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The effect of intracerebroventricular allopregnanolone on depressive-like behaviors of rats selectively bred for high and low immobility in the forced swim test

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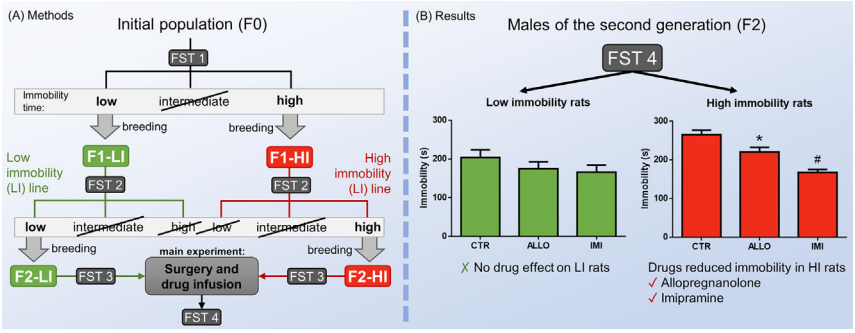
GRAPHICAL ABSTRACT



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ABSTRACT

Depression is a highly incapacitating disorder known to have a multifactorial etiology, including a hereditary genetic background. The neurosteroid allopregnanolone (ALLO) is a positive allosteric modulator of the $GABA_A$ receptor and has been shown to have an antidepressant-like effect in animals. This study aimed to assess the behavioral effect of ALLO in animals with different backgrounds of depressive-like activity. An initial population (F0) of male and female Wistar rats was screened for immobility behavior utilizing the Forced Swim Test (FST). Rats with extreme immobility scores were selected for either the High Immobility (HI) group or the Low Immobility (LI) group for breeding, giving origin to the subsequent generations F1 and F2. Guide cannulas were implanted in the lateral ventricle of F2 males for intracerebroventricular infusions of 5 μg/rat of ALLO, 5 μg/rat of imipramine (IMI) or vehicle (CTR), which occurred 24, 5 and 1 h prior to the test session of the drug FST. In the pre-drug FST, a statistically significant difference was observed between the immobility scores from the HI and LI groups of F2 rats. HI rats from F2 also showed significantly higher immobility time when compared to F0. In these HI animals, both IMI and ALLO significantly reduced immobility when compared to the CTR group. IMI-treated rats also showed lower immobility than the ALLO group. In the LI rats, no difference in immobility was found between treatments. In conclusion, two strains of rats with significantly different immobility profiles in

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the FST were obtained in a relatively short time, after only two generations. Infusions of both ALLO and IMI showed a strain-dependent antidepressant-like effect, being detected in the HI animals but not in the LI animals, which is in line with the clinical understanding that antidepressants have higher efficacy in more severe forms of depression.

1. Introduction

Depression is a very prevalent and incapacitating mental disorder that is estimated to affect over 300 million people worldwide, which corresponds to approximately 4.4% of the population [1]. A recent report from the World Health Organization has established depression as the leading cause of disability in the world [2]. Though diverse environmental aspects have been implicated in the causes of depression, its heredity is estimated to be around 40%, indicating the existence of an important genetic factor underlying its etiology [3]. This trait is also observed by studying close family members of depressed individuals, since first degree relatives of probands diagnosed with depression show an increased risk for affective disorders [4]. However, the specific genetic mechanisms of depression are yet to be discovered. Incomplete efficacy of available antidepressants may be due to variability in the neurobiology of depression [5], which is likely related to its genetic construct. Therefore, new antidepressant agents that act on novel neurotransmitter systems are still needed.

Allopregnanolone (3α-5α-tetrahydroprogesterone; 3α-5α-THP; ALLO) is a neurosteroid that has been widely implicated in the neurobiology of depression [6] due to its action as a potent allosteric modulator of the GABA type A receptor ($GABA_AR$) [7]. Allopregnanolone binds to the $GABA_AR$ in the first two transmembrane domains of the α1 subunit [8] and potentiates or directly activates the Cl^- channel, hyperpolarizing the post-synaptic GABAergic neurons [9]. The 5α-reductase inhibitor finasteride, which decreases the concentration of neurosteroids in the brain, including allopregnanolone, has been linked to depressive behavior in humans [10–12] and has been shown to induce depressive-like effects in the forced swim test in mice [13]. It has been reported that allopregnanolone levels are decreased in the cerebrospinal fluid and plasma of depressed individuals [14–16] and that successful treatment with antidepressants reverses its levels back to normal [14, 15].

Animal studies have corroborated this finding, since olfactory bulbectomized rats – a well-validated depression model – have decreased allopregnanolone levels when compared to sham-operated rats [17]. It has also been observed that overexpression of the 18 kDa translocator protein, which is crucial to neurosteroid synthesis, increases allopregnanolone levels and produces an antidepressant-like effect in mice [18]. The selective serotonin reuptake inhibitors (SSRIs) S-fluoxetine and S-norfluoxetine, when administered at doses lower than necessary for serotonin reuptake inhibition, normalize allopregnanolone levels and reverse depressive-like behaviors [19]. Furthermore, subchronic treatment with antidepressants increases allopregnanolone levels in rats and mice [20, 21].

Intracerebroventricular infusion of allopregnanolone in mice produces an antidepressant-like effect in the forced swim test [22]. In learned helplessness rats, hippocampal and amygdalar infusion of allopregnanolone also evoke an antidepressant-like effect [23]. When allopregnanolone is infused in the hippocampus [24] or in the nucleus accumbens [25, 26] of rats, it reduces the immobility time of rats in the forced swim test. This study aimed to generate two strains of rats selectively-bred for extreme high or low immobility times in the forced swim test and to verify the behavioral effects of allopregnanolone in each of these strains, in comparison to imipramine.

2. Materials and methods

2.1. Animals

Adult Wistar rats (40 males and 40 females in the initial population "F0") were obtained from the Animal House of Fundação Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). Following an initial behavioral screening of the F0 animals, those with high and low depressive-like behavior were selected and bred to produce the 60 males and 60 females of the first generation (F1). Upon reaching adulthood, these F1 animals also underwent selection and breeding to obtain the 60 males of the second generation (F2). Offsprings were limited to 10 pups per litter. All animals were housed in groups according to age. Adults were kept in groups of three in polypropylene cages (25 × 35 × 35 cm) with wood shavings as bedding. When surgery was performed, animals were subsequently maintained in isolated cages. Food and water were available *ad libitum*, and the animals were maintained in a temperature-controlled room (22 ± 2 °C) under a light–dark cycle (lights on from 5 AM to 5 PM). All *in vivo* experiments followed the guidelines of the International Council for Laboratory Animal Science and were approved by the Ethical Committee for the Use of Animals in Research of UFCSPA (process number 15–163). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Forced swim test

A slightly modified version of the Porsolt Forced Swim Test (FST) was used [27–29], in which the animals were individually introduced in opaque glass tanks (25 × 25 × 40 cm) filled with 28 cm of water at the temperature of 25 ± 1 °C. Two swim sessions were conducted: an initial 15-min training session followed 24 h later by a 5-min test session that was video recorded for behavioral analysis. Following both sessions, rats were removed from the tanks, carefully dried with towels, and warmed under a heated lamp for 10 min, being then returned to their home cages. Behaviors were analyzed using the Behavsoft™ software (patent pending) by a trained researcher that was blind to the different groups. Behavsoft™ was developed by our research group and allows viewing and analyzing the video recordings in a single interface using keyboard shortcut commands [30]. In addition to immobility time, detailed behaviors of mobility (climbing and swimming) and number of head shakes were also analyzed as in Ferigolo and colleagues (1998) [29]. Rats were submitted to non-drug FSTs at postnatal day (PND) 65 ± 5.

2.3. Behavioral selection and breeding

All animals of the first generation (F0) were submitted to the FST for screening of the immobility behavior. Rats that scored one standard deviation above or below the mean were selected for the High Immobility (HI; $n = 8$ males and 8 females) group or the Low Immobility (LI; n = 8 males and 8 females) group, respectively. Males and females from the HI or the LI selection were paired for breeding inside their groups and the subsequent generation (F1) was submitted to the FST screening process when adulthood was reached. Rats with either extremely high immobility (10 males and 10 females) or extremely low immobility (10 males and 10 females) were selected according to their strain (HI or LI) and bred inside their groups to give origin to the second and final generation (F2). Adult F2 animals were