Neuropsychopharmacology (2004) 29, 1597–1609

www.neuropsychopharmacology.org

Role of Neuroactive Steroid Allopregnanolone in Antipsychotic-like Action of Olanzapine in Rodents

Rajesh R Ugale[1], Khemraj Hirani[1], Micaela Morelli[2] and Chandrabhan T Chopde*[,1]

[1]University Department of Pharmaceutical Sciences, Nagpur University Campus, Nagpur, Maharashtra, India; [2]Department of Toxicology and Center of Excellence for Neurobiology of Dependence, University of Cagliari, Cagliari, Italy

Olanzapine increases brain allopregnanolone (ALLO) levels sufficiently to modulate neuronal activity by allosterically regulating $GABA_A$ receptors. Recently, we reported the antipsychotic-like profile of ALLO in rodents. The present study examined the hypothesis that olanzapine-induced elevation of endogenous neurosteroid ALLO is vital for its neuroleptic-like action. The conditioned avoidance response (CAR) and apomorphine-induced climbing behavioral paradigms were used in rodents. Administration of ALLO (1 μg, intracerebroventricular (i.c.v.)) or neurosteroidogenic agents such as the mitochondrial diazepam binding inhibitor receptor agonist, FGIN 1-27 (0.5 μg, i.c.v.) or the ALLO precursor, progesterone (10 mg/kg, i.p.) significantly potentiated olanzapine-induced blockade of CAR and apomorphine-induced climbing. In contrast, these agents failed to alter the antipsychotic-like effect of risperidone and haloperidol. On the other hand, inhibition of the endogenous biosynthesis of neurosteroids by the 3β-hydroxysteroid dehydrogenase inhibitor, trilostane (30 mg/kg, i.p.), the 3α-hydroxysteroid oxidoreductase inhibitor, indomethacin (5 mg/kg, i.p.), or the $GABA_A$ receptor antagonist bicuculline (1 mg/kg, i.p.) and dehydroepiandrosterone sulfate (DHEAS) (1 mg/kg, i.p.) blocked the effect of olanzapine, but not of risperidone and haloperidol. Socially isolated animals, known to exhibit decreased brain ALLO and $GABA_A$ receptor functions, displayed a shortening in the muscimol-induced loss of righting reflex and an increased susceptibility to apomorphine-induced climbing. Administration of olanzapine, but not of haloperidol and risperidone, normalized the duration of muscimol-elicited loss of righting reflex. Although all three antipsychotics proved capable of antagonizing the apomorphine-induced climbing, a dose almost five times higher of olanzapine was required in socially isolated animals. The data obtained suggest that enhancement of the GABAergic tone plays a key role in the antipsychotic-like effect exerted by olanzapine in rodents, likely as a consequence of augmented levels of neuroactive steroids, in particular ALLO, in the brain. The present findings provide the first specific behavioral evidence in support of the hypothesis that neuroactive steroid ALLO- mediated GABAergic modulation is essential for the antipsychotic-like action of olanzapine.
Neuropsychopharmacology (2004) **29,** 1597–1609, advance online publication, 21 April 2004; doi:10.1038/sj.npp.1300460

Keywords: neuroactive steroid; allopregnanolone; olanzapine; risperidone; haloperidol; $GABA_A$ receptors

INTRODUCTION

Olanzapine, a thienobenzodiazepine, is an atypical antipsychotic drug generally considered as being superior to classical antipsychotics (Robertson and Fibiger, 1996; Beasley *et al*, 1996; Madhusoodanan *et al*, 1999, 2001). Olanzapine exhibits anxiolytic activity (Moore *et al*, 1994) and improves memory and cognitive functions in rodents (Nowakowska *et al*, 1999). It displays affinity for many neuronal receptors including histamine H_1, serotonin $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, $5\text{-}HT_{2C}$, $5\text{-}HT_3$, and $5\text{-}HT_6$, dopamine D_1–D_5, muscarinic, α and glutamate receptors (Richelson and Souder, 2000). The multiple binding property of olanzapine contributes to its varied pharmacological and therapeutic effects (Roth *et al*, 1994; Bymaster and Falcon, 2000; Bymaster *et al*, 1996, 1999, 2001). However, the precise mechanism underlying its antipsychotic action remains unclear. The potent $5\text{-}HT_2$ receptor antagonism and weak D_1 and D_2 receptor antagonism have been suggested to play a key role in its atypical antipsychotic activity (Beasley *et al*, 1996). Furthermore, the potent antagonistic activity of olanzapine on $5\text{-}HT_3$ and $5\text{-}HT_6$ receptors may also contribute towards its antipsychotic effect (Bymaster *et al*, 2001). Emerging evidence suggests that in addition to dopaminergic and serotonergic antagonism, GABAergic neurotransmission may serve as a primary locus for some antipsychotic drugs, including olanzapine (Farnbach-Pralong *et al*, 1998; Carpenter *et al*, 1999; O'Connor, 2001). Recent studies have shown subdued GABAergic neurotransmission in schizophrenics (Akbarian

*Correspondence: Dr Professor CT Chopde, University Department of Pharmaceutical Sciences, Nagpur University Campus, Nagpur 440 033, Maharashtra, India, Tel: +91 712 2500355/2500324, Fax: +91 712 2563456/2500355, E-mail: chopdect@edumail.nic.in
Received 30 November 2003; revised 8 March 2004; accepted 15 March 2004
Online publication: 17 March 2004 at http://www.acnp.org/citations/Npp03170403550/default.pdf

et al, 1995; Lewis, 2000; Benes and Berretta, 2001), while antipsychotics-like clozapine and olanzapine were found to increase the GABA release in the nucleus accumbens (Drew *et al*, 1990; Osborne *et al*, 1994; O'Connor, 2001). Furthermore, the density of GABAergic interneurons decreases in corticolimbic regions coupled with a simultaneous increase in the density of postsynaptic GABA receptors in the same brain regions of schizophrenic subjects (Benes, 1995). Olanzapine exerts no direct action on the $GABA_A$ receptors as it shows very low affinity for these receptors (Bymaster *et al*, 1996; Schotte *et al*, 1996). However, chronic olanzapine administration remarkably decreases the density of $GABA_A$ receptors in rat hippocampus and temporal cortex (Farnbach-Pralong *et al*, 1998). Several studies have also indicated a close relationship between GABA and dopamine, involved in the precipitation of schizophrenia. Dopamine mediates the modulation of GABAergic neurotransmission in those brain regions, including nucleus accumbens and ventral tegmental area, involved in schizophrenia (Penit-Soria *et al*, 1987; Pennartz *et al*, 1992; Pirot *et al*, 1992; Pralong and Jones, 1993; Cameron and Williams, 1993; Law-Tho *et al*, 1994).

It has recently been demonstrated that both olanzapine and clozapine, but not risperidone and haloperidol, elevate brain cortical levels of the $GABA_A$-positive neuroactive steroid allopregnanolone (ALLO), leading to a putative increase in GABAergic tone (Barbaccia *et al*, 2001; Marx *et al*, 2000, 2003). Neuroactive steroids including ALLO are synthesized *de novo* in the brain from cholesterol or from peripheral steroids in adrenal or gonads (Purdy *et al*, 1991, 1992) and are present in the CNS at concentrations that may modulate $GABA_A$ receptor function (Paul and Purdy, 1992; Robel and Baulieu, 1995; Bixo *et al*, 1997; Rupprecht and Holsboer, 1999). ALLO increases the GABA-mediated chloride ion (Cl^-) flux through $GABA_A$ receptors and is 20-fold more potent than benzodiazepines and 200-fold more potent than barbiturates (Majewska *et al*, 1986; Morrow *et al*, 1987, 1990). High doses of ALLO increase GABAergic tone leading to suppression of dopaminergic transmission (Motzo *et al*, 1996; Van Kammen, 1977; Khisti *et al*, 1998) and elicit a behavioral profile similar to that of the D_2-receptor antagonist haloperidol (Khisti *et al*, 2002). ALLO exerts multiple effects in CNS including anxiolysis (Wieland *et al*, 1991), antistress (Zimmerberg and Blaskey, 1998), antidepressant (Khisti and Chopde, 2000; Khisti *et al*, 2000), and anticonvulsant activity (Frye, 1995), in addition to its neuroleptic-like activity (Khisti *et al*, 2002). Accumulating evidence suggests that physiological fluctuations in brain concentrations of neuroactive steroids contribute towards the genesis, development and course of neurological and psychiatric or affective disorders (George *et al*, 1994; Bixo *et al*, 1997; Hill *et al*, 2000). For example, a variation of ALLO concentration across the menstrual cycle are probably involved in the cyclic mood changes observed with premenstrual tension syndrome (Wang *et al*, 1996).

The paucity of behavioral data suggesting ALLO involvement in the antipsychotic action of olanzapine persuaded us to design different approaches with altered ALLO content. We studied the influence of various agents affecting neurosteroidogenesis and determined the essentiality of increased ALLO content by olanzapine as compared to risperidone and haloperidol. Animal behavioral paradigms widely used to predict antipsychotic activity (Arnt, 1982; Moore and Axton, 1988) were applied. In addition, neuroleptic activity was evaluated in protracted social isolation. This paradigm has been widely employed to investigate the influences of decreased cortical ALLO content and $GABA_A$ receptor function (Serra *et al*, 2000; Dong *et al*, 2001; Guidotti *et al*, 2001). The aim of this study was to provide the specific behavioral evidences to support the hypothesis that neuroactive steroid ALLO-mediated GABAergic modulation is essential for antipsychotic-like action of olanzapine.

MATERIALS AND METHODS

Subjects

All procedures were carried out under strict compliance with ethical principles and guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests; Government of India; New Delhi. Young healthy male, Sprague–Dawley rats (180–220 g) and Swiss albino mice (20–25 g) were used in the study. Rats or mice were group housed in separate cages (five per cage) except the mice that were employed for social isolation. Social isolates were kept individually (cage of $24 \times 17 \times 12$ cm) for 6 weeks before conducting the experiments. All animals were kept under standard 12 : 12-h light/dark cycle (lights on 0700 h) in a temperature controlled ($24 \pm 1^{\circ}C$) environment with *ad libitum* access to rodent chow (Lipton, India) and tap water.

Experiments were performed during the light cycle between 0900 and 1400 h to avoid circadian variation in the brain concentrations of neuroactive steroids (Robel *et al*, 1987). Each experimental group had separate sets of animals and the care was taken to ensure that animals used for one response were not employed elsewhere. Further, an individual animal of a group, subjected once for an experimental effect, was not used again under any condition. Animals were habituated to laboratory conditions for 1 week prior to beginning of experimental protocols to minimize, if any, nonspecific stress induced steroid increase.

Drugs and Administration

The neuroactive steroid ALLO, the mitochondrial diazepam binding inhibitory receptor (MDR) agonist *N*, *N*-di-*n*-hexyl-2-(4-fluorophenyl)-1H-indol-3-acetamide (FGIN 1-27), the $GABA_A$ receptor antagonist bicuculline, the $GABA_A$ receptor allosteric antagonist dehydroepiandrosterone sulfate (DHEAS), the specific $GABA_A$ receptor agonist muscimol, the ALLO precursor progesterone, and the dopamine receptors agonist apomorphine were purchased from RBI, USA. Atypical antipsychotics olanzapine and risperidone (Sun Pharmaceutical Advanced Research Center, Baroda, India), typical antipsychotic haloperidol (Searle, India), neuroactive steroid biosynthesis inhibitors trilostane (Sanofi Winthrop Development Center, UK), and Indomethacin (Zim Laboratories, India) were received as gifts.

ALLO, FGIN 1-27, olanzapine, risperidone, trilostane, indomethacin, DHEAS, and progesterone were dissolved in (45% w/v) solution of 2-hydroxypropyl-β-cyclodextrin and

diluted with 0.9% saline. For intracerebroventricular (i.c.v.) administration of drugs, dilutions were made with artificial cerebrospinal fluid (aCSF) of 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. Other drugs were dissolved or diluted in 0.9% saline.

All drugs were injected by intraperitoneal (i.p.) route with the exception of ALLO and FGIN 1-27 that were injected by i.c.v. route to circumvent their rapid metabolism in the liver and avoid peripheral effects, which could have interfered with behavioral studies, while apomorphine was injected by subcutaneous route (s.c.). At the most, two injections were given to each rat in conditioned avoidance response paradigm or to each mouse in climbing experiments before apomorphine treatment. Respective controls were maintained simultaneously by vehicle treatment at specific time intervals to study the effect of multiple injections or repeated handling stress for each experiment.

Intracerebroventricular Administration

The i.c.v. injections in rats (Sanchez-Blazquez *et al*, 1995; Bilsky *et al*, 1996; Khisti *et al*, 2002) and mice (Akwa *et al*, 2001; Hirani *et al*, 2002) were administered as described earlier. Rats were anaesthetized with pentobarbital sodium (50 mg/kg, i.p.) and fixed in a stereotaxic frame (Stoelting Co., IL, USA). A permanent 22-gauge stainless steel guide cannulae 313-G/Spc (Plastics One Inc., Virginia, USA) was implanted aseptically into the right lateral ventricle (coordinates from Paxinos and Watson, 1998; posterior −0.8 mm; lateral from midline +1.2 mm and ventral −3.5 mm; relative to bregma). For mice i.c.v. injections, guide cannulae (28 gauge) were stereotaxically implanted with the coordinates from Paxinos and Franklin (1997) (AP −0.22 mm; ML +1 mm and DV −2.5 mm; relative to bregma) under pentobarbital sodium (60 mg/kg, i.p.) anesthesia. The guide cannula was secured to the skull using mounting screws (Plastic One) and dental cement (Dental Products of India, Mumbai). A stainless steel dummy cannula was used to occlude the guide cannula when not in use. The animals were then allowed to recover for 1 week, during which they were habituated to the experimental protocols to minimize nonspecific stress. Injections were made using a Hamilton microliter syringe (Hamilton, Nevada, USA) connected to internal cannula (30 gauge) by polyethylene tubing and a volume of 5 μl was administered over a period of 1 min into the right lateral ventricle. The injection cannula was left in place for further 1 min before being slowly withdrawn to avoid back flow. At the end of all i.c.v. experiments, dilute India ink was injected (i.c.v.) and animals were killed immediately. Only data from animals showing uniform distribution of ink into lateral ventricles were used for statistical analysis.

Experiment 1: Dose-Related Effect of Antipsychotics

Conditioned avoidance response (CAR). Rats were trained individually to move from one compartment of a shuttle box (Techno Labs, Lucknow) into other upon presentation of the 10-s buzzer tone (conditioned stimulus; CS). If the rat failed to respond, the tone was further continued with an unconditioned stimulus (UCS) in the form of an electric shock (0.5 mA), delivered to the grid floor of the chamber for a period of 10 s. Each animal was subjected to a daily session of 10 trials separated by a 20-s intertrial interval. The trial terminated once the rat has moved into the other compartment during CS or UCS period. Crossings made during the CS period were recorded as avoidance responses and those made during UCS period were recorded as escape responses. All animals were trained for a week. Only those animals characterized by a high level of avoidance responding (>80%) were used for further experiments. Separate groups of trained rats ($n=7$ per group) were employed for individual dose effect of antipsychotics. They were treated i.p. with olanzapine (1–2 mg/kg), risperidone (0.3–0.5 mg/kg), haloperidol (0.1–0.3 mg/kg), or vehicle (0.2 ml/rat); 30 min thereafter, rats were placed individually in the shuttle box for the standard 10 trial session of CAR. The results are expressed as avoidance/10 trials.

Apomorphine-induced climbing behavior. Administration of apomorphine to mice results in a peculiar climbing behavior characterized initially by rearing and then full-climbing activity (Costall *et al*, 1978). The ability of a drug to antagonize apomorphine-induced climbing behavior in the mouse has been correlated with neuroleptic activity (Costall *et al*, 1978). For the purpose of the climbing test, mice were initially placed individually in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, mesh size 3 mm) for 60 min to acclimatize to the new environment. Separate groups of mice were used for each dose of individual antipsychotics. They were injected (i.p.) with olanzapine (0.5–2 mg/kg), risperidone (0.2–0.4 mg/kg), haloperidol (0.1–0.3 mg/kg), or vehicle (0.2 ml) 30 min before apomorphine (1 mg/kg, s.c.) administration. Animals were subsequently placed individually in wire mesh cages 10 min after apomorphine administration and their climbing behavior was scored at 5-min intervals for a period of 20 min as follows: 0 = four paws on the floor, 1 = one paw on the wall of cage, 2 = two paws on the wall of cage, 3 = three paws on the wall of cage, 4 = four paws on the wall of cage. Climbing scores across each time interval were then summed and expressed as climbing index, thus providing a maximum possible climbing index of 20.

Experiments 2 and 3: Influence of ALLO or Its Endogenous Increase on the Action of Antipsychotics

Two separate experimental designs were set with different groups of animals in each of the treatment protocols.

Experiment 2. Firstly, we examined the dose-related effects of ALLO or neurosteroidogenic drugs alone on escape and avoidance behaviors in rats and apomorphine-induced climbing behavior in mice. For CAR experiments, trained rats were divided into different groups ($n=7$) and injected aCSF (5 μl/rat, i.c.v.), ALLO (1–4 μg/rat, i.c.v.), an ALLO precursor progesterone (10–30 mg/kg, i.p.), or MDR agonist FGIN 1-27 (0.5–1 μg/rat, i.c.v.) that increases endogenous ALLO content. At 15 min after i.c.v. or 30 min after i.p. injection, rats were placed individually in the shuttle box for the standard 10 trial session of CAR to record escape and avoidance behaviors. Similarly, separate groups of mice

($n=7$) received identical treatment of ALLO or its enhancers as mentioned above and 15 min after i.c.v. or 30 min after i.p. administration; all mice were challenged with apomorphine (1 mg/kg, s.c.). The climbing behavior was assessed at 5-min interval for 20 min, starting 10 min after apomorphine injection.

Experiment 3. Secondly, the protocols were designed to examine whether the ALLO or neurosteroidogenic agents modulate the action of antipsychotics on CAR and apomorphine-induced climbing behaviors. Animals were treated with subeffective doses of ALLO (1 μg, i.c.v.), progesterone (10 mg/kg, i.p.), or FGIN 1-27 (0.5 μg, i.c.v.) and 15 min after i.c.v. or 30 min after i.p. injection, they received subeffective dose of antipsychotic or vehicle (0.2 ml). The subeffective doses of antipsychotics used in rats (olanzapine 1 mg/kg, risperidone 0.3 mg/kg, or haloperidol 0.1 mg/kg) for CAR experiments and in mice (olanzapine 0.5 mg/kg, risperidone 0.2 mg/kg, or haloperidol 0.1 mg/kg) for climbing test were selected on the basis of dose-related study from experiment 1. Following 30 min of antipsychotics, rats were subjected to CAR test for escape and avoidance behaviors, while mice were injected apomorphine (1 mg/kg, s.c.) for climbing test as described above.

Experiments 4 and 5: Influence of Neurosteroid Biosynthesis Inhibitors or Agents Capable of Reducing $GABA_A$ Receptor Activation on the Action of Antipsychotics

In order to study the influence of low neurosteroid content or decreased GABAergic activation on antipsychotic-like effect of olanzapine, risperidone, and haloperidol, following treatment protocols were designed.

Experiment 4. The *de novo* neurosteroid biosynthesis inhibitors indomethacin and trilostane were used either alone or in combination with effective doses of antipsychotics (olanzapine 2 mg/kg, risperidone 0.5 mg/kg, or haloperidol 0.3 mg/kg). Trilostane (30 mg/kg, i.p.), a 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibitor, was injected 2-h before i.p. injection of vehicle or antipsychotics. This dose of trilostane has been reported to significantly decrease the conversion of pregnenolone to progesterone (Potts *et al*, 1978, Korneyev *et al*, 1993). Indomethacin (5 mg/kg, i.p.), a 3α-hydroxysteroid oxidoreductase (3α-HSOR) enzyme inhibitor, was injected 20 min prior to antipsychotics or vehicle. This dose has been shown to inhibit the conversion of 5α-dihydroprogesterone (5α-DHP) to ALLO in rats (Beyer *et al*, 1999). At 30 min after antipsychotic or vehicle administration, each rat was subjected to 10 trial sessions to record escape or avoidance behaviors as described above.

Similarly for assessment of climbing behavior, different groups of mice were treated with indomethacin, trilostane, or vehicle in the doses and at time interval identical to that used in above CAR experiment. These agents were used either alone or in combination with effective doses of antipsychotics (olanzapine 1 mg/kg, risperidone 0.3 mg/kg, haloperidol 0.2 mg/kg) or vehicle. At 30 min after antipsychotic treatment, all mice received apomorphine (1 mg/kg, s.c.) and 10 min later, the climbing behavior was scored at 5-min interval for a period of 20 min.

Experiment 5. We studied the influence of reduced GABAergic tone on antipsychotic-like effect of olanzapine, risperidone, and haloperidol using separate groups of animals. Rats were injected i.p. $GABA_A$ receptor antagonist bicuculline (1 mg/kg), or the negative modulator of $GABA_A$ receptor DHEAS (1 mg/kg), 30 min prior to vehicle or antipsychotics (olanzapine 2 mg/kg, risperidone 0.5 mg/kg, or haloperidol 0.3 mg/kg). At 30 min after vehicle or antipsychotic injection, each rat was subjected to 10 trial session of escape and avoidance behavior as described above.

Similarly for climbing test, different groups of mice were treated with bicuculline, DHEAS, or vehicle in the doses and at time interval same as that used in the above CAR experiment. These agents were used either alone or in combination with effective doses of antipsychotics, olanzapine (1 mg/kg), risperidone (0.3 mg/kg), haloperidol (0.2 mg/kg), or vehicle. At 30 min after vehicle or antipsychotic treatment, all mice received apomorphine (1 mg/kg, s.c.) and 10 min later, the climbing behavior was scored at 5-min interval for a period of 20 min. Vehicle-treated groups were also maintained simultaneously.

Experiment 6: Influence of Social Isolation on Action of Antipsychotics

We evaluated the actions of different antipsychotics in protracted social isolation paradigm, reported to downregulate $GABA_A$ receptor and to selectively decrease cerebral cortical content of ALLO without altering pregnenolone and progesterone concentration (Matsumoto *et al*, 1999; Guidotti *et al*, 2001). Mice were housed either in groups of five per cage ($24 \times 17 \times 12$ cm) or socially isolated. For social isolation, each mouse was kept individually (isolated in cage of $24 \times 17 \times 12$ cm) for 6 weeks before conducting the experiments. Animals were kept under standard 12 : 12-h light/dark cycle (lights on 0700 h) in a temperature-controlled ($24 \pm 1^{\circ}$C) environment with *ad libitum* access to rodent chow (Lipton, India) and tap water.

Climbing test. Different cohorts of mice, both from group housed and isolates, were challenged with different doses of apomorphine (0.25–1 mg/kg, s.c.) or vehicle for climbing assessment. In a separate design, isolates or social mice were injected i.p. olanzapine (1–5 mg/kg), risperidone (0.3 mg/kg), haloperidol (0.2 mg/kg), or vehicle and 30 min thereafter challenged with apomorphine (1 mg/kg, s.c.) and following 10 min the climbing behavior was scored at 5-min interval for a period of 20 min as described above.

Righting reflex test. We examined whether reported downregulation of $GABA_A$ receptor alter the actions of different antipsychotics in social isolates by measuring the duration of muscimol-induced loss of righting reflex. Group-housed or isolated mice were injected i.p. olanzapine (0.05 or 0.1 mg/kg), risperidone (0.1–0.3 mg/kg), haloperidol (0.05–0.1 mg/kg), or vehicle 30 min before muscimol

(1.5 mg/kg) and duration of loss of righting reflex was measured. Loss of righting reflex was defined as the inability of mouse to right itself within 30 s and the return of righting response as the ability of a mouse to right itself twice in 1 min.

Statistical Analysis

The results of CAR were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. Climbing index data was analyzed using Kruskal–Wallis ANOVA followed by Mann–Whitney *U* test. Comparison between two groups was performed by unpaired two-tailed *t*-tests. For multiple comparisons, two-way ANOVA followed by Bonferoni test was utilized. A value of $p<0.05$ was considered to be statistically significant in all cases.

RESULTS

Experiment 1

CAR inhibition by antipsychotics in rats. As shown in Figure 1a, olanzapine (one-way ANOVA: $p<0.0001$; $F=187.4$; $df=3, 24$; $n=7$), risperidone (one-way ANOVA: $p<0.0001$; $F=183.2$; $df=3, 24$; $n=7$) and haloperidol (one-way ANOVA: $p<0.0001$; $F=223.8$; $df=3, 24$; $n=7$) produced dose-related suppression of CAR behavior. A dose of 1.5 mg/kg of olanzapine produced approximately 40% block ($p<0.001$) of avoidance responding, whereas 2 mg/kg almost completely (90%) abolished ($p<0.001$) the CAR. Administration of risperidone and haloperidol also produced around 80–90% inhibition of CAR at 0.5 mg/kg ($p<0.001$) and 0.3 mg/kg ($p<0.001$), respectively. At doses lower than 1 mg/kg of olanzapine, 0.3 mg/kg risperidone, and 0.1 mg/kg haloperidol, no significant effect ($p>0.05$) was observed. The doses of antipsychotics used here had no significant effect on escape performance.

Climbing behavior inhibition by antipsychotics in mice. Olanzapine (1–2 mg/kg, i.p.), risperidone (0.3–0.4 mg/kg, i.p.), and haloperidol (0.2–0.3 mg/kg, i.p.) exhibit dose-dependent antagonism ($p=0.0006$) to apomorphine-induced climbing behavior in mice (Figure 1b) with higher doses producing greater inhibition of climbing behavior. No apparent inhibition of climbing behavior was observed at doses lower than 0.5 mg/kg olanzapine, 0.2 mg/kg risperidone, or 0.1 mg/kg haloperidol.

Experiments 2 and 3: Influence of ALLO or Its Endogenous Increase on The Action of Antipsychotics

Experiment 2. As shown in Figure 2, the i.c.v. administration of ALLO was associated with dose-dependent inhibition of CAR behavior (one-way ANOVA: $p<0.0001$; $F=219.1$; $df=3, 24$; $n=7$), while it did not affect escape response. *Post hoc* comparison revealed that at a dose of 2–4 μg/rat, ALLO not only significantly ($p<0.001$) suppressed (60–90%, respectively) the CAR (Figure 2a) but also the apomorphine-induced climbing behavior in mouse ($p=0.002$) (Figure 2b). Lower doses were ineffective in blocking both behaviors. Furthermore, FGIN 1-27 (1 μg/rat or mouse, i.c.v.) and progesterone (20–30 mg/kg, i.p.)

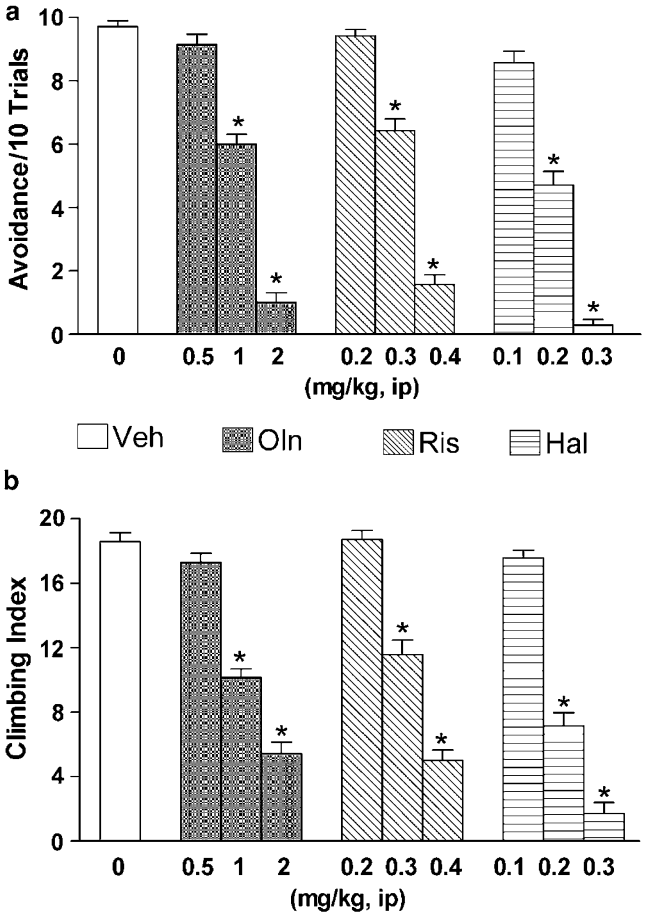

Figure 1 The effects of vehicle (Veh), olanzapine (Oln), risperidone (Ris), and Haloperidol (Hal) on (a) conditioned avoidance response (CAR) behavior in rats and (b) apomorphine-induced climbing behavior in mice. Animals were injected i.p. Oln, Ris, Hal, or Veh and 30 min thereafter, rats were assessed for CAR while mice were injected apomorphine (1 mg/kg, s.c.). For CAR inhibition, each trained rat was subjected to 10 trial sessions consisting of 20-s intertrial interval. For climbing behavior in mice, the total climbing scores was assessed at 5-min intervals for 20 min, starting 10 min after apomorphine administration. An index of 20 is the maximum possible for each mouse. Each column represents mean ± SEM for a group of ($n=7$) and significant at $^*p<0.001$ *vs* respective control.

effectively (60–90% respectively) blocked the CAR ($p<0.0001$) without significantly affecting the escape behavior. These doses also significantly ($p=0.002$) antagonized the apomorphine-induced climbing in mice. However, lower doses failed to affect these behaviors.

Experiment 3. As shown in Figure 3, the i.c.v. injection of subeffective dose of ALLO (1 μg/rat) that exert minimal or no effect potentiated the effect of low dose of olanzapine (1 mg/kg, i.p.) on CAR inhibition (two-tailed *t*-test: $p<0.0001$; $t=15.81$; $df=12$; $n=7$). Results showed that the effect of subeffective dose of olanzapine (1 mg/kg) significantly inhibited (nearly 90%) CAR in presence of ALLO (1 μg/rat) ($p<0.001$), as compared to the vehicle treatment (Figure 3a). The above drug treatment regimen in the rats had no effect on escape performance. Similarly, ALLO (1 μg/mouse, i.c.v.) potentiated the suppression of climbing behavior caused by olanzapine (0.5 mg/kg) (two-tailed *t*-test: $p=0.0001$; $t=5.628$; $df=12$; $n=7$). On the

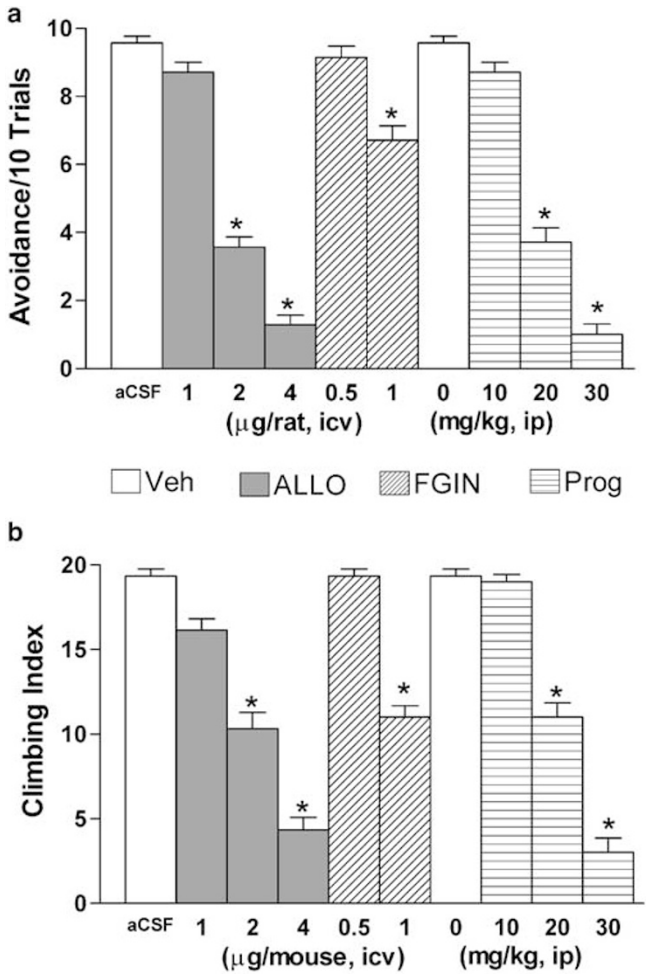

Figure 2 The effects of various doses of allopregnanolone (ALLO), FGIN 1-27, progesterone (Prog), or vehicle on (a) CAR in rats and (b) apomorphine-induced climbing behavior in mice. At 30 min after i.p. injection of Prog or vehicle and 15 min after i.c.v. injection of ALLO, FGIN1-27, or aCSF, rats were subjected to CAR behavior while mice received apomorphine (1 mg/kg, s.c.) climbing. For CAR inhibition, each trained rat was subjected to 10 trial session consisting of 20-s intertrial interval. For climbing behavior in mice, the total climbing scores was assessed at 5-min intervals for 20 min, starting 10 min after apomorphine administration. An index of 20 is the maximum possible for each mouse. Each column represents mean ± SEM for a group of ($n = 7$ rats or 6 mice) and significant at $^*p < 0.05$ *vs* respective control.

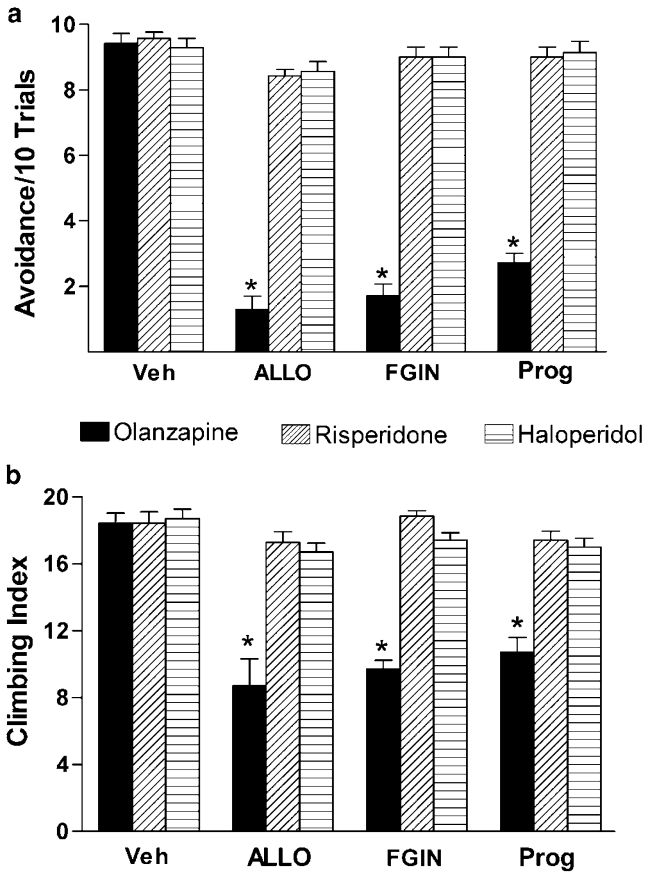

Figure 3 Effects of allopregnanolone (ALLO) or neurosteroidogenic agents on antipsychotic-induced antagonism of (a) CAR in rats and (b) apomorphine-induced climbing behavior in mice. Animals were injected i.c.v. ALLO (1 μg/mouse), FGIN 1-27 (0.5 μg/mouse), aCSF, or i.p. progesterone (Prog) (10 mg/kg) or vehicle. At 15 or 30 min after i.c.v. or i.p. injection, respectively, animals injected i.p. vehicle or antipsychotics. The subeffective doses of antipsychotics used were olanzapine 1 mg/kg, risperidone 0.3 mg/kg, or haloperidol 0.1 mg/kg in rats and olanzapine 0.5 mg/kg, risperidone 0.2 mg/kg, or haloperidol 0.1 mg/kg in mice. At 30 min thereafter, trained rats were subjected to CAR while mice were injected with apomorphine (1 mg/kg, s.c.). For CAR inhibition, each trained rat was subjected to 10 trial session consisting 20-s intertrial interval. For climbing behavior in mice, the total climbing scores was assessed at 5-min intervals for 20 min, starting 10 min after apomorphine administration. An index of 20 is the maximum possible for each mouse. Each column represents mean ± SEM for a group of ($n = 7$ rats or 7 mice) and significant at $^*p < 0.001$ *vs* respective control.

other hand, ALLO (1 μg/mouse, i.c.v.) did not significantly influence the effect of risperidone and haloperidol on CAR and climbing behavior (Figure 3a,b).

Administration of subeffective doses of FGIN 1-27 (0.5 μg/rat, i.c.v.) (two-tailed *t*-test: $p < 0.0001$; $t = 16.53$; $df = 12$; $n = 7$) or progesterone (10 mg/kg, i.p.) (two-tailed *t*-test: $p < 0.0001$; $t = 16.28$; $df = 12$; $n = 7$) significantly potentiated (80 or 70%, respectively) the olanzapine (1 mg/kg, i.p.) induced suppression of CAR (Figure 3a,b) without affecting the escape performance. Similarly, antagonistic effect of olanzapine on apomorphine-induced climbing was potentiated by FGIN 1-27 (0.5 μg/mouse, i.c.v.) (two-tailed *t*-test: $p < 0.0001$; $t = 10.84$; $df = 12$; $n = 7$) and progesterone (10 mg/kg, i.p.) (two-tailed *t*-test: $p < 0.0001$; $t = 7.13$; $df = 12$; $n = 7$). These agents however failed to significantly alter the effects of risperidone and haloperidol in both the behaviors (Figure 3b).

Experiments 4 and 5: Influence of Neurosteroid Biosynthesis Inhibitors or Agents Capable of Reducing $GABA_A$ Receptor Activation on Behavioral Actions of Antipsychotics

Experiment 4. As depicted in Figure 4, inhibitory effect of olanzapine (2 mg/kg, i.p.) on CAR was significantly blocked ($p < 0.001$) by the neurosteroid synthesis blockers indomethacin (5 mg/kg, i.p.) (two-tailed *t*-test: $p < 0.0001$; $t = 18.00$; $df = 12$; $n = 7$) or trilostane (30 mg/kg, i.p.) (two-tailed *t*-test: $p < 0.0001$; $t = 20.95$; $df = 12$; $n = 7$) (Figure 4a). Similarly, the inhibition of apomorphine-induced climbing

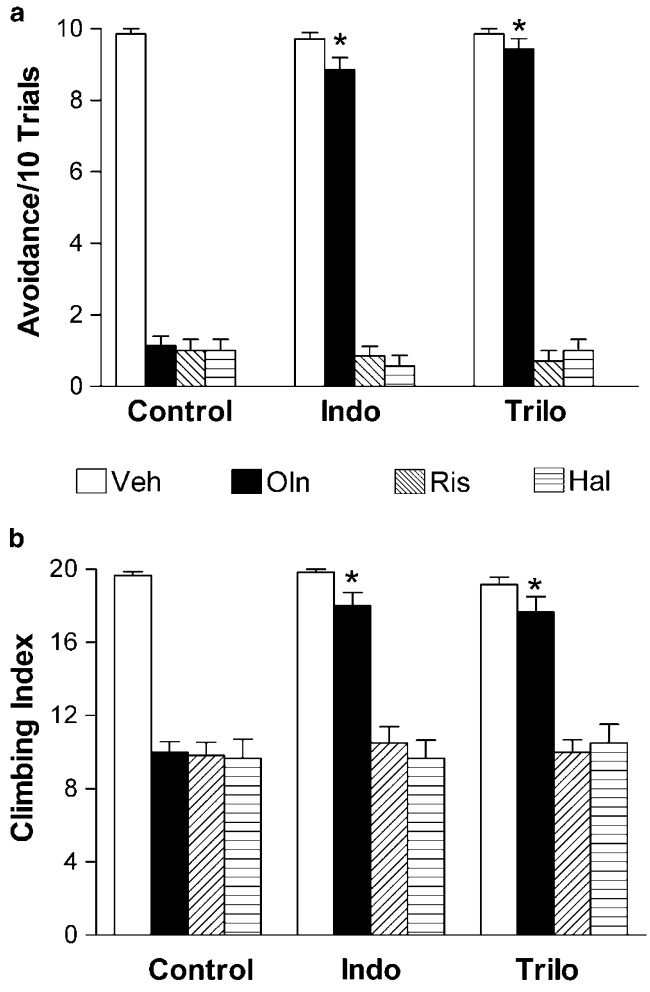

Figure 4 Influence of neuroactive steroid biosynthesis blockers on antipsychotic-induced inhibition of (a) CAR in rats and (b) antagonism of apomorphine-induced climbing behavior in mice. Animals were treated i.p. with Indomethacin (5 mg/kg) or trilostane (30 mg/kg) 30 or 120 min, respectively, before olanzapine (Oln), risperidone (Ris), haloperidol (Hal), or vehicle. At 30 min thereafter, rats were subjected to CAR behavior while mice were injected apomorphine (1 mg/kg, s.c.). For CAR behavior, each trained rat was subjected to 10 trial session consisting 20-s intertrial interval. For climbing behavior in mice, the total climbing scores was assessed at 5-min intervals for 20 min, starting 10 min after apomorphine administration. An index of 20 is the maximum possible for each mouse. Each column represents mean ± SEM for a group of ($n = 7$ rats or 6 mice) and significant at $^*p < 0.001$ *vs* vehicle + olanzapine-treated group.

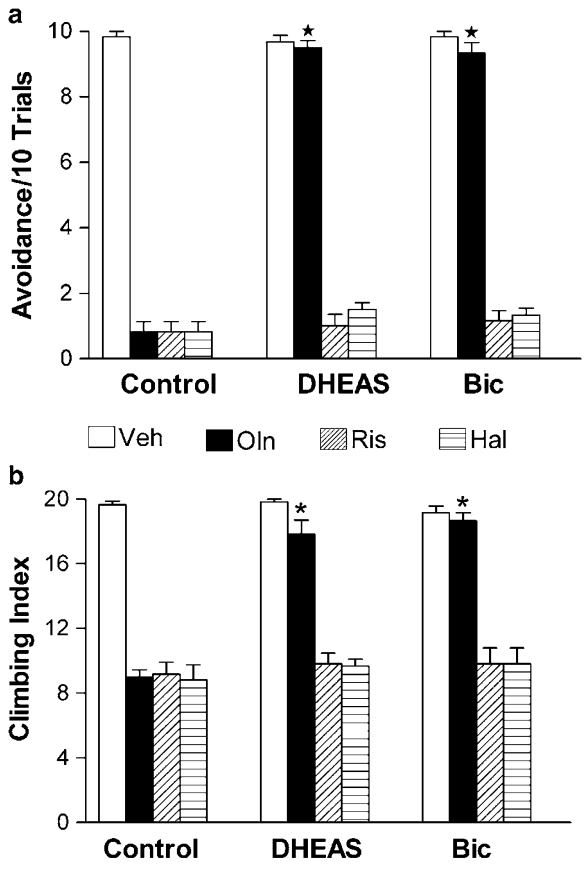

Figure 5 Effect of $GABA_A$ receptor antagonist bicuculline (Bic) and $GABA_A$ negative neuroactive steroid modulator dehydroepiandrosterone sulphate (DHEAS) on antipsychotic induced (a) CAR inhibition in rats and (b) blockade of apomorphine-climbing behavior in mice. Animals were injected i.p. bicuculline (Bic) (1 mg/kg), DHEAS (1 mg/kg), or vehicle 30 min prior to i.p. injection of olanzapine (Oln), risperidone (Ris), haloperidol (Hal), or vehicle. At 30 min thereafter, rats were subjected to CAR behavior while mice were injected apomorphine (1 mg/kg, s.c.). For CAR behavior, each trained rat was subjected to 10 trial session consisting 20-s intertrial interval. For climbing behavior in mice, the total climbing scores was assessed at 5-min intervals for 20 min, starting 10 min after apomorphine administration. An index of 20 is the maximum possible for each mouse. Each column represents mean ± SEM for a group of ($n = 6$ rats or 6 mice) and significant at $^*p < 0.0001$ *vs* vehicle + olanzapine-treated group.

produced by olanzapine (1 mg/kg, i.p.) was significantly reversed by indomethacin (two-tailed *t*-test: $p < 0.0001$; $t = 8.593$; df = 10; $n = 6$) and trilostane (two-tailed *t*-test: $p < 0.0001$; $t = 7.502$; df = 10; $n = 6$) (Figure 4b). However, the effects of risperidone and haloperidol on CAR and climbing behavior remained unaffected ($p > 0.05$). The neurosteroid inhibitors in the doses used here *per se* neither significantly affected avoidance responding and escape performance nor the climbing behavior.

Experiment 5. As shown in Figure 5, DHEAS (1 mg/kg, i.p.), the most potent negative modulator of $GABA_A$ receptors (two-tailed *t*-test: $p < 0.0001$; $t = 22.01$; df = 10; $n = 6$), reversed the inhibitory effects of olanzapine on CAR and climbing behavior (two-tailed *t*-test: $p < 0.0001$; $t = 9.010$; df = 10; $n = 6$). On the other hand, the effects of risperidone or haloperidol (Figure 5a,b) remained unchanged. Bicuculline (1 mg/kg, i.p.), the specific $GABA_A$ antagonist, reversed the effect of olanzapine on CAR (two-tailed *t*-test: $p < 0.0001$; $t = 18.75$; df = 10; $n = 6$) and climbing behavior (two-tailed *t*-test: $p < 0.0001$; $t = 10.77$; df = 10; $n = 6$) without altering the effects of risperidone and haloperidol. Further, Bicuculline or DHEAS treatment in the doses used here *per se* did not significantly alter the avoidance responding/escape performance in rats or climbing behavior in mice.

Experiment 6: Influence of Social Isolation on the Effects of Antipsychotics

As shown in Figure 6a, socially isolated subjects showed enhanced sensitivity ($p < 0.001$) in climbing test, as the

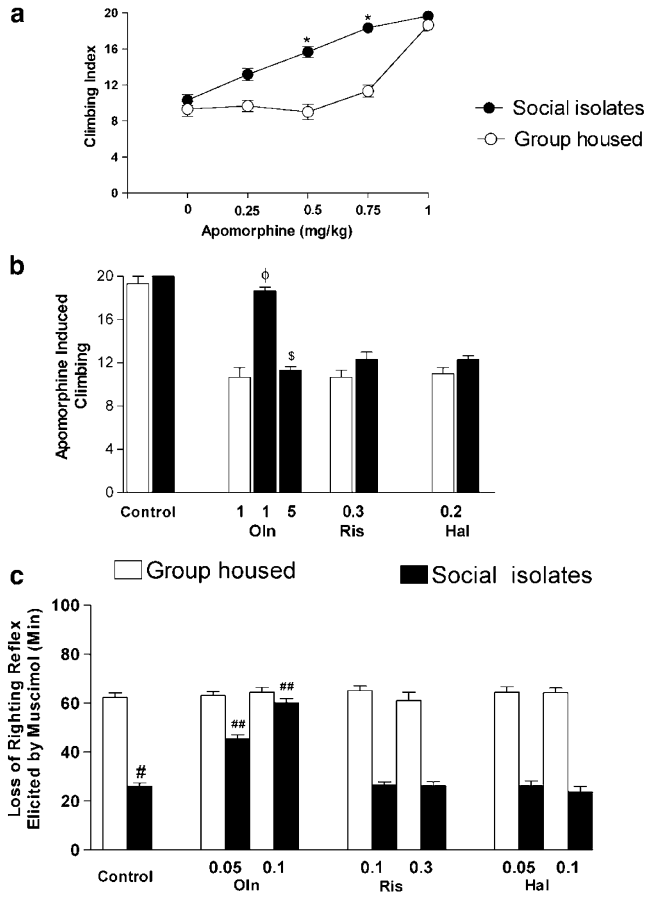

Figure 6 Effects of social isolation on mouse behaviors. (a) Apomorphine-induced climbing, (b) its antagonism by antipsychotic and (c) muscimol-induced loss of righting reflex and its modification by antipsychotics. Animals were housed in groups ($n=6$ per cage) or individually (socially isolated) for 6 weeks before the start of the experiments. (a) Apomorphine (0.25–1 mg/kg) or vehicle was injected s.c. and (b) olanzapine (Oln), risperidone (Ris), or haloperidol (Hal) was injected 30 min before apomorphine (1 mg/kg, s.c.). The total climbing scores was assessed at 5-min intervals for 20 min, starting 10 min after apomorphine administration. An index of 20 is the maximum possible for each mouse. (c) Oln, Ris, Hal, or Veh was administered 30 min prior to muscimol (1.5 mg/kg, i.p.) and duration of loss of righting reflex was measured. Each data represents mean ± SEM for a group of ($n=12$ mice) and significant at $^{*}p<0.01$ *vs* the respective control value, $^{\Phi}p<0.05$ *vs* Oln (1 mg/kg) in group-housed animals,$^{\$}$ $p<0.05$ *vs* socially isolated Oln (1 mg/kg)-treated social isolates, $^{\#}p<0.001$ *vs* vehicle-treated group-housed control, $^{\#\#}p<0.001$ *vs* vehicle-treated socially isolated mice.

lowest dose of apomorphine (0.75 mg/kg) produced 100% climbing score in isolates when compared to group-housed mice (1 mg/kg). Interestingly, a 4–5 times higher dose of olanzapine (two-tailed *t*-test: $p<0.0001$; $t=11.01$; df = 21; $n=12$) was required to antagonize apomorphine-induced (1 mg/kg, s.c.) climbing in social isolates as compared to group-housed subjects. On the other hand, no significant difference was observed in doses required to block apomorphine climbing by risperidone (0.3 mg/kg, i.p.) or haloperidol (0.2 mg/kg, ip) in either group-housed or socially isolated subjects.

Further, the duration of loss of righting reflex induced by muscimol (1.5 mg/kg, i.p.) was shortened in socially isolated mice ($p<0.001$) compared to group-housed animals. Olanzapine (0.05–0.1 mg/kg, i.p.) dose dependently normalized this decreased loss of righting reflex ($p<0.001$) in socially isolated mice, while risperidone and haloperidol had no such effect (Figure 6b).

The duration of loss of righting reflex induced by muscimol (1.5 mg/kg, i.p.) was shortened ($p<0.0001$) in social isolates as compared to group-housed mice. Olanzapine (0.05–0.1 mg/kg, i.p.) dose dependently normalized this decreased loss of righting reflex (ANOVA: $p<0.0001$; $F=78.46$; df = 2.66; $n=12$) in socially isolated mice, while risperidone and haloperidol had no such effect (Figure 6c).

DISCUSSION

Our data demonstrate that the neuroactive steroid ALLO, positive modulator of $GABA_A$ receptor, plays vital role in antipsychotic-like action of olanzapine but not of risperidone or haloperidol in rodents. The study employed young, healthy males to avoid gender and age-related variations of neurosteroids (Orentreich *et al*, 1984; Finn and Gee, 1993; Robel *et al*, 1995; Genazzani *et al*, 1998; Schumacher *et al*, 2003) that might affect the behavioral parameters *per se*. The present results replicate previous findings that olanzapine, risperidone, and haloperidol dose specifically inhibit avoidance response without affecting the escape behavior in rats (Moore *et al*, 1992; Wadenberg *et al*, 2001) and antagonized apomorphine-induced climbing (Costall *et al*, 1978; Strange, 2001). Similarly, exogenous injection (i.c.v.) of the neurosteroid ALLO and neurosteroidogenic agents like the MDR agonist FGIN 1-27 or i.p. injection of the neurosteroid precursor progesterone (Bitran *et al*, 2000; Paul and Purdy, 1992; Romeo *et al*, 1992; Rupprecht *et al*, 1993; Prasad *et al*, 1994; Lambert *et al*, 1995) also dose specifically antagonized these behaviors. Indeed, we and others (Khisti *et al*, 2002; Rupprecht *et al*, 1999) have recently demonstrated the neuroleptic-like properties of the neurosteroid ALLO and its precursor progesterone. Multiple studies indicated that the changes in the physiological rhythm of progesterone secretion, together with the normal metabolism of this steroid to ALLO, might contribute towards the genesis of several mental disorders. For example, postmenopausal women exhibit increased vulnerability to the onset of schizophrenic episodes (Hafner *et al*, 1993); whereas, during premenstrual phase, symptoms such as anxiety and discomfort are expressed (Guidotti and Costa, 1998; Bailey and Cohen, 1999). Severe psychotic symptoms and dysphoria occurring postpartum have been attributed to the withdrawal of progesterone secretions (Brockington and Meakin, 1994; Harris *et al*, 1994).

Next, we investigated whether the enhanced or reduced brain neuroactive steroids modulate the neuroleptic properties of olanzapine compared to that of haloperidol and risperidone. Interestingly, it was found that the central (i.c.v.) administration of ALLO or MDR agonist FGIN 1-27 and i.p. injection of progesterone, a peripheral precursor in their subeffective doses, augmented the effects of olanzapine on CAR and climbing behaviors. By contrast, these pretreatments failed to significantly affect the actions of risperidone and haloperidol in above behavioral paradigms. Further, necessity of ALLO in mode of action of different antipsychotics was studied using specific *de novo* neurosteroid

biosynthesis inhibitors. Trilostane and indomethacin, at the doses used in the study, *per se* did not significantly affect the avoidance/escape behavior or apomorphine-induced climbing. This insignificant effect on base line activity in these behaviors could be attributed to the fact that endogenous levels of ALLO in naïve male rats are below relevant physiological concentrations (Purdy *et al*, 1991). However, pretreatment with trilostane and indomethacin abolished the actions of olanzapine but not of risperidone and haloperidol, thereby implying the vital role of neuroactive steroids in the action of olanzapine. It may be recalled that while trilostane inhibits the enzyme 3β-hydroxysteroid oxidoreductase (3β-HSOR) (Potts *et al*, 1978, Korneyev *et al*, 1993) that catalyses the conversion of pregnanolone to progesterone, indomethacin inhibits the cytosolic isoform of enzyme 3α-HSOR (Beyer *et al*, 1999) that reduces the conversion of 5α-DHP to ALLO. Our results indicate that metabolism of progesterone to neurosteroids like ALLO is essential for antipsychotic-like effect of olanzapine. This strongly supports the assumption that progesterone-derived neuroactive steroids contribute to the actions of olanzapine but not of risperidone and haloperidol (Barbaccia *et al*, 2001; Marx *et al*, 2000, 2003). The recent reports support our observation that levels of GABAergic neuroactive steroid are significantly lower in schizophrenics (Kurumaji *et al*, 1997, 2000). Moreover, numerous evidences suggest that variations in the steady-state level of neuroactive steroids, especially ALLO in brain, may be relevant to pathophysiology of a range of psychiatric disorders (Guidotti *et al*, 2000, 2001). However, it is important to note that our results do not exclude the involvement of other GABAergic neuroactive steroids like THDOC since olanzapine (Marx *et al*, 2000) and clozapine (Barbaccia *et al*, 2001) modify levels of different brain neuroactive steroids including THDOC.

A large body of evidence indicates an inhibitory influence of $GABA_A$ receptors on the nigrostriatal (Bartholini, 1980) and the mesolimbic (Pacitti *et al*, 1982) dopaminergic systems. Olanzapine is known to block dopamine receptors with relatively weaker potency and the blockade of receptors, other than D_2, is thought to be responsible for its atypical antipsychotic profile (Bymaster *et al*, 1999). Moreover, ALLO represents the most potent positive modulator of $GABA_A$ receptor and is known to diminish basal and stress-induced dopaminergic transmission in the brain by its action on $GABA_A$ receptors (Motzo *et al*, 1996). Our observation that bicuculline pretreatment antagonized the effects of olanzapine on CAR- and apomorphine-induced climbing behaviors suggests that the antipsychotic-like action of olanzapine could be mediated via activation of $GABA_A$ receptors. Consequently it may be speculated that the influence of ALLO on dopaminergic neurotransmission might be mediated via $GABA_A$ receptors (Motzo *et al*, 1996; Khisti *et al*, 2002) perhaps through modulation of intracellular signaling mechanisms like protein kinase C (PKC) (Brunig *et al*, 1999; Brussard *et al*, 2000; Liu *et al*, 2000). That the activation or inhibition of PKC increases or decreases, respectively, dopamine release in striatum has already been demonstrated (Gimbalvo, 1988). Interestingly, interaction of ALLO with $GABA_A$ receptors prevents the PKC from phosphorylating $GABA_A$ receptor itself and prolongs the receptor ion channel open time (Twyman and Macdonald, 1992; Brussard *et al*, 2000). Further, PKC deficiency results in enhanced $GABA_A$ receptor sensitivity to allosteric modulators including ALLO or pregnanolone (Baulieu *et al*, 2001; Hodge *et al*, 2002). One line of evidence maintains that $GABA_A$ receptors participate in controlling the activity of dopaminergic neurons (Santiago and Westerink, 1992) and exerts tonic inhibition of dopamine release in rat striatum (Gruen *et al*, 1992; Smolders *et al*, 1995). It is noteworthy that olanzapine does not exercise a direct action on the $GABA_A$ receptors (Bymaster *et al*, 1996; Schotte *et al*, 1996) but alters the density of $GABA_A$ receptors in hippocampus and temporal cortex (Farnbach-Pralong *et al*, 1998). It is further stated that olanzapine or clozapine would assist substantially in reversing an under active GABAergic system in schizophrenia (Farnbach-Pralong *et al*, 1998). Thus, it seems reasonable to assume that olanzapine-induced elevations in ALLO increase the GABAergic tone or modulates the PKC function that may inhibit the hyperdopaminergic tone, leading to neuroleptic manifestation. However, ALLO does not display direct affinity towards D_2 receptors (Wadenberg *et al*, 2001), but influences the dopamine-mediated behaviors in rodents (Khisti *et al*, 2002). The possibility of ALLO and olanzapine interaction with dopaminergic neurotransmission, in light of the indirect GABAergic modulation is unclear at this stage and needs further investigations.

In order to better understand the neurosteroid olanzapine interaction and involvement of GABAergic transmission in the antipsychotic-like effect of olanzapine, we studied the effect of DHEAS, the $GABA_A$ receptor allosteric antagonist. Our results indicate that prior administration of DHEAS reversed the olanzapine-induced behavior in CAR and apomorphine climbing. This approach confirms and extends our hypothesis that modulation of $GABA_A$ receptors is the underlying mechanism in antipsychotic-like action of olanzapine but not of haloperidol and risperidone. Earlier reports have demonstrated an increase in DHEAS level, a putative stimulator of dopamine release (Murray and Gillies, 1997), also in schizophrenia (Oades and Schepker, 1994). Recently, it has been reported that treatment with the atypical antipsychotic clozapine, but not haloperidol, decreases the levels of DHEAS in rat cortex (Nechmad *et al*, 2003), thereby increasing GABAergic tone and leading to improvement of psychotic symptoms (Purdon *et al*, 2001). Indeed, monitoring of the DHEA/DHEAS levels following olanzapine treatment may help to determine their relevance in antipsychotic mode of action.

Physiological and pharmacologically induced fluctuations in brain concentrations of neuroactive steroids, such as those encountered during pregnancy and pseudopregnancy result in selective modulation of function and expression of $GABA_A$ receptors in cerebral cortex and hippocampus (Uzunova *et al*, 2003). A protracted social isolation paradigm was used to further investigate the role of $GABA_A$ receptors and of the neurosteroid ALLO in the actions of different antipsychotics. Social isolation of rodents for 6 weeks is known to downregulate the $GABA_A$ receptor and to selectively decrease ALLO levels without altering other neurosteroids in brain (Serra *et al*, 2000; Dong *et al*, 2001; Guidotti *et al*, 2001).

Isolation of animals engenders enhanced sensitivity of apomorphine-induced climbing response. The basis for

enhanced climbing susceptibility in socially isolated mice is unclear at this stage. However, this could possibly be attributed to increased dopamine levels in the brain secondary to the depletion of cortical ALLO (Dazzi *et al*, 2002) and decreased $GABA_A$ receptor functioning (Serra *et al*, 2000) in socially isolated animals. Further, different antipsychotics used in the study antagonized the apomorphine-induced climbing in both socially isolated and group-housed subjects. However, nearly five times the dose of olanzapine was required to inhibit the climbing response in social isolates as compared to that required in the group-housed animals. These results are interesting in light of the reports that risperidone and haloperidol have been shown to possess a high dopamine D_1/D_2 receptor blockade while olanzapine affinity for these receptors is relatively weak (Bymaster *et al*, 1996; Kapur *et al*, 1998, 1999; Tauscher *et al*, 1999). Another possibility is that olanzapine may exert its effect through other receptor mechanisms in a manner similar to ALLO. We suggest that the difference in efficacy in restoring the antagonism of apomorphine-induced climbing behavior by olanzapine and other antipsychotics in social isolates should be further investigated. Prolonged social isolation has been shown to affect the functional coupling between the recognition site for GABA and those for allosteric modulators such as muscimol, benzodiazepines (Dong *et al*, 2001). Thus, similar to previous studies (Guidotti *et al*, 2001), we observed that the duration of loss of righting reflex elicited by the $GABA_A$ receptor agonist muscimol was markedly shortened in socially isolated mice. Interestingly olanzapine, but not risperidone and haloperidol, normalized the duration of muscimol-induced decrease of righting reflex in socially isolated mice. Recently, the selective serotonin reuptake inhibitor (SSRI) fluoxetine has been shown not only to normalize the levels of brain ALLO content but also pentobarbital and muscimol-induced decrease of righting reflex in socially isolated mice (Matsumoto *et al*, 1999). Thus, it can be postulated that olanzapine normalized the endogenous cortical stores of ALLO that is reduced in socially isolated mice and thereby upregulated the $GABA_A$ receptors to normalize muscimol-elicited loss of righting reflex.

In conclusion, these results support the hypothesis that the antipsychotic-like effect of olanzapine is due to enhanced GABAergic tone or $GABA_A$ receptor function as a likely consequence of augmented brain content of neuroactive steroids especially ALLO (Majewska *et al*, 1986; Morrow *et al*, 1987, 1990). Furthermore, a recent report suggests that olanzapine increases midbrain allopregnanolone levels when infused in ventral tegmental area, and the behavioral effect of olanzapine is mediated through increase in ALLO, independent of progesterone or corticosterone (Frye and Seliga, 2003). Thus, these preclinical results provide the specific behavioral evidence that the neurosteroid ALLO contributes to the neuroleptic-like effect of olanzapine that may be clinically relevant. It is moreover possible that ALLO may play a role in mediating other therapeutic actions of olanzapine and its consequent reducing of symptoms. In addition to ALLO, olanzapine may also affect other neurosteroids and this possibility will require further investigations. Thus, the involvement of ALLO represents an important physiological modulator that contributes to the sensitivity of $GABA_A$ receptors to GABA (Guidotti *et al*, 2001). In turn, the latter will affect dopaminergic transmission (Khisti *et al*, 2002) and may play a key role in the control of psychiatric behavior by olanzapine. These findings further indicate that development of drugs targeted at neuroactive steroid mechanisms might provide a new direction in therapeutic intervention for psychiatric disorders.

ACKNOWLEDGEMENTS

This study was supported by the senior research fellowship grant from Council of Scientific and Industrial Research, New Delhi to KH (CSIR # 9/128(68)/2K2/EMR-I) and emeritus grant of All India Council of Technical Education, New Delhi to CTC (AICTE # 1-51/FD/EF (10)/2002-2003/I). We gratefully acknowledge the support from Regione Autonoma della Sardegna L.R 11/04/1996 n.19 and thank Dr Anne Farmer and Dr NK Subhedar for editorial assistance.

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