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Short communication

Flumazenil blocks the anxiolytic action of allopregnanolone

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Abstract

In the burying behaviour test allopregnanolone (0.5 mg/rat) reduced the cumulative time of burying, interpreted as a reduction in anxiety. The selective benzodiazepine antagonist, flumazenil (5 and 10 mg/kg), did not affect the burying behaviour when administered alone but effectively prevented the reduction produced by allopregnanolone. No scheme modified the ambulatory behaviour, thus suggesting that the effect of these treatments was selective on experimental anxiety. These results indicate that the anxiolytic actions of neurosteroids are most likely mediated via the stimulation of $GABA_A$ receptors. Data are discussed on the basis of such relationships.

Keywords: Flumazenil; Allopregnanolone; Anxiety; Burying behavior

1. Introduction

Recently it has been demonstrated that progesterone and some of its main metabolites, 5α-pregnanedione and 5α,3α-pregnanolone (allopregnanolone), induce anxiolytic actions when tested in various paradigms (Bitran et al., 1991; Weiland et al., 1991), including the burying behaviour test (Picazo and Fernández-Guasti, 1995). Moreover, it has been suggested that progesterone needs to be converted to its reduced metabolite (allopregnanolone) to diminish anxiety (Bitran et al., 1993). It has been speculated that such a reduction in anxiety is mediated via the activation of the $GABA_A$ receptor complex, which is known to be stimulated by these steroid hormones (Orchinik and McEwen, 1993). Additionally, it has been established that flumazenil may effectively block the actions of various benzodiazepines (Hunkeler et al., 1981) and thus it is proposed as a selective antagonist of this receptor complex.

Therefore, the aim of the present study was to test whether flumazenil was able to prevent the reduction in experimental anxiety produced by allopregnanolone.

2. Materials and methods

Female Wistar rats (200–250 g body weight) were used in this study. All females were ovariectomized under pentobarbital anaesthesia (40 mg/kg) 2 weeks before treatment. All animals were kept in a room under inverted and controlled (12-h light: 12-h dark, lights on at 22.00 h) light: dark cycle conditions, housed six per cage, and with ad libitum access to water and commercial rat chow all through the experiment.

Allopregnanolone (3α-hydroxy-5α-pregnan-20-one) was purchased from Sigma Chemical Co., dissolved in dichloromethane followed by corn oil and s.c. injected, in a volume of 0.2 ml/rat, 4 h before the tests. Allopregnanolone was injected at a dose of 0.5 mg/rat. Flumazenil (Hoffmann-LaRoche) was i.p. administered at two different doses, 5 and 10 mg/kg. This antagonist was dissolved in a drop of Tween 80 followed by saline solution and injected 30 min before the anxiety test. The steroid and antagonist doses were selected on the basis of previous studies (Picazo and Fernández-Guasti, 1995; Fernández-Guasti and Saldívar, 1990).

Experimental anxiety was measured in the burying behaviour test as previously described (Picazo and Fernández-Guasti, 1993; Treit et al., 1981). Briefly, a rat is placed in a box measuring 27 × 16 × 23 cm. In this paradigm, the cage floor is covered with fine sawdust and a prod of 7 cm long emerges 2 cm above

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the bedding material. The prod delivers an electric constant current of 0.3 mA. Immediately after the rat receives the electric shock, it displays burying behaviour. The cumulative burying behaviour time over a 10-min period is registered and is considered to directly reflect the level of experimental anxiety (Treit et al., 1981). After an animal was tested in the burying behaviour paradigm ambulatory behaviour was determined. Spontaneous ambulatory behaviour was recorded in a box measuring $43 \times 36 \times 19$ cm, which was placed over a sensitive plaque of an activity meter connected to a counter. In this model, each rat was put into the cage and the number of counts was recorded over a 10-min period. Results were compared using the Kruskal-Wallis analysis of variance followed by the Mann-Whitney *U*-test.

3. Results

Fig. 1 shows that flumazenil (10 mg/kg) per se did not modify the cumulative burying behaviour. This figure also shows a reduction in burying behaviour following allopregnanolone (5 mg/rat) administration as previously demonstrated (Picazo and Fernández-Guasti, 1995). The main finding of this study regards the blockade of the allopregnanolone-induced reduction of burying behaviour by flumazenil at the two different doses used (5 and 10 mg/kg).

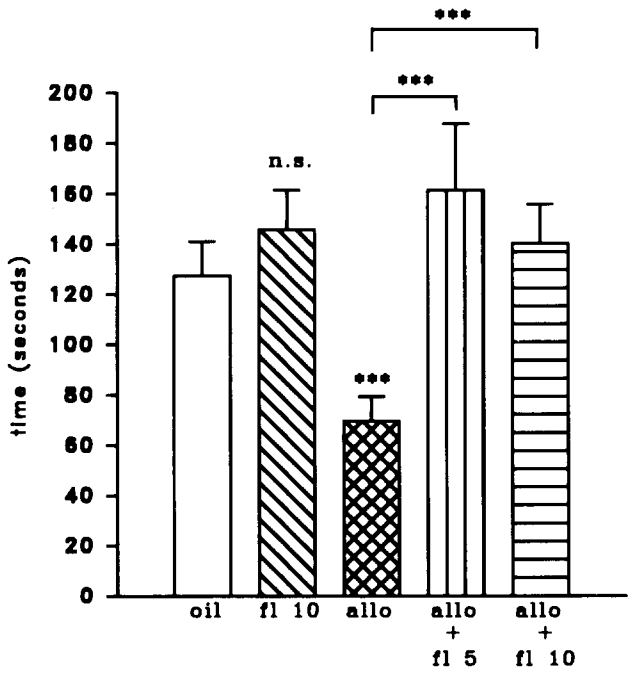

Fig. 1. Mean ± S.E. cumulative burying behaviour of ovariectomized rats treated with oil, flumazenil 10 mg/kg (fl 10), allopregnanolone 0.5 mg/rat (allo), allopregnanolone plus flumazenil 5 mg/kg (allo + fl 5) or allopregnanolone plus flumazenil 10 mg/kg (allo + fl 10). Kruskal-Wallis ANOVA, $H = 14.6186$, $df = 4$, $P < 0.005$. Asterisks over columns represent comparisons versus control group. Other comparisons are shown by brackets. Mann-Whitney *U*-test; $^{***}P < 0.01$.

The spontaneous ambulatory behaviour was not modified with either allopregnanolone, flumazenil or the combination of these compounds (Kruskal Wallis ANOVA $H = 1.3541$, $P = 0.8521$, $df = 4$; data not shown).

4. Discussion

The present result, showing that the reduction in burying behaviour produced by allopregnanolone is effectively antagonized by flumazenil, shows that this steroid reduces anxiety via the stimulation of the $GABA_A$ receptor complex.

Recently we have demonstrated a reduction in burying behaviour in the late prooestrus of the oestrous cycle (Fernández-Guasti and Picazo, 1992); this reduction seems to be related to the high levels of progesterone found during this phase. Additionally, we have found that allopregnanolone, a reduced progesterone metabolite produced in brain tissue, is more active than progesterone in reducing anxiety (Picazo and Fernández-Guasti, 1995) and thus might be responsible for the reduction in anxiety found in late prooestrus. In support of this idea, Bitran et al. (1993) have reported that the anxiolytic properties of progesterone are prevented by administering the 5α-reductase inhibitor, finasteride, which interferes with the conversion of progesterone to allopregnanolone.

It has been demonstrated that in the male rat there is a reduction in burying behaviour after ejaculation (Fernández-Guasti et al., 1989). This reduction seems to be mediated by changes in [^{3}H]flunitrazepam binding and is effectively blocked by selective antagonists (Fernández-Guasti and Saldívar, 1990). Our data show that the reduction in anxiety induced by the neurosteroid, allopregnanolone, is blocked by flumazenil. These results, taken together, suggest that the reduction in anxiety observed during certain phases of the oestrous cycle (Fernández-Guasti and Picazo, 1992) and during pregnancy (Picazo and Fernández-Guasti, 1993) could be blocked by $GABA_A$ receptor antagonists. Further experiments are required to test this idea.

These results strongly suggest that allopregnanolone and possibly other neurosteroids produce their antianxiety action through a membrane mechanism involving the stimulation of the GABA-benzodiazepine receptor in a similar manner to that of barbiturates (Majewska et al., 1986). Nevertheless, direct activation of the Cl^- conductance by this steroid, in the absence of GABA, has also been observed in electrophysiological studies (Harrison et al., 1987). Other experiments, however,

show that allopregnanolone enhances [^{3}H]flunitrazepam binding (Lan et al., 1990). The present data support this enhancement as mediating the anxiolytic effects of allopregnanolone in the rat.

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