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Antidepressant-like effect of the neurosteroid 3α-hydroxy-5α-pregnan-20-one in mice forced swim test

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Abstract

The present study aimed to examine the antidepressant-like effect of the neurosteroid 3α-hydroxy-5α-pregnan-20-one (3α, 5α THP) using the forced swim test in mice. Intracerebroventricular (ICV, 1 or 2 μg/mouse) or intraperitoneal (IP, 0.5, 1, or 2 mg/kg) administration of 3α, 5α THP dose-dependently reduced the duration of immobility in forced swim test without accompanying changes in ambulatory or rearing behaviors in the open-field test. The antidepressant-like effect of 3α, 5α THP (1 μg/mouse, ICV) was potentiated by prior administration of the $GABA_A$ receptor agonist, muscimol (0.5 mg/kg, IP) and blocked by prior administration of $GABA_A$ receptor antagonist, bicuculline (1 mg/kg, IP). Administration of the agonist at diazepam binding inhibitor receptors, 4′-chlorodiazepam (4′CD, 15 mg/kg, IP) or *N*,*N*-di-*n*-hexyl-2-(4-fluorophenyl)-indol-3-acetamide (FGIN 1–27, 1 or 2 μg/mouse, ICV), the 11β-hydroxylase inhibitor, metyrapone (150 mg/kg, IP and 1 or 2 μg/mouse, ICV) and the selective serotonin reuptake inhibitor (SSRI), fluoxetine (20 mg/kg, IP), which are known to increase the endogenous level of neurosteroids, also reduced the duration of immobility in forced swim test. The tricyclic antidepressant, imipramine (20 mg/kg, IP), which does not increase the 3α, 5α THP in the brain, also reduced the immobility time. While the antidepressant-like effect of fluoxetine, which is known to selectively increase the brain content of 3α, 5α THP, was either blocked partially by bicuculline (1 mg/kg, IP) or potentiated by muscimol (0.5 mg/kg, IP), the antidepressant-like effect of imipramine was not modified by bicuculline. These results demonstrate the antidepressant-like effect of the neurosteroid 3α, 5α THP, and suggest further evaluation for its development as a new class of antidepressant drug.

Keywords: Neurosteroid; 3α, 5α THP; Allopregnanolone; Metyrapone; 4′-Chlorodiazepam; FGIN 1–27; Fluoxetine; $GABA_A$-receptor; Antidepressant

1. Introduction

Neurosteroids are synthesized in the CNS either de novo from cholesterol or by in situ metabolism of blood-borne hormone precursors [2]. They are known to rapidly alter the excitability of CNS by bi-directional allosteric modulation of the $GABA_A$ receptor Cl^- ionophore complex (GRC) [21]. The reduced A-ring metabolite of progesterone 3α-hydroxy-5α-pregnan-20-one (3α, 5α THP) is a neurosteroid, which is one of the most potent endogenous modulator of GRC in the brain [39]. The mechanism by which 3α, 5α THP and related steroids modulate GRC is believed to be distinctly different from that of barbiturates and benzodiazepines [29]. In low (nanomolar) concentration, 3α, 5α THP potentiates GABA-activated Cl^- currents, whereas at higher concentrations (micromolar) it can directly open the Cl^- channel associated with $GABA_A$ receptor [29]. In certain physiological conditions like stress, pregnancy, and stages of the estrous cycle, endogenous levels of 3α, 5α THP can reach a concentration sufficient to modulate GRC [2,29]. Although the rapid effect of 3α, 5α THP on GRC continues to be the most thoroughly investigated mechanism, it also acts on other membrane targets including ligand-gated ion channels [29], voltage-gated Ca^{2+} channels [7], 5-HT_3 receptors [49], and nicotinic acetylcholine receptors [5].

Exogenous administration of 3α, 5α THP has been shown to produce a large number of behavioral effects such as antistress [26,53], anxiolytic [50], anticonvulsant [8], hyperphagic [6], and cataleptic [18,22]. Recent evidences suggest that 3α, 5α THP may play a vital role in depression. Decreased cerebrospinal fluid (CSF) levels of 3α, 5α THP have been shown in patients with major unipolar depression [42,45,48], and treatment with selective serotonin reuptake inhibitors (SSRIs) like fluoxetine or fluvoxamine not only restores the 3α, 5α THP levels but also produces beneficial

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effects [13,48]. Large fluctuations in the plasma levels of progesterone and its metabolites including 3α, 5α THP have been reported in physiological conditions like the menopause [1,30] or premenstrual syndrome [3,4,36,46] associated with various mood disorders including anxiety, dysphoria, tension, and depression [36]. It has been speculated that the local synthesis of 3α, 5α THP may protect the brain from drops in circulating steroid levels as they occur, for example, during the menstrual cycle or depressive states [12]. Exogenous administration of dehydroepiandrosterone (DHEA) [34], dehydroepiandrosterone sulfate (DHEAS), and pregnenolone sulfate (PS) [38] has been reported to produce antidespair effects in animals and also alleviates depression in patients [51 52]. However, to our knowledge the antidepressant-like effect of 3α, 5α THP has not been studied so far. In the present study we investigated the antidepressant-like effect of 3α, 5α THP in forced swim test in mice, an animal model of depression [33]. In addition, the effect of drugs like fluoxetine [47,48], metyrapone [37,43], 4′-chlorodiazepam (4′CD), and *N*,*N*-di-*n*-hexyl-2-(4-fluorophenyl)-indol-3-acetamide (FGIN 1–27) [41], which have been shown to increase the endogenous neurosteroids, was also examined in this test. To exclude the possible involvement of motor effect of 3α, 5α THP if any on the immobility time, it was screened in an open-field test.

2. Materials and methods

2.1. Subjects

Male Swiss mice weighing 20–25 g, housed five per cage in a temperature (25 ± 2°C) and light (12 L:12 D cycle; lights on at 0700 h)-controlled room, having free access to standard pelleted diet and water, were used. Animals were handled and acclimatized to laboratory conditions at least 12 h prior to experiments. All experiments were conducted between 0900 and 1500 h. The experiments described here comply with ethical principles and guidelines as provided by the committee for the purpose of control and supervision of experimental animals, Ministry of Environment and Forests, Government of India, New Delhi.

2.2. Drugs

3α, 5α THP, 4′CD, FGIN 1–27, muscimol, and bicuculline were purchased from Research Biochemicals International, Natick, MA. Fluoxetine, imipramine (Sun Pharmaceuticals, Mumbai, India) and metyrapone (Novartis, NJ) were provided as gift samples. All drugs except 3α, 5α THP, 4′CD, or FGIN 1–27 were dissolved in 0.9% saline. 3α, 5α THP, 4′CD or FGIN 1–27 were dissolved in 2-hydroxypropyl-β-cyclodextrin (45% w/v) solution. For intracerebroventricular (ICV) administration, 3α, 5α THP solution was diluted with the simulated CSF of the following composition: 0.2 M NaCl, 0.02 M NaH_2CO_3, 2 mM KCl, 0.5 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.8 mM $MgCl_2$, 0.5 mM Na_2SO_4, and 5.8 mM D-glucose. The direct injection method of Haley and McCormick [14] was followed for ICV administration of simulated CSF (10 μl) or 3α, 5α THP in conscious (unanesthetized) mice. Briefly, mouse was grasped firmly by the loose skin behind the head, the skin was pulled taut, and a 27½-gauge, 2.8-mm long hypodermic needle attached to a microliter syringe was inserted perpendicular through the skull into the brain, and 10 μl of solution was injected. The site of injection was 2 mm right of the midline of the mouse skull, on a line drawn through the anterior base of the ears. FGIN 1–27 and metyrapone solutions were also diluted with simulated CSF and injected by an ICV route. All other drugs were injected by an intraperitoneal (IP) or subcutaneous (SC) route.

At the end of all ICV experiments, dilute India ink was injected at the site of the neurosteroid injection and animals were killed immediately. The data of only those animals showing distribution of ink into the ventricles was utilized for statistical analysis.

2.2.1. Forced swim test

This test was a modification of the method of Porsolt et al. [33]. Unlike Porsolt's method for mice, which consists of direct immersion of animals after injecting drugs, we subjected the animals to a "pretest session" to avoid variations and for maintaining consistency in the immobility time between different groups. Briefly, mice were forced to swim individually for 15 min, in a glass cylinder (21 × 12 cm) containing fresh water up to a height of 9 cm at a temperature of 25 ± 2°C. This constituted the "pretest session". Twenty-four hours later, the animals were treated with either a drug (test group) or vehicle (control group), and each animal was again forced to swim in a similar environment for a period of 6 min in a "test session," and immobility time was recorded. Mice were judged to be immobile if they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Reduction in the duration of immobility by a drug was considered as its having antidespaired or antidepressant-like effects. Each experimental group consisted of seven mice, and was chosen by means of a completely randomized schedule.

2.3. Effect of exogenous administration of 3α, 5α THP

Experiments were initially done to assess whether the neurosteroid, 3α, 5α THP modifies the immobility time in animals that were previously exposed to a "pretest session." Groups of mice consisting of seven subjects each received by an ICV route 0.5, 1, or 2 μg doses of 3α, 5α THP in a constant volume of 10 μl, 15 min prior to the "test session." Control groups received an equivalent volume of simulated CSF (ICV), and the duration of immobility was measured during a 6-min forced swim test.

Table 1
Effect of ICV or IP administration of 3α, 5α THP on duration of immobility in forced swim test

Treatments	Dose (μg/mouse, ICV or mg/kg, IP)	Immobility time (s) (mean ± SEM)
Vehicle (ICV)	–	265.85 ± 7.44
3α, 5α THP (ICV)	0.5 μg	264.57 ± 4.67
	1 μg	164.71 ± 10.77*
	2 μg	146.83 ± 11.86*
Vehicle (IP)	–	231.17 ± 5.97
3α, 5α THP (IP)	0.5	179.00 ± 8.54**
	1	147.71 ± 4.84**
	2	130.33 ± 7.26**

Mice were injected (ICV or IP) with vehicle or 3α, 5α THP (ICV or IP). At 15 min after ICV injection or 30 min after IP injection duration of immobility in "test session" was measured. Data represent mean ± SEM immobility time in seconds of five to seven animals per group.

* $p < 0.001$, compared with vehicle (ICV/mouse)-treated group.

** $p < 0.001$, compared with vehicle (IP)-treated group.

Furthermore, the effect of IP administration of 3α, 5α THP (0.5, 1, or 2 mg/kg) was studied on immobility time. In these experiments separate groups of animals ($n = 7$) were injected with neurosteroid or vehicle 30 min prior to the "test session."

2.4. Effect of increased neurosteroid content

To evaluate the effect of accumulation of endogenous neurosteroid on the duration of immobility in the forced swim test, different classes of drugs were administered. Different groups of animals were injected intraperitoneally with the SSRI, fluoxetine (1, 5, or 20 mg/kg), 11-β-hydroxylase inhibitor metyrapone (50 or 150 mg/kg), or the agonist at the diazepam binding inhibitor receptors, 4′CD (1 or 15 mg/kg) 30 min prior to the "test session," and immobility time was recorded as described above. Similarly, the effect of vehicle treatments was also studied in separate animals. Separate groups of animals were injected with vehicle, metyrapone (1 or 2 μg/mouse, ICV), or FGIN 1–27 (1 or 2 μg/mouse, ICV), and were subjected to a "test session" 15 min after their administration.

2.5. Modulation by GABAergic drugs

These experiments were designed to assess whether the antidepressant-like effect of exogenously administered or endogenously increased 3α, 5α THP is mediated by its action at GRC. Groups ($n = 7$) of mice were administered the $GABA_A$ receptor agonist, muscimol (0.5 mg/kg, IP), the $GABA_A$ receptor antagonist, bicuculline (1 mg/kg, IP), or vehicle (0.9% saline), 30 min before the 3α, 5α THP (1 μg/mouse, ICV) or fluoxetine (5 mg/kg, IP), and the immobility time was recorded as described before in the "test session."

Separate groups of animals were injected with saline or bicuculline (1 mg/kg, IP) prior to imipramine (20 mg/kg, IP) to investigate whether the antidepressant action of a drug that does not increase the 3α, 5α THP level is mediated through $GABA_A$ receptors.

2.5.1. Open-field test

This test was performed to assess whether or not changes in immobility time were associated with changes in motor activity in an open-field test. The open-field apparatus consisted of a circular base (84 cm diameter; 30 cm high wall) having three concentric circles of 14, 28, and 42 cm radius, divided into 36 segments by radiating lines drawn from the center. Illumination was identical to that used for the forced swim test. The neurosteroid as well as the vehicle-treated mice were placed individually into the center of the arena, and the ambulations (number of partitions crossed with all four paws) and rearings (number of times mouse stood on its hind limbs) within a 5-min period were recorded. Separate groups of mice ($n = 7$) were treated with vehicle or 3α, 5α THP (1 and 2 μg/mouse, ICV or 0.5, 1, and 2 mg/kg, IP) and placed gently at the center of the apparatus 15 or 30 min, respectively, after ICV or IP injections.

2.6. Data analysis

The data is expressed as mean ± SEM and analyzed using one-way analysis of variance (ANOVA). Significant interactions were assessed by post hoc Dunnett's test. A value of

Table 2
Effect of metyrapone, 4′CD, FGIN 1–27 or fluoxetine on duration of immobility in forced swim test

Treatments	Dose (μg/mouse, ICV or mg/kg, IP)	Immobility time (s) (mean ± SEM)
Vehicle	–	256.71 ± 3.42
Metyrapone	50	246.86 ± 5.04
	150	202.43 ± 4.94*
Vehicle	–	245.57 ± 9.55
Metyrapone (ICV)	1	129.00 ± 8.11*
	2	110.43 ± 8.02*
Vehicle	–	262.29 ± 6.24
4′CD	1	243.43 ± 5.19
	15	91.71 ± 22.66*
Vehicle	–	258.43 ± 5.45
FGIN 1–27 (ICV)	1	149.14 ± 12.75*
	2	162.43 ± 6.87*
Vehicle	–	253.57 ± 9.38
Fluoxetine	1	254.86 ± 14.24
	5	253.57 ± 9.34
	20	97.14 ± 10.33*

Mice were injected with vehicle (IP or ICV), metyrapone (IP or ICV), 4′CD (IP), FGIN 1–27 (ICV), or fluoxetine (IP) and 15 min or 30 min, respectively, after ICV or IP administration the duration of immobility was measured in "test session." Data represent mean ± SEM immobility time in seconds of seven animals per group.

* $p < 0.001$, compared to respective vehicle-treated group.

Table 3
Effect of muscimol or bicuculline on the antidepressant-like effect of 3α, 5α THP and fluoxetine

Groups	Treatments	Dose (μg/mouse, ICV or mg/kg, IP)	Immobility time (s) (mean ± SEM)
A	Vehicle	–	235.83 ± 11.69
B	Muscimol	0.5	241.00 ± 9.49
C	Bicuculline	1	234.67 ± 9.67
D	Vehicle + 3α, 5α THP (ICV)	1 μg	164.71 ± 10.77*
E	Vehicle + fluoxetine	5	243.50 ± 7.67
F	Vehicle + fluoxetine	20	96.67 ± 12.38*
G	Vehicle + imipramine	20	107.83 ± 24.71*
H	Muscimol + 3α, 5α THP (ICV)	0.5 + 1	85.67 ± 11.07*
I	Muscimol + fluoxetine	0.5 + 5	88.33 ± 6.31*
J	Bicuculline + 3α, 5α THP (ICV)	1 + 1 μg	237.80 ± 2.82*
K	Bicuculline + fluoxetine	1 + 20	183.17 ± 25.45*,**
L	Bicuculline + imipramine	1 + 20	112.83 ± 16.15

Muscimol (IP), bicuculline (IP), or vehicle were injected 30 min prior to 3α, 5α THP (ICV), fluoxetine (IP), or imipramine (IP). At 15 min after 3α, 5α THP injection or 30 min after fluoxetine, imipramine, muscimol, or bicuculline administration the duration of immobility was measured in "test session." Data represent mean ± SEM immobility time in seconds of five to seven animals per group. Groups D vs. H, D vs. J, E vs. I, F vs. K, A vs. D, A vs. F, A vs. G and A vs. K.

* $p < 0.001$.

** $p < 0.05$.

$p < 0.05$ was considered to be statistically significant in all the cases.

3. Results

3.1. Forced swim test

3.1.1. Effect of 3α, 5α THP on the immobility time

One-way ANOVA revealed a significant effect of central (ICV), $F(3,25) = 57.83$, $p < 0.001$, or systemic (IP), $F(3,25) = 33.17$, $p < 0.001$, administration of 3α, 5α THP on immobility time in the forced swim test. Post hoc analysis revealed that ICV (1 or 2 μg/mouse) or IP (0.5, 1, or 2 mg/kg) administration of 3α, 5α THP significantly ($p < 0.001$) reduced immobility time compared to the respective vehicle-treated groups. The ICV dose 0.5 μg/mouse of 3α, 5α THP was, however, not significantly different from the vehicle-treated group. These results are shown in Table 1.

3.1.2. Effect of increased neurosteroid content

ANOVA showed significant main difference of 4′CD, $F(2,20) = 52.85$, $p < 0.001$, fluoxetine, $F(3,27) = 59.12$, $p < 0.001$, metyrapone (IP), $F(2,20) = 40.80$, $p < 0.001$, metyrapone (ICV), $F(2,20) = 84.82$, $p < 0.001$, FGIN 1–27 (ICV) $F(2,20) = 51.96$, $p < 0.001$, treatment on the immobility time in the forced swim test. A post hoc test revealed that fluoxetine (20 mg/kg, IP), 4′CD (15 mg/kg, IP), metyrapone (150 mg/kg, IP and 1 or 2 μg/mouse, ICV), or FGIN 1–27 (1 or 2 μg/mouse, ICV) significantly ($p < 0.001$) reduced immobility time compared to the respective vehicle-treated animals. The lower doses of fluoxetine (1 or 5 mg/kg, IP), metyrapone (50 mg/kg, IP), and 4′CD (1 mg/kg, IP) were not significantly different from the vehicle-treated animals. These results are shown in Table 2.

3.1.3. Modulation by GABAergic drugs

ANOVA showed a significant main effect of bicuculline treatment on the antidepressant-like effect of 3α, 5α THP, fluoxetine or imipramine in the forced swim test, $F(5,45) = 18.88$, $p < 0.001$. A post hoc test revealed that bicuculline (1 mg/kg, IP) significantly ($p < 0.001$) blocked the antidepressant-like effect of 3α, 5α THP (1 μg/mouse, ICV) but partially ($p < 0.05$) that of fluoxetine (20 mg/kg, IP). However, bicuculline failed to modulate the antidepressant-like effect of imipramine (20 mg/kg, IP) in the forced swim test. ANOVA showed a significant main effect of muscimol treatment on the antidepressant-like effect of 3α, 5α THP or fluoxetine in the forced swim test, $F(5,35) = 92.87$, $p < 0.001$. A post hoc test revealed that muscimol (0.5 mg/kg, IP) significantly ($p < 0.001$) potentiated the antidepressant-like effect of 3α, 5α THP (1 μg/mouse, ICV) or fluoxetine (5 mg/kg, IP). The doses of muscimol or bicuculline used here per se did not modify the duration of immobility in the forced swim test (Table 3).

Table 4
Effect of 3α, 5α THP on the ambulatory and rearing behaviors in open-field test

Treatments	Dose (μg/mouse, ICV or mg/kg, IP)	Ambulations ± SEM	Rearing ± SEM
Vehicle (ICV)	–	82.71 ± 20.42	6.71 ± 0.61
3α, 5α THP (ICV)	1	95.17 ± 16.57	6.50 ± 0.79
	2	98.00 ± 7.46	6.86 ± 0.80
Vehicle (IP)	–	87.00 ± 14.15	6.71 ± 0.90
3α, 5α THP (IP)	0.5	91.33 ± 8.56	7.57 ± 1.12
	1	89.00 ± 8.69	7.14 ± 1.52
	2	86.17 ± 13.02	7.29 ± 1.07

At 15 min after ICV or 30 min after IP injection of neurosteroid the ambulatory and rearing behavior was recorded in open-field test. Data represent mean ± SEM ambulations or rearings of five to seven animals per group.

3.2. Open-field test

Central or systemic administration of 3α, 5α THP (1 and 2 μg/mouse, ICV or 0.5, 1, and 2 mg/kg, IP) produced no significant difference in ambulatory, $F(2,19) = 0.33$, or $F(2,18) = 0.04$, and rearing, $F(2,19) = 0.07$, or $F(2,20) = 0.15$, behavior compared with vehicle-treated controls (Table 4).

4. Discussion

Numerous observations exist to indicate the significance of 3α, 5α THP in different neuropsychiatric disorders including stress [26,53], anxiety [50], epilepsy [8], cognitive dysfunction [25], and catalepsy [18,22]. Recently, it has been suggested that 3α, 5α THP may play a vital role in depression. A reduced concentration of 3α, 5α THP and 3α, 5β THP (5β-pregnan-3α-ol-20-one) with a concomitant increase in 3β, 5α THP (5α-pregnan-3β-ol-20-one) levels has been reported in patients suffering from major unipolar depression [42,45,48]. Moreover, treatment with SSRI like fluoxetine has been shown not only to relieve the depression but also to restore the neurosteroid disequilibrium observed in such depressed patients [42,48]. In the present study, we have demonstrated for the first time that the neurosteroid 3α, 5α THP, a positive allosteric modulator of GRC, displays antidepressant-like effects as is evident from the reduction in the immobility time when administered by either systemic or central routes in mice. The antidepressant effect of 3α, 5α THP was also confirmed by us in a learned-helplessness model [23] of depression (unpublished observation). This effect of 3α, 5α THP was not associated with any motor effects, as it did not affect ambulatory or rearing behavior in the open-field test. To assess the effect of increased levels of neurosteroids including 3α, 5α THP in the brain, drugs belonging to different categories like the 11β-hydroxylase enzyme inhibitor, metyrapone [37,43], the agonist at the diazepam binding inhibitor receptors, 4′CD or FGIN 1–27 [41], and the SSRI, fluoxetine [47,48] were given. Metyrapone, 4′CD, FGIN 1–27, or fluoxetine treatment reduced the duration of immobility in the forced swim test. Metyrapone has been shown to exert beneficial effect in depressed patients [28,37,43]. Evidence was recently presented that metyrapone can cause pronounced increase in the level of neuroactive steroids like pregnenolone; progesterone; 11-deoxycortisol; 5α DHDOC (5α-pregnan-21-ol-3, 20-dione); 3α, 5α THDOC (5α-pregnane-3α, 21-diol-20-one); 5α DHP (5α-pregnan-3, 20-dione); 3α, 5α THP; 3α, 5β THP; 3β, 5α THP and DHEA, which could contribute to its antidepressant-like effect [37,43]. This effect of the 11β-hydroxylase inhibitor, metyrapone, has been explained due to overdrive of corticotropin as a consequence of inhibition of cortisol synthesis [43]. However, if metyrapone is administered by a systemic route, the antidepressant-like effect observed in the present study may be mediated through the inhibition of glucocorticoids or mineralocorticoids released from adrenals glands during stress. This issue was addressed by administration of metyrapone via an ICV route, and it was found that metyrapone also reduced the duration of immobility when administered centrally. This effect of metyrapone (ICV) along with other reports about the direct action of metyrapone in the brain [15,16], suggest the involvement of central neurosteroids in the antidepressant action of metyrapone. Similarly, acute administration of the agonist at diazepam binding inhibitor receptors, including 4′CD and FGIN 1–27, have also been shown to stimulate neurosteroidogenesis by increasing the levels of pregnenolone [41], which is the parent hormone in the biosynthesis of all the neurosteroids including progesterone and its 3α- and 5α-reduced metabolites. The effect of 4′CD or FGIN 1–27 to increase the level of brain neurosteroids has been shown to be independent of the peripheral source, because adrenalectomy or castration failed to alter the action of these drugs to increase the brain content of neurosteroids [19]. However, 4′CD has been reported to modify the gating of the GABA receptor in higher doses [35]. To rule out such a possibility, the effect of FGIN 1–27, a specific and potent agonist at the diazepam binding inhibiting receptor, was studied in the forced swim test. Unlike 4′CD, FGIN 1–27 has been reported not to affect the gating of the GABA receptor [40]. Central administration of FGIN 1–27 (1 or 2 μg/mouse, ICV) in the present study reduced the duration of immobility in mice. Administration of SSRIs like fluoxetine or fluvoxamine to unipolar depressed patients for 8–10 weeks has been reported to not only ameliorate depressive symptoms but also restore the reduced levels of 3α, 5α THP in the CSF [48]. Fluoxetine has been suggested to increase 3α, 5α THP levels by inhibiting the enzyme 3α-hydroxysteroid oxidoreductase, which is responsible for the metabolism of 3α, 5α THP to 5α DHP [47,48]. A recent report on the SSRIs suggest that these drugs increase 3α, 5α THP production through an increased efficiency of conversion of 5α DHP to 3α, 5α THP [11]. It has been shown that peripheral administration of SSRIs like fluoxetine (5, 10, or 20 mg/kg) or paroxetine (20 mg/kg) to adrenalectomized or castrated rats, increase the brain steady-state content of 3α, 5α THP selectively without altering other neurosteroids [47]. Fluoxetine has also been reported to normalize the brain content of 3α, 5α THP in socially isolated mice, independent of its action on 5-HT uptake [24]. However, in the present study, unlike the higher dose (20 mg/kg) of fluoxetine, the lower dose (5 mg/kg) alone did not reduce immobility time. In fact, at this lower dose a very small but significant increase in the endogenous level of 3α, 5α THP in the brain has been demonstrated [47]. However, a rise of this magnitude in the level of 3α, 5α THP by fluoxetine may not be substantially adequate to produce an antidepressant-like effect. In general, these results support the as-

sumption that an increase in the levels of neurosteroids including 3α, 5α THP produces an antidepressant-like effect in patients with major depression [42,43,45]. Although we are suggesting the role of neurosteroids in the antidepressant-like action of the metyrapone, 4′CD, or fluoxetine, interpretation of these findings must remain tentative until the level of neurosteroids are measured after systemic and central administration of these agents in mice, which requires further study.

It has been shown that (a) depressed patients have reduced plasma [31] and/or CSF levels of GABA [9 10] and glutamic acid decarboxylase [17]; (b) repeated administration of antidepressants alters the number of GABA receptors in the rat brain and increases the hippocampal flux of GABA [32]; (c) progabide, a GABAergic drug, exerts antidepressant activity in humans and antidepressant-like effects in animals [20]. These findings suggest that a GABAergic mechanism may be involved in depressive phenomenon and antidepressant action. The neurosteroid 3α, 5α THP is an endogenous potent positive allosteric modulator of GRC [29]. Thus, the possible involvement of GABAergic mechanism in the antidepressant-like effect of 3α, 5α THP was investigated. In the present study, prior administration of the $GABA_A$ receptor agonist, muscimol, or the $GABA_A$ receptor antagonist, bicuculline, respectively potentiated or blocked the antidepressant-like effect on 3α, 5α THP. Furthermore, the antidepressant-like effect of fluoxetine was potentiated by prior administration of muscimol or partially blocked by prior administration of bicuculline. This shows that the antidepressant-like effect of fluoxetine, apart from facilitation of serotonergic transmission, may partially be due to activation of GRC through increased 3α, 5α THP levels. However, the antidepressant-like effect of imipramine was not blocked by bicuculline. The inability of bicuculline to block the antidepressant-like effect of imipramine, which is reported to have no effect on brain 3α, 5α THP content [11,47], suggests that antidepressant action elicited by imipramine, unlike fluoxetine, is not associated to their effect on the $GABA_A$ receptor. In fact, it has been shown that the antidepressant-like effect of desipramine (a metabolite of imipramine) remained unaffected by prior treatment with bicuculline [27]. This, along with our observations with muscimol, further suggests that the partial antagonism of antidepressant-like action of fluoxetine by bicuculline is not associated to the other intrinsic pharmacological properties of bicuculline [44].

In summary, central or peripheral administration of the neurosteroid 3α, 5α THP exhibits an antidepressant-like effect in the forced swim test by its action at the GRC. In addition, drugs like metyrapone [37,43], 4′CD [41], or fluoxetine [47,48], which increase the endogenous levels of neurosteroids including 3α, 5α THP, also reduced the immobility in the forced swim test. The findings of this study have immense therapeutic importance, as reduced levels of 3α, 5α THP are reported in depressed patients [42,45,48] and SSRIs like fluoxetine or fluvoxamine have been shown to produce beneficial effect by selectively increasing the levels of 3α, 5α THP [42,48]. Thus, the neurosteroid 3α, 5α THP has tremendous potential for development as a new class of antidepressant and be considered for further evaluation.

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