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Increases in plasma corticosterone and stretched-attend postures in rats naive and previously exposed to the elevated plus-maze are sensitive to the anxiolytic-like effects of midazolam

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Abstract

A single exposure to the elevated plus-maze test (EPM) reduces or abolishes the anxiolytic efficacy of benzodiazepines on a second trial. This phenomenon known as one-trial tolerance (OTT) is considered to be due to a shift in the emotional state of the animals across the test/retest sessions. Activation of the hypothalamic–pituitary–adrenocortical (HPA) axis has been considered to be an adaptive response to stressful or challenging situations such as height and openness of the EPM. This work looks at the phenomenon of OTT to the benzodiazepine compound midazolam through the conjoint examination of the novel ethological measures of the EPM and adrenocortical response of rats exposed to single and repeated sessions of the EPM. The results obtained confirmed that the approach/avoidance conflict on the first trial of the EPM is very sensitive to the anxiolytic effects of benzodiazepines. Moreover, stressful stimuli present upon initial exposure to the EPM render the standard measures of the EPM resistant to the anxiolytic effects of benzodiazepines on retest. However, the increases in plasma corticosterone and in risk assessment behavior observed in rats submitted to single or repeated sessions in the EPM were reversed by pretreatment with midazolam. The administration of metyrapone, a glucocorticoid synthesis blocker, decreased risk assessment but did not affect locomotion and anxiety-like behaviors. It is suggested that the detection of the dangerous environment through the stretched-attend postures in the second trial leads to the known low level of exploration and the consequent OTT upon retest. Moreover, in view of the similarity between the risk assessment and plasma corticosterone patterns in both naive and experienced rats, this hormone–behavior profile may be crucial for the expression of OTT to benzodiazepines in rodents exposed to the EPM.

Keywords: Anxiety; Fear; Plasma corticosterone; Elevated plus-maze; Midazolam; One-trial tolerance

Introduction

The elevated plus maze test (EPM) as an animal model of anxiety is based on the measures of behavioral categories that reflect the conflict resulting from the natural tendency of the animals to approach and avoid dangerous situations (Pellow et al., 1985; Anseloni et al., 1995; Anseloni and Brandão, 1997; Albrechet-Souza et al., 2005). As the test involves mixes of conditioned, proximal and distal aversive stimuli, the nature of the threat and of the appropriate response (emission or suppression of an action) has a bearing on drug responses (Handley and McBlane, 1993). In this context, while benzodiazepines (BZD) injected in rats upon initial exposure to the EPM increase the percentage of entries and the time spent in the open arms of the maze, a single previous undrugged experience in the EPM renders these compounds inefficacious (Bertoglio and Carobrez, 2000; Carvalho et al., 2005; Cruz-Morales et al., 2002; File and Zangrossi, 1993; Holmes and Rodgers, 1998). This phenomenon, known as 'one-trial tolerance' (OTT),

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appears to be dependent on learning from the first trial of the location of relative safety in the EPM (Holmes and Rodgers, 1998; Rodgers and Shepherd, 1993). This phenomenon has also been associated to a qualitative shift in emotional state (Cruz-Morales et al., 2002; File and Zangrossi, 1993; Rodgers and Shepherd, 1993). However, it is still open to investigation how to reconcile this hypothesis with the notion that the aversion (or preference) for open vs. closed zones is mostly not due to specific fear of the open arms, but a preference for one zone or the other (Falter et al., 1992; Treit et al., 1993; Becker and Grecksch, 1996; Lamberty and Gower, 1996).

Activation of the hypothalamic–pituitary–adrenocortical (HPA) axis represented by an increase in the plasma corticosterone has been considered to be part of the stress reaction and is triggered either by innate or conditioned fear stimuli (File et al., 1994; Rodgers et al., 1999). The first report looking at the adrenocortical activation as a response to the aversive stimuli of the EPM described freezing, defecation and increases in plasma corticosteroids as behavioral and physiological expressions of fear in animals submitted to the EPM (Pellow et al., 1985). The increase in plasma corticosterone in rats submitted for the first time to the EPM test was confirmed by other studies (File et al., 1994; Rodgers et al., 1999; Mikics et al., 2005). Although marked increase in plasma corticosterone has been associated with the EPM test, this hormonal response upon re-exposure of BZD-treated animals to this test in the context of the OTT phenomenon was not yet studied. If the assumption is made that the amount of aversion an animal experiences in the EPM is negatively correlated with its tendency to explore the open arms, then a simple relationship might be expected between this activity and increase of plasma corticosterone. That is, as exploration of open arms decreases, this measure should increase. To examine this hypothesis we looked at changes of plasma corticosterone along with the conventional and novel ethological categories in rats treated with saline and midazolam and submitted to single or repeated trials in the EPM. The choice of midazolam in this study was based on the fact that this benzodiazepine compound has been found to cause clear "anxiolytic-like effects" without changing the locomotor activity of rats in the closed arms of the maze (Anseloni and Brandão, 1997; Cruz-Morales et al., 2002; Albrechet-Souza et al., 2005). The conventional analysis of the exploratory behavior in the EPM has been extended to incorporate the so-called novel ethological categories which have disclosed additional dimensions to plus-maze behavior patterns, for example, vertical activity, directed exploration (head dipping and end-arm exploration), decision making and risk assessment (Rodgers and Cole, 1993). We thought that inclusion of measures of risk assessment (primarily, stretched-attend postures) in the test and retest sessions of the EPM would be valuable in identifying the nature of the emotional state of the animals during OTT. The stretched-attend postures are generally viewed as a measure of anxiety, but factor analysis and pharmacological studies suggest that it has a different significance than open arm exploration (Cole and Rodgers, 1994; Anseloni and Brandão, 1997). It has been proposed to belong to the category of information-gathering behaviors displayed in potentially threatening situations, the function of which is to optimize the most adaptive behavioral strategy (Blanchard et al., 1993). Furthermore, risk assessment measures have proved extremely valuable in identifying anxiolytic-like actions of drugs (e.g., 5-HT1A receptor ligands) not detected by conventional scoring methods (e.g., Rodgers and Cole, 1993; Rodgers et al., 1999, Griebel et al., 1997; Setem et al., 1999). To further explore the correlation between hormonal response and risk assessment behavior a second experiment also assessed the effects of metyrapone, a glucocorticoid synthesis inhibitor, on the exploratory behavior of animals submitted to single or repeated sessions of the EPM.

Materials and methods

Animals

Seventy-five male Wistar rats, weighing 230–260 g, from the animal house of the Campus of Ribeirão Preto of the University of São Paulo, were used. These animals were transported to a room adjacent to the test laboratory 72 h before the test. They were housed in groups of four per cage under a 12:12 dark/light cycle (lights on at 07:00 h) at 23±1 °C and given free access to food and water. The experiments reported in this article were performed in compliance with the recommendations of the SBNeC (Brazilian Society for Neuroscience and Behavior), which are based on the US National Institutes of Health *Guide for Care and Use of Laboratory Animals*.

EPM testing

The EPM device was made of wood and consisted of two open arms (50×10 cm) and two enclosed arms of the same size, with 50 cm high walls. The maze was configured such that arms of the same type were opposite each other, and the whole maze was raised 50 cm from the floor.

All testing was conducted during the light phase of the LD cycle, between 09:00 and 11:00 h. Rats were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration. The behavior of the animals was recorded by a video camera positioned above the maze that allows the discrimination of all behaviors, with the signal relayed to a monitor in another room via a closed circuit TV camera. The maze was cleaned thoroughly after each test using damp and dry cloths.

An observer trained in measuring ethological plus-maze parameters subsequently scored the videotapes. The behavioral categories were scored using ethological analysis software (Observer) developed by Noldus (Netherlands). This software allowed measurement of the number of entries and the time spent in both arms of the maze. Using separate location and behavior keys, this software allows the real-time scoring of videotapes of all behavioral categories by direct keyboard entry to a PC. This software only records the next behavior after a "stop" key is pressed, thus allowing for the recording of duration and frequency of entries into each type of arm.

The performance of each animal in the maze was analyzed, taking the standard measurements recorded in each section of the maze into account (closed and open arms), comprising the frequency of open and closed arm entries (an arm entry or exit being defined as all four paws into or out an arm, respectively), total arm entries and the amount of time spent by the animals in each section of the maze. These data were used to calculate the percentage of time spent in open arms. In addition, the frequencies of the following "novel ethological categories" were measured: (1) head dipping: dipping of the head below the level of the maze floor, (2) stretched-attend postures: when the animal stretches to its full length with the forepaws (keeping the hind paws in the same place) and turns back to the anterior position, (3) end-arm exploration: the number of times the rat reached the end of an open arm. These categories were defined according to previous studies (Anseloni and Brandão, 1997; Rodgers and Cole, 1993).

Procedure

Midazolam (Roche Products Limited, Brazil) (0.5 mg/kg) was dissolved in saline solution (0.9%) shortly before use. Selection of midazolam dose and the time