Anxiolytic-like effects of YL-IPA08, a potent ligand for the translocator protein (18 kDa) in animal models of post-traumatic stress disorder

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Abstract

Recently, the translocator protein (18 kDa) (TSPO), previously called peripheral benzodiazepine receptor (PBR) and both the starting point and an important rate-limiting step in neurosteroidogenesis, has received increased attention in the pathophysiology of post-traumatic stress disorder (PTSD) because it affects the production of neurosteroids, reinforcing the hypothesis that selective TSPO ligands could potentially be used as anti-PTSD drugs. As expected, we showed that chronic treatment with YL-IPA08 [N-ethyl-N-(2-pyridinylmethyl)-2-(3,4-ichlorophenyl)-7-methylimidazo [1,2-a] pyridine-3-acetamide hydrochloride], a potent and selective TSPO ligand synthesized by our institute, caused significant suppression of enhanced anxiety and contextual fear induced in the inescapable electric foot-shock-induced mouse model of PTSD and the time-dependent sensitization (TDS) procedure. These effects were completely blocked by the TSPO antagonist PK11195. Furthermore, YL-IPA08 could increase the level of allopregnanolone in the prefrontal cortex and serum of post-TDS rats, and these effects were antagonized by PK11195. In summary, the findings from the current study showed that YL-IPA08, a potent and selective TSPO ligand, had a clear anti-PTSD-like effect, which might be partially mediated by binding to TSPO and the subsequent synthesis of allopregnanolone.

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Introduction

Post-traumatic stress disorder (PTSD) is a debilitating anxiety disorder characterized by intrusive re-experiences of traumatic events, avoidance of situations and stimuli that could serve as reminders of these events, and feeling jumpy or easily startled. The selective serotonin reuptake inhibitors (SSRIs) sertraline and paroxetine are the only medications currently approved by the Federal Drug Administration for the treatment of PTSD, but their effects are modest, and in some PTSD populations, including American male combat veterans, they were found to be ineffective. In addition, there are adverse effects (include cognitive dysfunction, weight gain, sexual dysfunction, sedation, dependence and withdrawal) that should be considered (Haddad, 1998; Nelson and Philbrick, 2012; Sheeler et al., 2012). Although benzodiazepines (BZDs) (e.g. diazepam) have proved to be effective in anxiety disorders (Baldwin and Nair, 2005) and have been widely prescribed after trauma, there is no consistent evidence that they can stop the development of PTSD or are at all effective in the treatment of PTSD (Gelpin et al., 1996; Viola et al., 1997; Davidson, 2004). Other drawbacks of BZDs include the risk of developing a dependence and withdrawal syndrome (Pinna et al., 1997). Accordingly, considerable effort has been invested in the search for better drugs for more effective treatment of PTSD.

During the last decade, the down-regulation of neurosteroid biosynthesis has been implicated as a possible contributor to the aetiology of PTSD (Pinna et al., 2006a; Rasmussen et al., 2006; Brinton, 2013). King A–reduced neurosteroids are endogenous metabolites of the hormone progesterone and potent positive allosteric modulators of γ-aminobutyric acid type A (GABAA) receptors, which mediate the effects of the inhibitory neurotransmitter GABA in the mammalian nervous system (Belelli and Lambert, 2005). Allopregnanolone (Allo), a progesterone metabolite, is a potent positive...
allosteric modulator of the action of GABA at GABAA receptors. In clinical studies, decreases of Allo in the serum, plasma, and cerebrospinal fluid (CSF) content are associated with several psychiatric disorders, such as depression, anxiety, PTSD, schizophrenia and impulsive aggression (Uzunova et al., 1998; Rasmusson et al., 2006). Preclinical studies using socially isolated mice as an animal model of PTSD have also demonstrated that corticolimbic Allo levels become markedly decreased in association with the development of anxiety-like behaviours, resistance to sedation and extreme aggression (Pibiri et al., 2008; Nin et al., 2011; Pinna and Rasmusson, 2012). It is important to point out that SSRIs such as fluoxetine or norfluoxetine were able to reverse the decrease of brain neurosteroid levels (such as Allo) and to correct behavioural deficits expressed by socially isolated mice even at dosages that are 50-fold lower than those required to cause an effective 5-HT reuptake inhibition (Pinna et al., 2003, 2004a, 2009; Pinna, 2010). On the basis of these considerations these drugs, originally termed ‘SSRI’ antidepressants, were renamed the more appropriate term ‘selective brain steroidogenic stimulants (SBSSs)’, which more accurately defines the pharmacological mechanisms (Pinna et al., 2006a, 2009; Pinna and Rasmusson, 2012). Taken together, these data suggest that a deficit of GABAergic neurotransmission, likely caused by the down-regulation of brain neurosteroid biosynthesis, must be among the molecular mechanisms considered in the aetiology of PTSD. Thus, the novel SBSSs, devoid of SSRI activity, but potent neurosteroidogenic agents, should be developed for the treatment of psychiatric disorders that result from the down-regulation of neurosteroid expression, including major depression, anxiety and in the prevention of PTSD.

Recently, the role of the 18 kDa translocator protein (TSPO), both the starting point and an important rate-limiting step in neurosteroidogenesis, has received increased attention in the pathophysiology of the stress-response and stress-related disorders (Beurdeley-Thomas et al., 2000; Pinna et al., 2006a; Pinna and Rasmusson, 2012). In the central nervous system, TSPO is mainly located in the glial cells and mediates the translocation of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in the synthesis of steroids and neurosteroids. TSPO density has been found to be altered in anxiety disorders such as panic disorder (Papadopoulos et al., 2006a, b) as well as in stress responses (droogleever Fortuy et al., 2004; Veenman and Gavish, 2006). Studies of PTSD that have investigated platelet TSPO levels in veteran populations during or after chronic war stress have reported lower TSPO levels in these populations (Gavish et al., 1996; Veenman and Gavish, 2006; Dell’Osso et al., 2010). We have recently found that AC-5216, a selective TSPO ligand that has entered phase II clinical research, exerted an anti-PTSD-like effect (Qiu et al., 2013). Collectively, the evidence presented above supports the hypothesis that TSPO may be involved in the pathophysiology of PTSD and that ligands of TSPO are promising candidates for the treatment of PTSD in the future.

YL-IPA08 [N-ethyl-N-(2-pyridinylmethyl)-2-(3,4-ichlorophenyl)-7-methylimidazol [1,2-a] pyridine-3-acetamide hydrochloride, Fig. 1], a new TSPO ligand, was designed and synthesized by our institute. Our previous studies found that YL-IPA08 showed a relatively higher affinity for TSPO (IC$_{50}$=0.23 nm) and appears to be more potent than AC-5216 (IC$_{50}$=0.65 nm), with more negligible binding to central benzodiazepine receptors compared to AC-5216 (706.18 vs. 67.4 nm), indicating that YL-IPA08 may be a potent and selective TSPO ligand. We also found that YL-IPA08 showed notable antidepressant and anxiolytic-like effects in several animal models but does not cause the side effects that are typically associated with conventional BZDs, such as myorelaxant effects, memory impairment or the potential of hexobarbitone-induced sleep (Zhang et al., 2013).

Given all the factors mentioned above, it is reasonable to hypothesize that YL-IPA08 may be effective in the treatment of PTSD. Thus, we first assessed the effects of YL-IPA08 in alleviating the enhanced anxiety and fear response induced in the inescapable electric foot-shock-induced mouse model of PTSD and the time-dependent sensitization (TDS) procedure, a rat PTSD animal model. In an effort to explore the role of TSPO in mediating the anti-PTSD effect of YL-IPA08, we then tested whether blocking the TSPO ligand with a TSPO antagonist, PK11195; either alone or in combination with YL-IPA08 treatment, affected the behavioural effect of YL-IPA08 in post-TDS rats. Furthermore, the changes of Allo in the serum and prefrontal cortex after chronic YL-IPA08 administration were also assessed in TDS-treated rats.

**Method**

**Animals**

Both male ICR mice (18±2 g, used in the electric foot-shock procedures) and male Sprague–Dawley rats (180±10 g, used for TDS experiments) were purchased from...
the Beijing SPF Laboratory Animal Technology Company (China). The animals were maintained under non-reversed 12 h light/12 h dark cycle conditions (lights on from 07:00 to 19:00) at a constant room temperature (23±1 °C) and relative humidity (45%), with food and water freely available. The experiments were conducted according to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). The institutional committee on animal care and use approved the experimental procedures, and all efforts were made to minimize animal suffering and to reduce the number of animals used for the experiments.

**Drugs and treatments**

Sertraline (Ser) and PK11195 were purchased from Sigma (USA). YL-IPA08 (Purity ≥ 99%) was synthesized by the Department of Medicinal Chemistry at our institute. Ser (15 mg/kg) or YL-IPA08 was dissolved in saline and administered by intragastric gavage (i.g.) in a volume of 20 ml/kg (mice) or 2 ml/kg (rats). PK11195 (1, 3 mg/kg) was suspended in a saline solution containing 2% DMSO and 0.8% Tween 80 and was injected i.p. in a volume of 2 ml/kg. Behavioural tests were performed 1 h after the Ser or YL-IPA08 administration.

**Behavioural experiments**

**Long-term behavioural effects of YL-IPA08 after electric foot-shock procedures in mice**

The experimental procedure was conducted as described previously (Zhang et al., 2012; Qiu et al., 2013). For the training session, a Plexiglas chamber (20×10×10 cm) with a stainless steel grid floor (9 mm interval) was used. Electric foot-shocks were delivered through the grid floor by an isolated shock generator (Med Associates Inc., USA). Each mouse was placed in the chamber, and after a 5-min adaptation period, a total of 15 intermittent inescapable foot-shocks (intensity: 0.8 mA, interval: 10 s, and duration: 10 s) were delivered for 5 min. Control animals were placed in the same chamber for 10 min without electric foot-shocks. From the first day after the foot-shock procedure, either Ser (15 mg/kg) or YL-IPA08 (0.1, 0.3, and 1 mg/kg i.g., respectively) was administered once a day between 8:00–9:00. The drug doses and the administration time were selected according to our previous studies. Each mouse was successively tested for contextual freezing measurement, locomotor activity test and staircase test paradigm. The outline of the design of the treatment schedule and behavioural tests is shown in Fig. 2a.

**Contextual freezing measurement.** This test was based on studies that showed that the freezing response upon re-exposure to the shock context is a measure of the conditioned associative fear memory, reflecting the response to trauma-related cues as a symptom of PTSD (Maier, 1990; Siegmund and Wotjak, 2007). The test was performed as described previously, with minor modifications (Zhang et al., 2012). All animals were exposed to the reminder situation, i.e. the same chamber where the foot-shocks had been delivered, but with no further foot-shocks, for 5 min on day 15. The total cumulative freezing time was measured and analysed automatically by computer software (Med Associates Inc., Video Freeze SOF-843., USA).
Locomotor activity test. To evaluate whether the reversion of contextual freezing by YL-IPA08 is dependent on an effect on locomotor activity, we assessed the number of line crossings and rearing in mice. Mice were placed in the corner of a plastic box (36x29x23 cm) in which the base was divided into equal sectors for a 5-min acclimation period, and then the number of crossings (all four paws placed into a new square) and rearing (both front paws raised from the floor) were recorded over the next 5 min.

Staircase test. The staircase was made from polyvinylchloride and consisted of five identical steps (2.5 cm high, 10 cm wide and 7.5 cm deep). The heights of the walls were constant (12.5 cm above the stairs) along the entire length of the staircase. On day 18 after the foot-shock, each mouse was placed individually on the floor of the box, with its back to the staircase. During a 3-min period, the number of rearings and number of steps climbed were recorded. A step was considered climbed only if the mouse placed all four paws on the stair. The number of steps descended was not counted.

Long-term behavioural effects of YL-IPA08 after TDS in rats

The TDS procedure was performed as described previously. Briefly, after a 1-wk acclimatization period, each rat was immobilized inside a disposable clear polyethylene rodent restraint cone for 2 h (day one). The large end of the cone was closed with tape. The bag size was adjusted according to the size of the animal to achieve complete immobilization. A hole in the small end of the cone allowed the rats to breathe freely. After the restraint complete immobilization. A hole in the small end of the cone allowed the rats to breathe freely. After the restraint period, the rats were individually placed in a clear acrylic cylinder (24 cm diameter, 50 cm height) filled with water (24°C) to 2/3 of its height and forced to swim for 20 min. Following a 15-min recuperation period, the rats were then exposed to diethyl ether until loss of consciousness. The rats were allowed to recover for a week and were then subjected to a brief re-stress on day seven (20 min swim stress). The control rats were kept in a room adjacent to the TDS rats during the treatment and were handled twice for several minutes each time.

From the first day of the TDS procedure, YL-IPA08 (0.03, 0.1 and 0.3 mg/kg i.g., respectively) was administered once per day between 8:00–9:00. After establishing the effective dose (0.3 mg/kg) at which YL-IPA08 exhibits the maximum anti-PTSD effect in the TDS procedure, we further extended our study in another set of experiments to explore the anti-PTSD role of the TSPO ligand by the combined administration of YL-IPA08 (0.3 mg/kg) and PK11195 (1, 3 mg/kg, i.p.). PK11195 was administered 30 min before testing. In addition, the doses of PK11195 were determined by preliminary studies in which this compound had no effect on the behavioural changes related to PTSD when given alone at the indicated doses (Kita et al., 2004; Kita and Furukawa, 2008). To minimize effects of multiple testing, tests were ordered from least to most stressful (Fig. 3a). The originality of our protocol lies in the fact that the same cohort of animals was tested using the different behavioural paradigms according to the procedure described previously (Zhang et al., 2012).

Locomotor activity test. Locomotor activity was assessed using the open field test thirteen days after TDS. Rats were placed in the corner of a plastic box (76x76x46 cm) in which the base was divided into equal sectors for a 5-min acclimation period. Subsequently, the number of crossings and rearing were recorded for 5 min (Zhang et al., 2012).

Contextual fear paradigm. The contextual fear paradigm was conducted after the end of the fourteen-day drug treatments. Each rat was exposed to the conditioning context (180 s in the conditioning chamber [60x21x30 cm] without any stimulation). Immediately after exposure, a foot-shock (0.8 mA, 4 s) through a stainless steel grid floor (Med Associates Inc. USA) was administered. Twenty-four hours after the initial foot-shock (day 15 after TDS), the rat was placed in the conditioning chamber where it had previously been foot-shocked, and the contextual fear response was then evaluated by measuring the duration of freezing behaviour over a 5-min interval.

Elevated plus maze test. The elevated plus maze (EPM) test is one of the most widely used assessments for evaluating PTSD-associated anxiogenic-like behaviour in rodents (Li et al., 2009; Zhang et al., 2012). The apparatus consisted of four branching arms (60×12 cm) with two open arms and two closed arms with dark walls (14 cm high). The arms were connected by a centre platform (10x10 cm), and the maze was 50 cm above the ground. Eighteen days after TDS, an individual rat was placed in the central platform facing the closed arms. For analysis purposes, open-arm activity was quantified as the time spent on the open arms relative to the total time spent in both arms (open/total×100) and the number of entries into open arms relative to the total number of entries into any arm (open/total×100). Rats were scored as entering an open or closed arm only when all four paws passed over the dividing line. The maze was cleaned with a 5% ethanol/water solution after each test to remove any confounding olfactory cues and dried thoroughly between sessions.

Effects of YL-IPA08 on the Allo level in post-TDS rats

The rats used in the TDS test were sacrificed by decapitation, and their brains were removed, rinsed of blood and carefully dissected to remove the prefrontal cortex, which is important in behavioural regulation. The brain regions were extracted in 1 ml extraction buffer/100 mg tissue and then homogenized in ice-cold lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1% NP40,
Fig. 3. Treatment schedules and order of behavioural tests for the PTSD model of TDS (a). The post-TDS rats showed a significant increase in contextual freezing and a decreased percentage of both time spent in and entries into open arms in the elevated plus maze test. Repeated administration of YL-IPA08 ameliorated these behavioural deficits (b, d); these behavioural effects of YL-IPA08 treatment could be antagonized by PK11195 treatment (c, e). Daily administrations of either Ser or YL-IPA08 were begun on the first day after the TDS procedure. PK11195 was administered 30 min before testing. Data are presented as mean±S.E.M (n=10). **p<0.01, ***p<0.001, compared with TDS (−) group; #p<0.05, ##p<0.01, ###p<0.001 compared with the saline-treated TDS (+) group; and &p<0.05 vs. YL-IPA08 (0.3 mg/kg)-treated group.
10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml leupeptin and 0.5 mM sodium vanadate. The tissue homogenate solutions were centrifuged at 14000 g for 25 min at 4 °C, and then the supernatants were collected. For serum Allo measurement, blood samples were centrifuged (860 g, 20 min) at 4 °C, and the supernatants were collected. All samples were maintained at −80 °C until further use. The Allo levels of the samples were detected by ELISA kits according to the manufacturer’s protocol (RayBiotech, USA), and the optical density values were read at 450 nm in the ELISA plate reader.

Statistical analysis

All data were expressed as the means ± S.E.M. Student’s t-test compared the difference between two groups (i.e. control non-shocked mice vs. shocked mice, shocked mice vs. shocked + Ser treatment mice; non-TDS rats vs. post-TDS rats, post-TDS rats vs. TDS + Ser treatment rats), with Bonferroni corrections performed where necessary to control for Type I error. Long-term behavioural effects of YL-IPA08 after electric foot-shocks in mice or TDS in rats were analysed by one-way analysis of variance (ANOVA), and a two-way ANOVA was used to examine the antagonism effects of PK11195, and both were followed by Dunnett’s test. For all of the tests, differences with p < 0.05 were considered to be significant.

Results

Long-term behavioural effects of YL-IPA08 after electric foot-shocks in mice

There was no significant effect on the number of line crossings and rearings between the control non-shocked mice and the shocked mice. Daily oral administration of either Ser (15 mg/kg) or YL-IPA08 also did not significantly affect the number of line crossings and rearings. These results indicate that neither foot-shocks nor repeated YL-IPA08 treatment affected locomotor activity in this animal model (figures are not shown).

Electric foot-shocks caused a significant increase in the contextual freezing response (Student’s t-test) compared to the non-shocked mice, indicating that the electric foot-shock model was successfully developed. Ser (15 mg/kg) administration also significantly reduced the freezing behaviour (Student’s t-test). However, since multiple tests were undertaken in this analysis, we used the Bonferroni correction to identify these effects. After this correction, the significant difference remained, which demonstrates the predictive validity of this model. Chronic treatment with YL-IPA08 showed main effects on the contextual freezing response in mice exposed to foot-shocks (one-way ANOVA, F[3,36]=3.166). Further post-hoc analysis revealed that YL-IPA08 (0.1 and 0.3 mg/kg) decreased the contextual freezing response (Dunnett’s test, compared with foot-shock vehicle group) on day 15 (Fig. 2b). These results demonstrate a persistent fear response in mice to the context associated with the traumatic events and indicate that repeated treatment with YL-IPA08 alleviated the contextual freezing behaviour in stressed mice.

In the staircase test, mice that had been previously exposed to foot-shocks exhibited an increased number of rearings but failed to demonstrate a significant change in the number of steps (Student’s t-test). These results indicate that the animals still avoided the aversive-like compartment and exhibited a fear response to the context associated with the traumatic events. We also found that repeated administrations of either YL-IPA08 (0.3 and 1 mg/kg, one-way ANOVA, F[3,36]=2.873) or Ser (15 mg/kg, Student’s t-test) decreased the number of rearings without affecting the climbing behaviour in the staircase test (Fig. 2c), suggesting that YL-IPA08 significantly ameliorated PTSD-associated anxiogenic-like behaviours.

Long-term behavioural effects of YL-IPA08 after exposure to TDS

To examine the possibility that TDS and/or the drug treatments influenced the baseline locomotor activity, we investigated the level of spontaneous locomotor activity for each group. The results showed that there was no significant difference between the control non-TDS rats and the post-TDS rats. As positive controls, Ser also did not significantly affect the number of line crossings and rearings. And after Bonferroni correction, the significant difference also remained.

Daily oral administration of YL-IPA08 did not show an evident effect on the number of line crossings and rearings. Two-way ANOVA analyses showed that the administration of YL-IPA08 either alone or in combination with PK11195, a TSPO antagonist, did not affect the locomotor activity (figures are not shown).

Compared to vehicle treatment, exposure to TDS significantly increased the contextual freezing response (Student’s t-test). A 15-day chronic co-administration with Ser alleviated the enhanced contextual freezing in rats exposed to TDS (Student’s t-test). And after Bonferroni correction, the significant difference also remained. A similar effect was also observed with repeated YL-IPA08 (0.1 and 0.3 mg/kg) treatment (one-way ANOVA, F[3,36]=8.002) (Fig. 3c).

Two-way ANOVA analyses showed significant effects of PK11195 alone [F(2,53)=7.108], YL-IPA08 alone [F(1,53)=13.971] and PK11195 and YL-IPA08 interaction [F(2,53)=6.949]. As shown in Fig. 3d, treatment with PK11195 alone (1, 3 mg/kg) had no effect on the contextual freezing time of post-TDS rats. YL-IPA08 (0.3 mg/kg) treatment significantly decreased the contextual freezing time (vs. vehicle group), but pre-treatment with PK11195 (1 mg/kg) did not counteract the effect of YL-IPA08 (0.3 mg/kg) (vs. YL-IPA08 group), although a
higher dose of PK11195 (3 mg/kg) could reverse the effects of YL-IPA08 treatment vs. YL-IPA08 group).

As shown in Fig. 3e, Student’s t-test revealed that TDS-exposed animals showed significant reductions in the percentage of time spent in open arms and in the number of entries into open arms in the EPM test (Student’s t-test). One-way ANOVA analyses revealed that chronic co-administration with YL-IPA08 (0.1 and 0.3 mg/kg) significantly increased the percentage of time spent in (F[3,36]=2.773) and the number of entries into open arms (F[3,36]=3.606), as did repeated administration of Ser.

In addition, a two-way ANOVA revealed significant differences between PK11195 alone [F(2,53)=2.820], YL-IPA08 alone [F(1,53)=3.134] and PK11195 and YL-IPA08 interaction [F(2,53)=3.159] on the percentage of time spent in open arms (F[3,36]=3.606), as did repeated administration of Ser.

In addition, a two-way ANOVA revealed significant differences between PK11195 alone [F(2,53)=4.042, p<0.05], YL-IPA08 alone [F(1,53)=3.417] and PK11195 and YL-IPA08 interaction [F(2,53)=2.917] on the percentage of entries into the open arms in the EPM test. Post-hoc comparisons further showed that the effect of YL-IPA08 treatment (0.3 mg/kg, i.p.), which by itself had no effect on the percentage of either time spent in or entries into the open arms (Fig. 3f). These results indicate that YL-IPA08 ameliorated the PTSD-associated anxiogenic-like behaviour induced by TDS exposure, but these effects could be blocked by PK11195, suggesting that these effects might be mediated by TSPO activation.

Effects of YL-IPA08 on the Allo levels in post-TDS rats

The levels of Allo in the serum and prefrontal cortex of post-TDS rats were measured at the end of the EPM test. As shown in Fig. 4a, statistical analysis revealed that the TDS procedure significantly reduced the Allo level in both the serum and in the prefrontal cortex (Student’s t-test), which was clearly reversed by the chronic administration of YL-IPA08 at 0.3 mg/kg (one-way ANOVA, serum: F[3,36]=2.5937; prefrontal cortex: F[3,36]=3.710). In addition, we did not find an increase in the Allo level after chronic Ser administration in either serum or the prefrontal cortex (Student’s t-test).

In addition, a two-way ANOVA revealed significant differences between PK11195 alone [F(2,53)=10.15], YL-IPA08 alone [F(1,53)=3.417] and PK11195 and
YL-IPA08 interaction \( F(2,53)=10.15 \) on the level of the Allo in the prefrontal cortex. Significant effects were also observed between PK11195 alone \( F(2,53)=4.042 \), YL-IPA08 alone \( F(1,53)=3.417 \) and PK11195 and YL-IPA08 interaction \( F(2,53)=2.917 \) on the level of the Allo in the serum. Post-hoc comparisons further showed that both the increased Allo in the prefrontal cortex and serum induced by YL-IPA08 (0.3 mg/kg, i.g.) were antagonized by PK11195 (3 mg/kg, i.p.) at a dose that did not alter the Allo level (Fig. 4b).

Discussion

In the present study, we showed that chronic treatment with YL-IPA08 caused significant suppression of the enhanced anxiety and contextual fear induced in the inescapable electric foot-shock-induced mouse model of PTSD and the TDS procedure in rats; these effects were completely blocked by the TSPO antagonist PK11195. Furthermore, YL-IPA08 treatment increased the levels of Allo in the prefrontal cortex and serum of post-TDS rats and these effects were antagonized by PK11195.

Previous studies have demonstrated that mice exposed to repeated situational reminders followed by electric foot-shocks acquired conditioned fear, and these mice showed an innate aversive freezing behaviour (Zhang et al., 2012). Our previous study found that the selective TSPO ligand AC-5216 reversed the PTSD-associated behaviour deficits in this model (Qiu et al., 2013). In the present study, repeated YL-IPA08 treatments significantly increased the time spent in the aversive-like context, indicating that YL-IPA08 alleviated the fear of the stressed animals to the context associated with the traumatic event. Moreover, the aversive procedure did not affect the animals’ spontaneous locomotor activity or the number of climbed steps in the staircase test. These results are in accordance with the studies of Pynoos et al. (1996), who showed that foot-shocks associated with situational reminders did not affect the motor activity of male mice in an open field test performed 3–6 weeks after the first foot-shock (Pynoos et al., 1996). The present study indicates that aversive foot-shocks followed by repeated reminders are a reliable long-lasting animal model for PTSD and that YL-IPA08 treatment showed a therapeutic effect in this animal model within a certain dosage range.

Accumulating evidence demonstrates that the TDS model is a proven PTSD animal model that utilizes intense stressors, aversive challenges and situational reminders of a traumatic event in an attempt to model long-term effects on behavioural, autonomic and hormonal responses observed in humans with PTSD (Uys et al., 2003; Khan and Liberzon, 2004). The data presented here demonstrate that contextual freezing was significantly enhanced in rats exposed to TDS but that chronic administration of YL-IPA08 successfully reversed these adverse effects. EPM, a model using the natural fear of rodents to avoid open and elevated places, has been well validated in detecting responses to external stressful stimuli. The present study also shows that TDS exposure produces representative anxiety-like behaviour, as evidenced by the fact that TDS-exposed animals significantly decreased the percentage of time spent in and the number of entries into the open arms, and YL-IPA08 treatment reversed these behavioural changes and alleviated anxiety in rats after TDS exposure. We also found that neither the TDS procedure nor the drug treatments significantly influenced the locomotor activity in rats, suggesting that the behavioural changes observed in this study were not due to the changes in the basal locomotor activity.

It should be stated that, in our present study, we did not find dose-dependent effect of YL-IPA08. But our laboratory also found that AC-5216 exerted an anti-PTSD-like effect in a similar dose-response manner (Qiu et al., 2013). Interestingly, YL-IPA08 exhibited a similar dose-response curve antidepressant effect of YL-IPA08 in tail-suspension tests (Zhang et al., 2013). One possibility is that there might be a critical range of TSPO activity in modulating changes in PTSD. In addition, the sensitivity of the animal model should also be considered.

In an effort to better understand the anti-PTSD mechanisms of YL-IPA08, we then tested whether blocking the TSPO ligand affected the behavioural effects of YL-IPA08 treatment in the TDS procedure. The current findings show that PK11195 administration could reverse all of the behavioural effects induced by YL-IPA08 treatment in this animal model. These results are consistent with our previous study in which the antidepressant-like and anxiolytic-like effects of YL-IPA08 were completely blocked by PK11195 (Zhang et al., 2013). Combining our previous and present studies, these findings indicate that the anti-PTSD effects of YL-IPA08 treatment might be mediated by TSPO activation.

Allo is the most abundant brain neurosteroid acting at GABA receptors and is the most potent and selective positive endogenous modulator of the action of GABAA at brain GABA receptors (Puia et al., 1990; Lambert et al., 1995, 2003). The physiological relevance of endogenous Allo not only potentiates the inhibitory signals resulting from the release of GABA acting on GABA receptors, but it also plays a facilitatory permissive role in fine-tuning the efficacy of direct receptor activators such as muscimol or that of other positive allosteric modulators of GABA action at GABA receptors (Pinna et al., 2000; Guidotti et al., 2001; Belelli and Lambert, 2005). The down-regulation of Allo in the CNS and the peripheral nervous system was associated with the symptoms of PTSD (Vaiva et al., 2004; Rasmussen et al., 2006). Clinical studies demonstrated that the CSF Allo levels in pre-menopausal women with PTSD were 40% of those observed in healthy comparison subjects and were inversely correlated with PTSD re-experiences and comorbid depressive symptoms (Uzunova et al., 1998). In fact, the CSF Allo levels were the lowest in those patients with PTSD and comorbid depression. In addition, the ratio of
Allo to its steroid precursor, 5α-dihydroprogesterone, was decreased among the PTSD patients, suggesting a block in Allo synthesis (Rasmussen et al., 2006; Pinna and Rasmusson, 2012). Several experiments have demonstrated that corticolumbic Allo plays a pivotal rather than incidental role in the regulation of contextual fear responses and aggression. Taken together, these data suggest that a deficit of GABAergic neurotransmission that is likely caused by the downregulation of brain Allo biosynthesis must be among the molecular mechanisms considered in the etiology of PTSD.

Drugs like SBSSs (such as S-norfloxetine), which rapidly increase corticolumbic Allo levels, but fail to inhibit serotonin reuptake, normalize the exaggerated contextual fear responses resulting from social isolation. Interestingly, the socially isolated mouse fails to respond to the anxiolytic action of BZDs (diazepam and zolpidem) that fail to modulate GABAA receptors containing α4 and α6 subunits (Pinna et al., 2000, 2006b; Nin et al., 2011). Taken together, these data suggest that TSPO ligands that stimulate Allo biosynthesis might have an advantage over BZDs in the treatment of psychiatric disorders in which neurosteroid down-regulation and changes in GABAA receptor expression are operative (Pinna and Rasmusson, 2012).

As discussed above, it is possible that the normalization of brain Allo levels may underlie the therapeutic effects of YL-IPA08 treatment in PTSD. Therefore, we studied the level of Allo in post-TDS rats after the administration of YL-IPA08 to substantiate this hypothesis. Our results showed decreased Allo levels in the prefrontal cortex and serum in post-TDS rats, which is consistent with preclinical studies (Pinna et al., 2003, 2006a; Nin et al., 2011). We also found that the lowered Allo levels were reversed by YL-IPA08 treatment in post-TDS rats, and that these effects were antagonized by PK11195, further suggesting that the anti-PTSD effects of YL-IPA08 were mediated by binding to TSPO and the subsequent synthesis of Allo.

It is important to note that several preclinical studies observed that SSRIs such as fluoxetine or norfluoxetine were able to reverse the decrease in brain neurosteroid levels (such as Allo) and correct the behavioural deficits expressed by socially isolated mice (Pinna and Rasmusson, 2012), even at dosages that are 50-fold lower than those required to cause an effective 5-HT reuptake inhibition (Pinna et al., 2003, 2004b, 2009; Pinna, 2010). Clinical studies also demonstrated that treatment with fluoxetine and fluvoxamine normalized the CSF Allo content of the depressed patients studied (Uzunova et al., 1998). Based on these reports, how chronic administration of Ser exerts its anti-PTSD effect may be due to increased Allo concentrations. It appears that the present data, which did not find an increase in the Allo levels after chronic Ser administration in post-TDS rats, are somewhat controversial. But it should be stated that, we took the samples at only one time point (24h after the last injection of the long-term treatment drugs). It has been reported that SSRIs (such as fluoxetine) are able to rapidly (within minutes) increase corticolumbic Allo levels. However, chronic administration of this drug did not inhibit the increases in the cortical and plasma concentrations of Allo induced by acute foot-shock stress (Serra et al., 2002), which is consistent with our findings. It is therefore possible that we might have missed the time of transitory Allo elevation.

Several lines of evidence suggest that the Allo levels in corticolumbic structures play a prominent role in the acquisition and expression of conditioned fear. In our present study, a transitory Allo elevation after Ser may be involved in the enhancement of the contextual fear responses and anxiety-like behaviours observed in these two PTSD animal models, and may have affected acquisition of fear and consolidation ahead of time of determining contextual fear conditioning. Therefore, we cannot discard the idea that a Ser stimulation of brain Allo biosynthesis might be operative in the anti-PTSD actions of this drug, although further experiments are needed to clarify the exact molecular mechanisms.

In summary, the findings from the current study show that YL-IPA08, a potent and selective TSPO ligand, has a clear anti-PTSD-like effect that might be partially mediated by binding to TSPO and the subsequent synthesis of Allo. The results of these investigations not only advance our knowledge of the theories of PTSD but also have clinical implications for the treatment of this mental disorder.

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Statement of Interest

None.

References


