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Alterations in GABAergic function following forced swimming stress

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Abstract

Forced swimming induces alterations in the GABA brain concentration and could change the sensitivity of the GABA/benzodiazepine receptor–chloride ionophore complex to benzodiazepines. This change in sensitivity could be explained by the allopregnanolone release that takes place during stress. The current study was carried out to determine whether forced swimming is able to modify the anti-anxiety effect of diazepam and to explore the possible relation of this change to allopregnanolone, the GABA concentration or/and the GABA/benzodiazepine receptor density.

Unstressed and stressed mice, injected with the vehicle or diazepam, were evaluated in the exploratory behavior test. Diazepam induced clear anxiolytic actions at all doses in unstressed animals, but such an effect was not observed in stressed animals. The injection of allopregnanolone 24 h before the anxiety test blocked the effect of this benzodiazepine. Forced swimming decreased GABA concentrations in the hippocampus and the thalamus–hypothalamus region, besides decreasing the [^{3}H]flunitrazepam labeling in both the hypothalamus and amygdala.

These results show that forced swimming abolishes the anti-anxiety effect of diazepam.

Keywords: Stress; Forced swimming; GABA; Mice; Anxiety; Diazepam

1. Introduction

The effects of environmental stress on the central gamma-amino-butyric acid (GABA) mediated neurotransmission have been extensively studied in animals by using biochemical and behavioral techniques. Acute stressors have been reported to either increase (Saulskaya and Marsden, 1995; Harvey et al., 2002) or decrease (Sherman and Gebhart, 1974; Otero Losada, 1988) brain GABA levels. Other studies have reported an augmented function of the brain GABA system following heavy swim stress (Soubrie et al., 1980; Skerritt et al., 1981; Akinci and Johnston, 1993, 1997). Several independent laboratories have also demonstrated that stress alters the functional properties of GABA/benzodiazepine receptor–chloride ionophore complex and/or the number of such sites in the nervous system (Schwartz et al., 1987; Otero Losada, 1988; Drugan et al., 1989; Rago et al., 1989; Montpied et al., 1993). In this sense, stressful handling of rats decrease the convulsive activity of several GABA/benzodiazepine receptor ligands such as bicuculline (Drugan et al., 1985), picrotoxin and pentylenetetrazole (Soubrie et al., 1980; Abel and Berman, 1993; Pericic et al., 2001). Therefore, it is unquestionable that the GABAergic activity is modified by severe stress, and that such change can be related to alterations in the pharmacological profile of drugs interacting with the GABA/benzodiazepine complex.

Forced swimming is one of the main stressful factors employed to understand changes at GABA/benzodiazepine receptor (Deutsch et al., 1994; Pokk et al., 1996; Marin et al., 1996; Pericic et al., 2000, 2001; Avital et al., 2001). For instance, this stressor is able to attenuate the anti-seizure efficacy of flurazepam (Deutsch et al., 1990). Based on the observation that acute stress influences the GABA/benzodiazepine receptor functionality, the main purpose of this

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investigation was to test the capability of forced swimming to modify the behavioral effect of diazepam. To this end, stressed mice injected with several doses of this agent were observed in the exploratory behavior test and the results were compared with those of unstressed animals.

Evidence shows that the progesterone $GABA_A$-modulatory metabolite 5α-pregnan-3α-ol-20-one (allopregnanolone) is released and rapidly metabolized after an acute stress session such as forced swimming (Purdy et al., 1991). It has been confirmed that this release occurs in both rats (Barbaccia et al., 1996, 2001) and mice (Mele et al., 2004) when using other stressful stimuli other than swimming. Accordingly, it has been reported that acute stress increases the peripheral-type benzodiazepine receptor density, and such receptor plays a major role in steroidogenesis (Weizman and Gavish, 1993; Cavallaro et al., 1992). Some authors have found that high doses of allopregnanolone (Gulinello et al., 2001) as well as its abrupt withdrawal (Smith et al., 1998) increase the synthesis of α_4 mRNA (one of the subunits that constitute the $GABA_A$ receptor). Interestingly, several studies have demonstrated that the presence of the α_4 subunit in the $GABA_A$ receptor decreases its sensitivity to benzodiazepines (Wisden et al., 1991; Knoflach et al., 1996; Wafford et al., 1996). From this evidence, it is likely that forced swimming could produce isomeric changes in the $GABA_A$ receptor complex that, at the same time, could alter the pharmacological response to benzodiazepines. Trying to mimic the abrupt release of allopregnanolone that supposedly takes place after stressing, we administered a high dose of this hormone to unstressed mice and evaluated their behavior 24 h later in the exploratory behavior test.

As mentioned, acute stress seems to modify the GABA levels and the functionality of the GABAergic transmission. In order to explore this putative consequence, both GABA concentration and GABA/benzodiazepine receptor density were analyzed in several brain areas of mice previously stressed by forced swimming. Hence, mice were sacrificed and their brain analyzed by means of autoradiographic techniques and high performance liquid chromatography (HPLC). Since to stress mice showed the lowest anxiety levels 24 h after swimming in the exploratory behavior test (Briones-Aranda et al., 2002), all experiments were carried out at this time.

The brain regions studied were chosen because they have been implicated in both anxiety and stress regulation (Shibata et al., 1989; Bowers et al., 1998; Koyama et al., 1999; Jardim and Guimaraes, 2001; Herman et al., 2002; Cook, 2004).

2. Materials and methods

2.1. Animals

Swiss Webster adult male mice (25–30 g) were used. Mice were bred in our laboratory, housed in groups of 10 animals each in plastic cages (44×21×21 cm) and submitted to 12:12 h inverted light cycle (10:00 off, 22:00 on). Food and water were available ad libitum at all times. All procedures were conducted in accordance with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) as approved by the Institutional Ethics Committee of CINVESTAV-IPN, México.

2.2. Forced swimming stress

This process was carried out by using a modified version of the animal model proposed by Porsolt et al., (1977a,b) as a validated tool to screen agents with antidepressive activity. This paradigm consists of an escape-proof Plexiglas cylinder (25 cm high, 10 cm in diameter) containing 15 cm of water at 25 °C where each mouse was forced to swim. After the stressing session (15 min), the animal was dried, warmed up, and returned to its home cage. In all cases the water in the tank was changed every two sessions.

2.3. Anxiety paradigm

The avoidance exploratory behavior test is a broadly used procedure to study experimental anxiety and to screen drugs with potential anxiolytic activity. This model consists of an acrylic cage (44×21×21 cm) divided into a small, darkened compartment (1/3 of total size) and a large and highly illuminated (560 lx light intensity) compartment (2/3). A little opening (13×15 cm) separated the dark area from the bright one. In this test each mouse was introduced (only once) into the bright area and the number of transitions throughout the opening was registered for 10 min. Thus, an increase in the number of transitions was interpreted as an anxiolytic effect (Crawley and Goodwin, 1980). After each session the test cage was carefully cleaned with a moist cloth.

2.4. Activity test

For controlling results in the anxiety test due to alterations in motor activity, a spontaneous ambulatory behavior test was conducted after the anxiety test. Hence, the animal was placed into an acrylic cage (60×40×40 cm) that had a checkerboard pattern (20×20 cm) on the floor, and the total number of squares crossed by the mouse was manually registered for 10 min.

2.5. Drugs

The drugs used in this study were: diazepam and allopregnanolone (Sigma, St. Louis, Mo., U.S.A.). All drugs were injected i.p. at a total volume of 4 ml/kg. Diazepam was dissolved in propylene glycol 40%. Allopregnanolone was dissolved in beta-cyclodextrin (5%). Doses and latencies were chosen considering previous