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Short communication

Chronic antidepressants reverse cerebrocortical allopregnanolone decline in the olfactory-bulbectomized rat

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Abstract

Olfactory bulbectomy is one of the most validated models of depression. We demonstrate that bilateral removal of the olfactory bulbs in rats produced a significant decline of allopregnanolone content in a select cerebrocortical area which was reversed by chronic (3-week) treatment with three different classes of antidepressant (desipramine, fluoxetine, and sertraline, and venlafaxine). The effects of the chronic antidepressant treatments on allopregnanolone cortical content are observed at a time which typically coincides with the drug's abilities to reverse the behavioral deficits of the bulbectomy syndrome. We therefore propose that normalization of allopregnanolone cerebrocortical levels may contribute to the antidepressant-like profile of these drugs in the olfactory-bulbectomized rat model of depression.

Keywords: Allopregnanolone; Olfactory-bulbectomized rat; Antidepressant

1. Introduction

The neuroactive steroid allopregnanolone (3α-hydroxy-5α-pregnan-20-one) is perceived as the most potent positive endogenous allosteric modulator of γ-aminobutyric acid type A ($GABA_A$) receptors (Majewska et al., 1986; Paul and Purdy, 1992). In low nanomolar concentrations, allopregnanolone can potentiate GABA-evoked chloride ion channel conductance and thereby impact neuronal excitability in a rapid nongenomic manner affecting certain aspects of behavior and the response to stress (Lambert et al., 1995; Majewska et al., 1986; Paul and Purdy, 1992). Furthermore, endogenous allopregnanolone has been recently demonstrated to occupy a critical presence in the mechanism of maintaining physiological GABAergic tone (Guidotti et al., 2001; Matsumoto et al., 1999).

An increasing body of evidence has accumulated from both clinical and animal studies implicating allopregnanolone in the pathophysiology of major depression and in depressive-like animal behavior (Frye and Walf, 2002; Khisti and Chopde, 2000; Khisti et al., 2000). Chronic antidepressant therapy, in correlation with depressive symptom improvement, was shown to normalize allopregnanolone levels which were significantly decreased in the cerebrospinal fluid (CSF) and plasma of patients with major depression, thus suggesting a deficiency of allopregnanolone levels as an etiological hallmark of the depression pathology (Romeo et al., 1998; Stroehle et al., 1999; Uzunova et al., 1998). Moreover, these studies suggest a putative role of allopregnanolone in the mechanism of action of clinically established antidepressants, such as the selective serotonin reuptake inhibitors, which is further supported by the demonstration that the selective serotonin reuptake inhibitor fluoxetine elevates allopregnanolone content in rat brain (Uzunov et al., 1996).

Recently, we demonstrated a strong region-specific temporal dysregulation of allopregnanolone brain levels in the olfactory-bulbectomized rat model of depression (Uzunova et al., 2003). This model is perhaps the most predictive animal model with which to screen for drugs with potential antidepressant activity (Cryan et al., 2002; Kelly et al. 1997). The reliability and face validity of the olfactory-bulbectomized rat model of depression is bol-

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stered by the fact that the bulbectomy syndrome is reversed almost exclusively by chronic, but not acute, antidepressant treatment (Cryan et al., 2002). Therefore, considering this notable highlight of the model and taking into account the observed bulbectomy-induced alteration of allopregnanolone brain levels, we decided to investigate the effect of repeated antidepressant treatments on the cerebrocortical content of allopregnanolone in the olfactory-bulbectomized rat model of depression. We measured allopregnanolone levels in cerebral cortices of sham-operated and olfactory-bulbectomized rats treated for 3 weeks with the noradrenaline reuptake inhibitor desipramine, the selective serotonin reuptake inhibitors fluoxetine and sertraline, and the mixed serotonin/noradrenaline reuptake inhibitor venlafaxine.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (200–220 g) (Iffa Crédo F-69592 L'Arbresle, Cedex-France) were housed in a humidity-controlled (50–60%) and temperature-controlled (22–24 °C) animal room under a 12/12 h day/night cycle (light on at 7:00 a.m.). Animals were housed in plastic cages in groups of four per cage with free access to food and water. All animals were handled daily for 1 week prior to surgery and daily postsurgery to alleviate any stress that the handling procedures may have had on the animals. Surgery took place 1 week after arrival of the animals in the laboratory. All protocols were in accordance with the Swiss animal protection law for the care and use of laboratory animals and performed under license No. 1756 (BS). All possible measures were taken to minimize pain or discomfort to the experimental animals. Bilateral olfactory bulbectomy or sham surgery were carried out as described previously (Uzunova et al., 2003). Animals were allowed 2 weeks to recover from the surgical procedure prior to commencing the antidepressant treatment. Following 3 weeks of treatment, 24 h after the last treatment, the animals were sacrificed by decapitation and their brains were rapidly dissected and frozen on dry ice. All samples were stored at −80 °C until being assayed. The cortical region used in this experiment includes all aspects of dorsoparietal and occipital cortex dorsal to the commissura and anterior to the level of the pituitary.

2.2. Drugs

All drugs were administered p.o. in 2 ml/kg saline solution. The doses of the antidepressants used (desipramine, 10 mg/kg; fluoxetine, 30 mg/kg; sertraline, 20 mg/kg; and venlafaxine, 20 mg/kg) were based on previous in-house experience demonstrating the drugs' ability to reverse the bulbectomy-induced behavioral deficits.

2.3. Allopregnanolone determination

Quantitative determination of allopregnanolone levels in brain tissue was conducted by a highly sensitive and specific analytical procedure combining normal phase high-performance liquid chromatography and gas chromatography/electron impact mass spectrometry as described in detail previously (Uzunova et al., 2003).

2.4. Statistical analysis

ALLO levels are expressed as picogram per gram brain tissue (pg/g) and represent the mean ± standard error of the mean (S.E.M.) from at least 10 different animals per group. Data were analyzed using a two-way analysis of variance (two-way ANOVA) with drug treatment and lesion (bulbectomy or sham surgery) as factors. Any overall significance was further analyzed using the Fisher LSD post hoc test [SigmaStat for Windows (Version 2.03)]. Values of $P < 0.05$ were considered as statistically significant.

3. Results

As depicted in Fig. 1, our results revealed that 5 weeks after bilateral removal of the olfactory bulbs, allopregnanolone content in the selected cerebrocortical region of the vehicle-treated rats was significantly decreased as compared to the sham-operated vehicle-treated animals [92.3 ± 6.8 (± S.E.M.) vs. 145.6 ± 10.7 pg/g tissue, respectively].

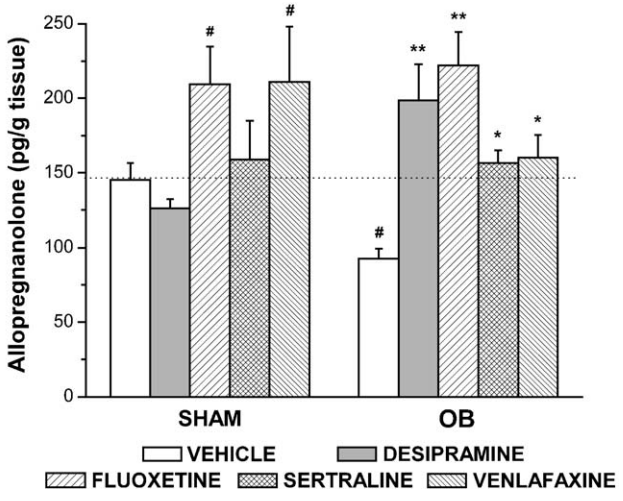

Fig. 1. Cerebrocortical allopregnanolone content in olfactory-bulbectomized (OB) and sham-operated (SHAM) rats following chronic (3-week) antidepressant or vehicle (saline) treatment. Data represent the mean ± S.E.M. of at least 10 animals per group; *$P < 0.05$ as compared to vehicle-treated olfactory-bulbectomized animals; **$P < 0.001$ as compared to vehicle-treated olfactory-bulbectomized animals; #$P < 0.05$ as compared to vehicle-treated SHAM animals (two-way ANOVA followed by Fisher LSD Method).

Notably, all four antidepressants tested reversed, in a statistically significant manner, the bulbectomy-induced decrease of allopregnanolone cerebrocortical content. Two-way ANOVA revealed a significant effect of drug [$F(4, 111) = 5.889$, $P < 0.001$] and a significant drug × lesion interaction [$F(4,111) = 3.480$, $P = 0.01$]. Chronic desipramine and fluoxetine displayed the strongest effects on allopregnanolone levels elevating them by 115% and 139%, respectively. Less pronounced but statistically significant were the effects of chronic sertraline and venlafaxine which increased allopregnanolone content by 69% and 73%, respectively.

4. Discussion

The objective of the present study was to assess the effect of various chronic antidepressant treatments on allopregnanolone content in the cerebral cortex of olfactory-bulbectomized rats. We have demonstrated that 5 weeks after bilateral removal of the olfactory bulbs, the abundance of allopregnanolone in the cerebral cortex of the vehicle-treated animals was significantly reduced by approximately twofold as compared to the sham-operated rats. Taking into account the well-established permissive influence exercised by endogenous allopregnanolone on $GABA_A$ receptor function (Guidotti et al., 2001; Matsumoto et al., 1999), we believe that a sustained twofold drop in cerebrocortical allopregnanolone content will likely result in a significantly compromised physiological GABAergic tone in the selected brain structure.

We have further demonstrated in this study that 3 weeks of chronic treatment with the noradrenaline reuptake inhibitor desipramine, the selective serotonin reuptake inhibitors fluoxetine and sertraline, and the mixed serotonin/noradrenaline reuptake inhibitor venlafaxine reversed the bulbectomy-induced decline in cerebrocortical allopregnanolone content. Interestingly, while all chronic antidepressant treatments increased allopregnanolone cerebrocortical content within the olfactory-bulbectomized group, only chronic fluoxetine and venlafaxine treatments produced elevated allopregnanolone levels in the sham-operated animals as well. To the best our knowledge, this is the first demonstration of the ability of chronic venlafaxine to elevate cortical allopregnanolone content in rat brain. The observed effect of fluoxetine was not unexpected as earlier studies have already demonstrated a potentiation of allopregnanolone brain levels following protracted (6-day) fluoxetine treatment in normal unstressed animals (Guidotti and Costa, 1998).

It should be noted that in contrast to fluoxetine and venlafaxine, chronic desipramine and sertraline failed to modulate allopregnanolone levels in the sham-operated animals while increasing allopregnanolone content only in the bulbectomized rats. This interesting and novel observation leads us to suggest that an underlying bulbectomy-induced neurobiological deficit producing lower allopregnanolone cerebrocortical levels may be required in order for the effects of desipramine and sertraline to become operative in increasing and hence normalizing the content of allopregnanolone.

As the effects of the four chronic antidepressant treatments on allopregnanolone cerebrocortical levels are observed at a time which typically coincides with the drugs' ability to reverse the behavioral aspects of the bulbectomy syndrome, we propose that normalization of allopregnanolone levels may contribute to the antidepressant-like effect of the drugs in the olfactory-bulbectomized rat model of depression via an upregulation of GABAergic tone. The most widely studied bulbectomy-induced behavioral deficit which responds selectively to chronic antidepressant treatment is hyperactivity in a novel and brightly lit (''open field'') environment (Harkin et al., 2003; Kelly et al., 1997). We therefore plan to study whether restoration of normal cortical allopregnanolone levels would coincide with attenuation of the bulbectomy-induced hyperactive response in the ''open field'' following chronic antidepressant treatment. Finally, this study further reinforces the putative association of the formation of the bulbectomy syndrome with an alteration in cerebrocortical allopregnanolone levels.

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