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The effect of intra-nucleus accumbens administration of allopregnanolone on δ and γ2 $GABA_A$ receptor subunit mRNA expression in the hippocampus and on depressive-like and grooming behaviors in rats

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ABSTRACT

Alterations in $GABA_A$ receptor expression have been associated with the allopregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α,5α-THP) antidepressant-like effect in rats. The present study aimed to verify the effect of bilateral, intra-nucleus accumbens core (intra-AcbC) administration of the neurosteroid allopregnanolone on behaviors in the forced swim and grooming microstructure tests and in the δ and γ2 $GABA_A$ receptor subunit mRNA expression in right and left hippocampus of rats. The results of this study showed that bilateral, intra-AcbC allopregnanolone administration (5 μg/rat) presented antidepressant-like activity in the forced swim test concomitant with an increase in climbing. Allopregnanolone at doses of 1.25 and 5 μg/rat also decreased the percentage of correct transitions in the grooming microstructure test. Both δ and γ2 $GABA_A$ subunit expressions increased in the rat hippocampus after allopregnanolone intra-AcbC treatment. Our findings point to asymmetrical $GABA_A$ receptor expression changes in the hippocampus of animals treated with allopregnanolone. Further investigation should evaluate the antidepressant-like effect of allopregnanolone not only in other directly infused regions but also with respect to changes in other brain areas of the limbic system to understand allopregnanolone's mechanism of action.

1. Introduction

Neuroactive steroids are important endogenous modulators of depressive and anxiety-related behaviors (Schüle et al., 2011). Indeed, allopregnanolone and other neurosteroids, such as dehidroepiandrosterone, pregnenolone (Schüle et al., 2011) and pregnenolone-derivatives (Bianchi and Baulieu, 2012) have been implicated with antidepressant-like effect in rodents. Allopregnanolone (3α-5α-tetrahydroprogesterone; 3α-5α-THP; ALLO), a positive modulator of the $GABA_A$ receptor (Paul and Purdy, 1992), is decreased in the cerebrospinal fluid (Uzunova et al., 1998) and plasma (Romeo et al., 1998; Strohle et al., 1999) of depressive patients. Furthermore, clinically effective treatment with antidepressants is correlated with increased levels of allopregnanolone in the plasma of depressive individuals (Romeo et al., 1998; Strohle et al., 1999).

Likewise, preclinical studies show that allopregnanolone is decreased in specific brain areas of the olfactory bulbectomized rat model of depression compared with sham-operated rats (Uzunova et al., 2003). Moreover, there is an increase in allopregnanolone levels in mouse and rat brain after subchronic antidepressant treatment (Griffin and Mellon, 1999; Nechmad et al., 2003). The intracerebroventricular administration of allopregnanolone shows an antidepressant-like effect in the forced swim test (Khisti et al., 2000) and learned helplessness animal models of depression (Shirayama et al., 2011). The idea that allopregnanolone is one of the most important neurosteroids involved in the antidepressant effect of drugs has been confirmed by the administration of finasteride. This 5α-reductase inhibitor, decreases the conversion of progesterone to 5α-dehydroprogesterone and subsequently to allopregnanolone, and increases immobility behaviors in the forced swim test (Beckley and Finn, 2007).

Limbic brain regions are implicated in mood disorders and studies point to the nucleus accumbens as an important area for emotional control (Sheline, 2003). The nucleus accumbens has extensive input from and output to other limbic areas, such as the hippocampus (Goto and O'Donnell, 2001; Nauta et al., 1978). Because there is an inverse correlation between hippocampal allopregnanolone levels and depressive-like behavior in female rats it has been supposed that the hippocampus is implicated in the antidepressant effect of this neurosteroid (Frye and Walf, 2002, 2004). Indeed, intra-hippocampal infusion of allopregnanolone

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reduces the immobility of rats in the forced swim test (Nin et al., 2008). Furthermore, when administered into the nucleus accumbens, allopregnanolone also reduces immobility duration in rats (Molina-Hernandèz et al., 2005), leading to the conclusion that there might be more than one brain areas related to the antidepressant effects of neurosteroids or that the allopregnanolone infusion in a certain limbic area may evoke GABAergic system alteration in the hippocampus.

$GABA_A$ receptor function is modulated by GABA and also by benzodiazepines, barbiturates, convulsants and neurosteroids, including allopregnanolone (Compagnone and Mellon, 2000). After intrahippocampal allopregnanolone administration, mRNA expression of the γ2 $GABA_A$ receptor subunit increases in the hippocampus of rats during the forced swim test (Nin et al., 2008), and expression of the δ subunit increases in the CA1, CA3 and dentate gyrus regions of the hippocampus after social isolation in mice (Serra et al., 2006). The socially isolated mouse model evokes behavioral deficits that are comparable to those of depression (Pinna et al., 2003, 2004). These deficits are associated with decreases in allopregnanolone levels in several brain areas (Pinna et al., 2003, 2006) and can be normalized with antidepressant treatment (reviewed in Pinna, 2010). Different stress models in rodents increase not only corticosterone, but also depressive-like behaviors in the forced swim test that are reversed by antidepressant treatment (Rayen et al., 2011; Viana et al., 2008; Weathington et al., 2011). Grooming is an important part of rodent behavior that can be induced by exposure to a new environment. This behavior represents a complex sequence of patterns sensitive to GABAergic drugs (Barros et al., 1992; Nin et al., 2012), and grooming is a predictor of stress-like behavior (Kalueff and Tuohimaa, 2005).

The regulation of negative emotions in normal humans depends on hemispheric asymmetries: left frontal activation is related to the voluntary suppression of negative emotions, and the right frontal area is connected to spontaneous negative emotional responses (Jackson et al., 2000). Several changes in brain asymmetry for certain brain areas have been detected with neuroimaging in mood disorder patients (Soares and Mann, 1997), including patients with major depressive disorders (Lacerda et al., 2003). Patients with bipolar disorder and depression show motor, perceptual and emotional functional disturbances associated with interhemispheric asymmetries in many brain regions, reinforcing the hypothesis that an asymmetric component may be critical for mood regulation (Caligiuri et al., 2004; Jackson et al., 2003; Tranel et al., 2002). There are hemispheric asymmetries in biochemical markers in animals subjected to unpredictable tones and shocks (Orman and Stewart, 2007), which may trigger coping problems in these animals that are associated with stressful stimuli (Sullivan, 2004). In a study from our laboratory, in rats, we observed that following allopregnanolone treatment, antidepressant-like effects and higher mRNA expression of the γ2 $GABA_A$ subunit the right hippocampus compared to the left hippocampus were detected (Nin et al., 2008).

Our present study was designed to determine the antidepressant and antistress-like effects of intra-nucleus accumbens infusion of allopregnanolone in rats and to quantify the hippocampal δ and γ2 $GABA_A$ receptor subunits expression in these animals, correlating the behaviors observed with the biochemical data.

2. Methods and materials

2.1. Animals

Male Wistar rats (250–280 g; 90–110 days; $n=42$) were obtained from the Animal House of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). Before surgery, the animals were housed in groups of five in polypropylene cages with wood shavings as bedding. After surgery, the animals were maintained in isolated cages ($25\times35\times35$ cm in height). 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 7 am–7 pm). All *in vivo* experiments followed the guidelines of the International Council for Laboratory Animal Science and were approved by the Ethical Committee for Research of UFCSPA (557/08). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Treatments

Immediately before administration, allopregnanolone (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in a 20% (w/v) 2-hydroxypropyl-β-cyclodextrin (Fluka-Sigma-Aldrich, St. Louis, MO, USA) solution prepared in artificial cerebrospinal fluid (ACSF) (NaCl 147 mM; $CaCl_2$ 2.3 mM; KCl 4 mM; $MgCl_2$ 0.9 mM; pH 7.1–7.3) to obtain a concentration of 1.25 μg/μL, 2.25 μg/μL and 5 μg/μL. These doses showed antidepressant-like effects when infused in the hippocampus (Nin et al., 2008), and similar doses do not affect locomotion when infused in the nucleus accumbens and in the hippocampus of rats (Molina-Hernandèz et al., 2005; Rodríguez-Landa et al., 2009). The vehicle, infused in the control rats (0 μg/rat), was the 20% 2-hydroxypropyl-β-cyclodextrin ACSF solution. The animals were randomly divided into four subgroups. Allopregnanolone (1.25, 2.5, or 5 μg/rat) or vehicle were infused in each hemisphere in a volume of 0.5 μL per rat. The solutions were infused at a constant rate of 0.25 μL/min through a microperfusion pump (CMA/102. Acton; Harvard Apparatus, Holliston, MA, USA) connected to a 27-gauge needle that was introduced 0.2 mm below the end of the guide cannula. To avoid reflux, the injection needles were removed from the guide cannula 2 min after the end of the infusions. Allopregnanolone or vehicle solution was bilaterally administered three times: 24, 5 and 1 h before the test section of the behavioral tests. The same animals were used for all of the behavioral and biochemistry experiments. Each experimental group included 10–11 rats for the behavioral data and 6–9 rats for the biochemical data.

2.3. Surgery procedure

The rats were anesthetized with xylazine HCl (5 mg/kg) and ketamine HCl (100 mg/kg) i.p. and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Bilateral cannulae were placed in the nucleus accumbens core (anteroposterior: +1.6 mm from bregma; lateral: ±1.8 mm from bregma; vertical: −6.6 mm from dura mater according to Paxinos and Watson, 1998). The nucleus accumbens core was chosen because it is an area related to selection and integration of the limbic inputs that acquire control over motivation, discrete cues and learning (Ito and Hayen, 2011) which are considered important in depression. Although the surgery procedure to introduce the cannulae may provoke injuries, it is minimal, since the cannulae trespasses vertically the beginning of the hippocampus, leaving most part of it intact (Paxinos and Watson, 1998). The endpoints of the cannulae were 0.2 mm under the target, and the infusion areas are presented in Fig. 1. The cannulae were fixed to the skull with two screws and dental cement. The surgeries were performed 7 ± 2 days prior to the training day component of the tests.

2.4. Grooming microstructure test

Forty-five minutes after the last treatment dose, the animals were subjected to the behavioral tests. For grooming analyses, the animals were placed inside a cylinder with a diameter of 20 cm surrounded by 30-cm high white walls and a transparent floor. A camera placed 10 cm below the apparatus floor was used to record animals' behaviors and provide a detailed record of activity. The test comprised of two sections 24 h apart: training (15 min) and test (15 min), both of which were performed between 1 and 5 pm. In both sections, each rat was placed in the center of the cylinder. Only the test section was recorded. The testing room was illuminated by a dim light (25 W), and the