral symptoms.

2. Neurosteroids modulation of $GABA_A$ receptors function

Biosynthesis of neurosteroids in the brain is independent from adrenals, ovaries, and testis (40-44). Neurosteroids are functionally active in modulating gene expression and neurotransmitter systems (45-52). Allopregnanolone exerts pharmacological actions, such as anticonvulsant, anxiolytic, antidepressant, and even sedative-hypnotic (53-60). These pharmacological actions are similar to those elicited by barbiturates and benzodiazepines (52; 61; 62). Allopregnanolone potently (nM affinity), positively, and allosterically modulates the action of GABA at $GABA_A$ receptors (45-51). The endogenous physiological relevance of allopregnanolone is substantiated by its facilitation and fine-tuning of the efficacy of direct $GABA_A$ receptor activators and positive allosteric modulators of GABA action at $GABA_A$ receptors (43; 47; 48; 63). The demonstration that allopregnanolone potentiates GABA responses via two binding sites in the $GABA_A$ receptor that, respectively, mediate the potentiation and the direct activation of the $GABA_A$ receptor by allopregnanolone has been

pivotal in neurosteroid pharmacology (64). Also, $GABA_A$ receptors incorporating α4, α6, and δ subunits in combination with γ and β subunits show higher affinity (nM range) for allopregnanolone (45; 46; 51; 64; 65). Relevant for pharmacological strategies to overcome behavioral deficits resulting from $GABA_A$ receptor signal transduction deficits, allopregnanolone allosteric modulation of the action of GABA at $GABA_A$ receptors is much less selective than that of benzodiazepines, which are relatively inactive at α4- or α6-containing $GABA_A$ receptors (4; 45; 46; 66).

3. Neurosteroid biosynthesis in corticolimbic neurons

A study of the neuronal localization of the neurosteroidogenic enzymes, 5α-reductase type I and 3α-hydroxysteroid dehydrogenase (3α-HSD), has recently showed that these enzymes are not expressed in GABAergic cortical interneurons or glial cells (67). Of note, 5α-reductase and 3α-HSD were highly expressed and co-localized in a region-specific way in primary GABAergic and glutamatergic neurons, including pyramidal neurons, granular cells, reticulo-thalamic neurons, medium spiny neurons of the striatum and nucleus accumbens, and Purkinje cells in the cerebellum (67). This suggested that allopregnanolone synthesized in glutamatergic cortical or hippocampal pyramidal neurons or in granular cells of the dentate gyrus may be secreted in: 1) a paracrine manner which would allow allopregnanolone to reach $GABA_A$ receptors located in the synaptic membranes of other cortical or hippocampal pyramidal neurons, or 2) an autocrine fashion which would allow allopregnanolone to act locally by binding post-synaptic or extra-synaptic $GABA_A$ receptors located on the same dendrites or cell bodies of the cortical or hippocampal pyramidal neuron in which it was produced (67). Alternatively, allopregnanolone might not be released, but may instead diffuse laterally into synaptosome membranes of the cell bodies or dendritic arborization of glutamatergic neurons in which it is produced to attain intracellular access to specific neurosteroid binding sites of $GABA_A$ receptors (67; 68). In the amygdala, for example, this would functionally baffle the effects of concomitant excitatory inputs to glutamatergic projection neurons during exposure to unconditioned stress during fear conditioning or to conditioned stressors during extinction.

On the other hand, allopregnanolone produced in primary output GABAergic neurons from the reticular thalamic nucleus may secrete allopregnanolone simultaneously with GABA to concomitantly act at post-synaptic $GABA_A$ receptors inserted in glutamatergic thalamocortical neurons (69). Very similarly, allopregnanolone synthesized by striatal medium spiny GABAergic neurons and cerebellar Purkinje cells may activate post-synaptic $GABA_A$ receptors located on cell bodies or dendrites of neurons in the deep cerebellar nuclei (67).

The clarification of allopregnanolone site of synthesis and action across several brain regions has been pivotal to our understanding of the possible mechanisms by which allopregnanolone is secreted and acts at $GABA_A$ receptors. These studies underscore the functional role of allopregnanolone in fine tuning the strength of GABAergic neurotransmission under physiological conditions and how deficits in allopregnanolone biosynthesis may result in abnormal behavior.

4. Social isolation induces a selective neuron-specific decrease of 5α-reductase in corticolimbic neurons

Exposure of rodents to protracted social isolation stress for 4-8 weeks induces a decrease in allopregnanolone biosynthesis in several corticolimbic structures as a result of a

downregulation of the mRNA and protein expression of 5α-reductase type I (35; 70-73; reviewed in 38). Socially isolated mice show a 70% reduction in the synthesis rate of allopregnanolone and 5α-DHP biosynthesis compared to group-housed mice (35; 72).
Allopregnanolone and 5α-DHP are unevenly distributed and expressed in various brain structures (48; 74). The rodent olfactory bulb shows the highest concentrations of 5α-DHP and allopregnanolone followed by the frontal cortex, hippocampus, amygdala, striatum, and cerebellum (74). Interestingly, the largest decrease of 5α-reductase was found in brain regions regulating emotional behavior, including the amygdala and hippocampus, followed by the olfactory bulb and the frontal cortex (74). The expression of 5α-reductase failed to change in the cerebellum and striatum (74; 75). Decreased 5α-reductase was specifically found in cortical pyramidal neurons of layers V-VI, in hippocampal CA3 pyramidal neurons and glutamatergic granular cells of the dentate gyrus, and in the pyramidal-like neurons of the basolateral amygdala (75). However, 5α-reductase fails to change in GABAergic neurons of the reticular thalamic nucleus, central amygdala, cerebellum, and in the medium spiny neurons of the caudatus and putamen (75). In these brain areas, we confirmed that the decrease of 5α-reductase resulted in a reduction of allopregnanolone levels (74; 76; 77). Social isolation failed to change the expression of 3α-HSD, the mRNA expression of diazepam binding inhibitor, and the expression of the 18 kDa translocase protein (TSPO), which is involved in the transport of cholesterol across the inner mitochondrial membrane and activation of neurosteroidogenesis (reviewed in 72). Thus, the downregulation of 5α-reductase appears to be the main factor responsible for the reduction of corticolimbic allopregnanolone levels.

5. GABAergic neurotransmission deficits resulting from allopregnanolone downregulation

Allopregnanolone biosynthesis downregulation as a result of social isolation stress or pharmacological decrease of allopregnanolone induced by inhibiting 5α-reductase with the potent competitive 5α-reductase inhibitor SKF 105,111 decreases GABAergic neurotransmission as demonstrated by reduced loss of righting reflexes induced by $GABA_A$ receptor active ligands. The effects of SKF on the muscimol-, pentobarbital-, benzodiazepine-, or alcohol-induced loss of righting reflex loss can be reversed by the systemic or intracerebroventricular administration of allopregnanolone (43; 48). Likewise, social isolation or SKF-induced decrease of allopregnanolone results in facilitation of the seizure activity induced by several drugs that decrease $GABA_A$ receptor function, including picrotoxin (63). Administration of allopregnanolone at doses that have virtually no effects on group-housed control mice normalized the increased susceptibility to picrotoxin-induced seizures in SKF-treated or social isolated mice (63). The protracted social isolation or SKF treatment-induced allopregnanolone biosynthesis downregulation appeared to be the primary reason for the $GABA_A$ receptor signal transduction deficits observed in these mice. In fact, seizures induced by kainic acid or strychnine in socially isolated mice are similar to those induced by these agents in group housed mice.

6. Behavioral effects induced by allopregnanolone downregulation in corticolimbic areas

The decrease of allopregnanolone biosynthesis in socially isolated mice has been associated with several behavioral deficits that resemble behavioral abnormalities observed in patients

with PTSD (16; 17; 30; 38). Hence, this mouse model can be used to study the behavioral responses elicited by treatment with neurosteroidogenic agents, the SBSSs. This new class of drugs includes the SSRI antidepressants that have been shown to elicit a potent neurosteroidogenic activity selectively at low doses as their principal action.

Allopregnanolone has emerged as an important biomarker of emotional behavioral deficits (16; 35-38; 72). This was demonstrated by experiments using socially isolated mice to induce a downregulation of allopregnanolone biosynthesis. We have established a fundamental role for allopregnanolone in the regulation of anxiety-like and aggressive behavior as well as contextual fear conditioning, (16; 37; 63; 74; 77). When mice are socially isolated for a period varying from one to eight weeks, there is a time-dependent increase in aggressive behavior over the first four weeks of isolation, which is inversely correlated with a time-dependent decrease of corticolimbic allopregnanolone levels (35). Likewise, socially isolated mice exposed to a classical fear conditioning paradigm showed enhanced conditioned contextual but not cued fear responses compared with group housed mice (74). The time-related increase of contextual fear responses correlated with the downregulation of 5α-reductase mRNA and protein expression observed in the frontal cortex, hippocampus, and amygdala (74). Socially isolated mice also exhibited impaired and incomplete fear extinction (74). Of note, socially isolated mice also exhibit higher levels of anxiety-like behavior, determined by the elevated plus maze and in the open field (16; 39).

Allopregnanolone plays a *pivotal* rather than incidental role in the regulation of contextual fear responses and aggression. In fact, pharmacological treatment with allopregnanolone dose-dependently decreased aggression in a manner that correlated with an increase in corticolimbic allopregnanolone content (35). Allopregnanolone also normalized the exaggerated contextual fear responses and anxiety of socially isolated mice (74). Further, administration of the potent 5α-reductase competitive inhibitor SKF 105,111 to normal group-housed mice (43; 48; 47) rapidly (~1 h) decreased levels of allopregnanolone in the olfactory bulb, frontal cortex, hippocampus, and amygdala by 80-90% (73; 74) in association with a dose-dependent increase of conditioned contextual fear responses (74). Administering allopregnanolone doses that normalized hippocampus allopregnanolone levels reversed the effects of SKF 105,111 on conditioned contextual fear responses (74). These results are in agreement with results of many other investigators who have observed that allopregnanolone elicits anxiolytic and antidepressant effects (39; 54; 78-84).

7. Social isolation induces changes in $GABA_A$ receptor subunit expression

Postmortem studies suggest that altered corticolimbic GABAergic neurotransmission, GABA receptor binding and receptor subunit composition, as well as GABA synthesis and transport may be associated with various psychiatric disorders, including anxiety disorders, schizophrenia, and depression (85-88).

The regional distribution of $GABA_A$ receptor subunit subtypes plays an important role in the pharmacology of $GABA_A$ receptor ligands that bind to selective and specific $GABA_A$ receptor subunits (89-90). Recent studies showed that α1-containing $GABA_A$ receptors mediate the sedative properties of specific GABAergic ligands, such as diazepam, in the same way α2 and probably α3 subunits mediate the anxiolytic effects of benzodiazepines, and α5 subunits appear to be involved in learning and cognition (89; 90). High affinity binding of benzodiazepine to $GABA_A$ receptors requires the interaction of α and γ subunits (89; 90).

In socially isolated mice, we found changes in the mRNA and protein expression of several $GABA_A$ receptor subunits in the frontal cortex and hippocampus (91). The mRNA levels encoding α1, α2, and γ2 $GABA_A$ receptor subunit subtypes were reduced (~50%), while the mRNAs encoding α4 and α5 subunits were increased (~130%) compared to levels measured in group-housed mice (91). Protein levels of α1 and α5 determined in synaptic membrane preparations in the frontal cortex and hippocampus confirmed the former results. Using a laser microdissection technique coupled with nested RT-PCR amplification, we found that α1 mRNA levels were decreased by 50% in layer I neuropil, whereas the expression of α1 subunit mRNA in the pyramidal neurons of layer V was unchanged as a result of social isolation. Thus, changes in $GABA_A$ receptor subunits within one brain area are region-specific (91).

Changes in $GABA_A$ receptor subunit subtype composition are expected to result in altered pharmacological responses to various $GABA_A$ receptor ligands in socially isolated mice. As expected, socially isolated mice showed resistance to the sedative and anxiolytic properties of diazepam and zolpidem, positive allosteric $GABA_A$ receptor modulators that bind with high affinity to α1, α2, α3 or α5 subunit-containing $GABA_A$ receptors (diazepam) and to α1 subunit-containing $GABA_A$ receptors (zolpidem) (91). The α1 subunit of the $GABA_A$ receptor plays a primary role in mediating the sedative pharmacological effects of diazepam and zolpidem (92). Hence, their altered pharmacological response could result by a decrease in α1 subunit-containing $GABA_A$ receptors. Likewise, a decreased γ2 subunits support the formation of $GABA_A$ receptors in which this subunit might be substituted. Given that γ2 subunits are a necessary prerequisite for the formation of benzodiazepine-sensitive $GABA_A$ receptors (89; 90), the lack of anxiolytic activity of diazepam may result from the formation of benzodiazepine-insensitive $GABA_A$ receptors in neuronal circuits that regulate anxiety (39; 91).

Increases of α4 subunit-containing $GABA_A$ receptor expression in the frontal cortex appeared to be irrelevant to the behavioral or pharmacological alterations observed in socially isolated mice. GABA agonists such as THIP or the allosteric modulator, allopregnanolone, show selectivity and increased potency, respectively, for $GABA_A$ receptors containing α4/δ-subunits. These compounds comparably decrease locomotor activity in group-housed and socially isolated mice (91). In contrast to diazepam, allopregnanolone dose-dependently induces potent anxiolytic actions in socially isolated mice (16; 39).

Interestingly, the expression of $GABA_A$ receptor subunits is susceptible to changes in brain neurosteroid levels. In particular, expression of α4–containing subunits increases during progesterone withdrawal or following blockade of 5α–reductase (93). Likewise, in socially isolated mice, allopregnanolone levels decrease in several corticolimbic structures that concomitantly show changes in $GABA_A$ receptor subunit mRNA and protein expression. It would be important to determine whether social isolation directly affects the expression of $GABA_A$ receptor subunit composition or whether such changes are mediated by decreasing the levels of 5α–DHP and its binding at nuclear progesterone receptors or by allopregnanolone biosynthesis downregulation.

8. Selective brain steroidogenic stimulants (SBSSs) improve behavioral deficits in socially isolated mice

Behavioral deficits induced by social isolation in rodents include aggressive behavior (94-96). Aggression is correlated with the downregulation of corticolimbic allopregnanolone

biosynthesis (35). Upregulation of allopregnanolone levels in socially isolated mice by systemic administration or local microinfusion of allopregnanolone induces a dose-dependent amelioration of aggressive behavior of a resident mouse to a same-sex intruder (35; 77). Thus, the decrease of corticolimbic allopregnanolone levels appears to be involved in the expression of aggression.

As indicated above, SSRI antidepressants potently increase the levels of allopregnanolone in rodents and depressed humans. The effects of paroxetine and fluoxetine on allopregnanolone levels were independent from pregnenolone or progesterone levels that failed to change (34; 76). Racemic fluoxetine (R- and S-isomers) normalized the righting reflex loss induced by pentobarbital in mice by increasing corticolimbic allopregnanolone levels (35-37). Of note, at the doses used, fluoxetine failed to change the behavior and allopregnanolone levels of group housed mice (35; 36). Importantly, inhibition of serotonin synthesis by treatment with p-chlorophenylalanine failed to block the behavioral effects of fluoxetine, suggesting that increasing corticolimbic allopregnanolone levels is part of the pharmacological actions of fluoxetine (76).

These observations led us to hypothesize that fluoxetine could improve the behavioral abnormalities of socially isolated mice by enhancing corticolimbic allopregnanolone biosynthesis rather than by inhibiting serotonin reuptake. This hypothesis was investigated using the R- and S-stereoisomers of fluoxetine and norfluoxetine as pharmacological tools. We expected that these drugs would stereospecifically upregulate corticolimbic allopregnanolone content but have no stereoselectivity with regard to inhibition of 5-HT reuptake. We additionally thought that doses of fluoxetine and norfluoxetine stereoisomers that increase corticolimbic allopregnanolone content might differ from those that inhibit 5-HT reuptake. Indeed (16; 35-38), fluoxetine dose-dependently and stereospecifically normalized the duration of pentobarbital-induced sedation and reduced aggressiveness, fear responses, and anxiety-like behavior at the same submicromolar doses that normalized the downregulation of brain allopregnanolone content in socially isolated mice. Interestingly, the S-stereoisomers of fluoxetine or norfluoxetine appeared to be 3 to 7 fold more potent than their respective R-stereoisomers and S-norfluoxetine was about 5-fold more potent than S-fluoxetine. Importantly, the effective concentrations (EC_{50}s) of S-fluoxetine and S-norfluoxetine that normalize the brain allopregnanolone content are 10- (S-fluoxetine) and 50-fold (S-norfluoxetine) lower than their respective EC_{50s} needed to inhibit 5-HT reuptake (35-38). Remarkably, the SSRI activity of S or R-fluoxetine and of S or R-norfluoxetine was devoid of stereospecificity (35; 36). Hence, this study demonstrated that neither the behavioral action nor the normalization of corticolimbic allopregnanolone content by S-fluoxetine and S-norfluoxetine is related to their intrinsic SSRI activity.

9. A novel promising therapy for anxiety disorders and PTSD

In the pathophysiology of depression and PTSD, a GABAergic neurotransmission dysfunction could at least in part be involved in the symptomatology of these disorders. Decreased GABA levels and reductions in $GABA_A$ and $GABA_B$ receptor binding and/or sensitivity have been found in depressed patients (97; 98). In PTSD, decreased frontal lobe benzodiazepine receptor binding (99; 100) and decreased plasma GABA levels (101) have been demonstrated. These changes were most consistently and profoundly observed among treatment resistant patients. Benzodiazepines have not been found to effectively treat PTSD (10-12) and SSRIs sertraline and paroxetine are the only medications currently approved by

the Federal Drug Administration (FDA) for the treatment of PTSD. However, their effect sizes are modest (102-105), or even ineffective (106). In patients who cannot adequately synthesize allopregnanolone and in whom administration of an SSRI (or SBSS) is ineffective, the administration of an allopregnanolone analog (e.g. 107, 108), such as ganaxolone may offer a therapeutic alternative. A multisite Phase II trial of the efficacy and safety of ganaxolone in PTSD is currently been tested. Other medications that increase plasma allopregnanolone levels by a different mechanisms than the SSRIs also may be effective in PTSD (109-111).

The findings that the socially isolated mouse expresses decreased levels of allopregnanolone, as well as changes in the expression of several $GABA_A$ receptor subunits in corticolimbic structures that regulate cognition, anxiety, PTSD, and depression suggests that the *socially isolated mouse model* may be useful in investigating new molecules designed to improve behavioral deficits characterized by $GABA_A$ receptor signal transduction dysfunction (reviewed in 16; 38; 73).

Hence, as in PTSD patients, the socially isolated mouse fails to respond to sedative and anxiolytic benzodiazepines. Our studies demonstrate that allopregnanolone or S-norfluoxetine -at nonserotonergic doses- infused into the basolateral amygdala potently increase allopregnanolone biosynthesis in target corticolimbic areas including the hippocampus, basolateral amygdala, and frontal cortex (77) and exert a strong anti-anxiety, anti-fear, and anti-aggression effect (35-38; 72; 77).

Neurosteroids lack $GABA_A$ receptor subunit selectivity and the functional $GABA_A$ receptor binding characteristics of benzodiazepines. Thus, this suggests that allopregnanolone, its analogs, or molecules that stimulate allopregnanolone biosynthesis might be advantageous over benzodiazepines in a scenario of neurosteroid downregulation and changes in $GABA_A$ receptor subunit subtypes. Despite benzodiazepines, allopregnanolone activates $GABA_A$ receptors incorporating α4, α6, and δ subunits in combination with γ and β subunits (64-66). Thus, allopregnanolone or SBSSs improve anxiety, fear, and aggressiveness when benzodiazepines fail. Of note and in contrast to benzodiazepines, both allopregnanolone and SBSS molecules decrease anxiety, fear, and aggression at concentrations that fail to be sedative (16; 35; 39; 77).

New SBSS molecules that fail to exert any significant SSRI activity but increase corticolimbic allopregnanolone levels and thereby improve behavioral symptoms in mouse models of anxiety and depression. The high potency and stereospecificity of these drugs in reducing behavioral deficits and in normalizing brain allopregnanolone content suggest that they may affect specific targets for regulating neurosteroidogenesis. The finding that protracted social isolation affects the expression of 5α-reductase in corticolimbic structures, but fails to change the expression of 3α-HSD, as well as the finding that brain progesterone levels don't change in socially isolated mice suggest that a mechanism involving 5α-reductase is responsible for the decrease of corticolimbic allopregnanolone content. This is further supported by the fact that 5α-reductase is the rate-limiting step-enzyme in allopregnanolone biosynthesis from progesterone (73). Hence, these data suggest that fluoxetine and norfluoxetine mediate upregulation of corticolimbic allopregnanolone levels by a direct action on 5α-reductase. However, *in vitro* studies by Griffin and Mellon (112) showed that fluoxetine, paroxetine, and sertraline failed to activate 5α-reductase and instead, directly activated 3α-HSD by decreasing its K_m for 5α-DHP, thereby facilitating an accumulation of allopregnanolone (112). The hypothesis that neurosteroidogenic antidepressants activate 3α-

HSD is also suggested by the finding that fluoxetine accelerates the rate of allopregnanolone accumulation during incubation of brain slices with 5α-DHP (34). Furthermore, progesterone levels in group-housed and socially isolated mice are not affected by fluoxetine administration, suggesting that the SSRI/SBSSs impact neurosteroidogenesis downstream from progesterone (34; 76). On the other hand, experiments by Trauger and collaborators (113) were inconsistent with the hypothesis that fluoxetine and paroxetine directly activate 3α-HSD. The finding that low doses of the S isomers of fluoxetine or norfluoxetine increase corticolimbic levels of allopregnanolone in socially isolated mice, but fail to change levels in group-housed mice, suggests that 5α-reductase and/or 3α-HSD may become more susceptible to the effects of SBSSs during isolation (reviewed in 38). Investigations at the molecular enzymatic level will clarify whether social isolation and neurosteroidogenic agents change the kinetics of 5α-reductase and/or 3α-HSD.

Other feasible pharmacological targets to enhance neurosteroidogenesis include the translocase protein (18 kDa) or TSPO, previously called mitochondrial peripheral benzodiazepine receptor or PBR (114). TSPO represents the starting point and an important rate-limiting step in neurosteroidogenesis. It gives access to neurosteroids in the brain by regulating the entry of cholesterol into the inner mitochondrial membranes and its conversion to pregnenolone by P450scc, which is located in the inner mitochondrial membrane (29; 114). A cascade of enzymatic processes then take place in the cytosol, resulting in the production of neuroactive steroids, including pregnenolone sulfate, DHEAS (though apparently not in human brain (115)], THDOC, and allopregnanolone (reviewed in 31).

New molecules that bind with high affinity to TSPO have been recently investigated. These drugs are able to exert important anxiolytic effects but are devoid of the unwanted side effects associated with benzodiazepines, including over-sedation and tolerance (28; 29). In mouse models, TSPO agents have been shown to potently increase pregnenolone levels in the hippocampus and cortex, as well as to induce anxiolytic effects (116-119). TSPO ligands include XBD173 and etifoxine, which have proven to be highly efficacious anxiolytic and antidepressant drugs in a number of behavioral tests (29; 30). The anxiolytic and antidepressant effects of these agents were related their ability to increase neurosteroid biosynthesis, as confirmed by studies in which key enzyme blockers for neurosteroid biosynthesis, including finansteride and trilostane (56; 30), were used. TSPO ligands have recently showed promising therapeutic effects in clinical studies (29; 30).

10. Closing remarks

The new class of drugs, the SBSSs (selective brain steroidogenic stimulants) -whose mechanism of action involves the stimulation of neurosteroidogenesis with the goal of increasing brain allopregnanolone levels- has emerged as a new therapeutic strategy for the treatment of psychiatric disorders associated with a downregulation of brain allopregnanolone biosynthesis. These disorders include anxiety, depression, and PTSD.

In comparison to benzodiazepines, the SBSSs are more efficacious as well as devoid of the unwanted side-effects induced by benzodiazepines. Allopregnanolone pharmacology involves the allosteric modulation of GABA action at $GABA_A$ receptors, which is broader than that of benzodiazepines, which fail to modulate $GABA_A$ receptors containing α4 and α6 subunits. Hence, selective stimulation of allopregnanolone biosynthesis may avoid the therapeutic hindrances caused by the formation of benzodiazepine-resistant $GABA_A$

receptors with altered subunit composition, such as may occur in stress-related psychiatric disorders (reviewed in 120). Thus, novel SBSS drugs that specifically increase corticolimbic allopregnanolone biosynthesis appear to be a novel promising pharmacological class of future drugs for the treatment of anxiety disorders, depression, and PTSD.

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12. References

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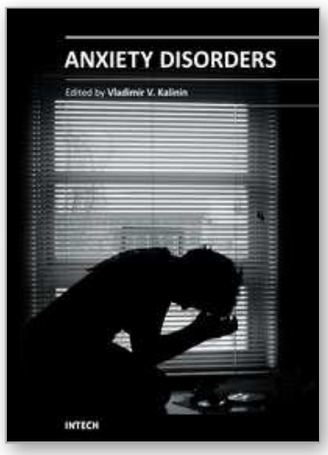

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During the last 2-3 decades drastic research progress in anxiety issues has been achieved. It concerns mostly the study of different subtypes of anxiety and their treatment. Nevertheless, the data on anxiety pathogenesis is less elaborated, although here a multidimensional approach exists. It includes neurochemistry, pathophysiology, endocrinology and psychopharmacology. Again, we are able to recognize the multifarious sense of anxiety, and the present collective monograph composed of 16 separate chapters depicting the different aspects of anxiety. Moreover, a great part of book includes chapters on neurochemistry, physiology and pharmacology of anxiety. The novel data on psychopathology and clinical signs of anxiety and its relationship with other psychopathological phenomena is also presented. The current monograph may represent an interest and be of practical use not only for clinicians but for a broad range of specialists, including biochemists, physiologists, pharmacologists and specialists in veterinary.

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