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Prototypical anxiolytics do not reduce anxiety-like behavior in the open field in C57BL/6J mice

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ABSTRACT

Understanding and effectively treating anxiety disorders are a challenge for both scientists and clinicians. Despite a variety of available therapies, the efficacy of current treatments is still not optimal and adverse side effects can result in non-compliance. Animal models have been useful for studying the underlying biology of anxiety and assessing the anxiolytic properties of potential therapeutics. The open field (OF) is a commonly used assay of anxiety-like behavior. The OF was developed and validated in rats and then transferred to use in the mouse with only limited validation. The present study tests the efficacy of prototypical benzodiazepine anxiolytics, chlordiazepoxide (CDP) and diazepam (DZ), for increasing center time in the OF in C57BL/6J (B6) mice. Multiple doses of CDP and DZ did not change time spent in the center of the OF. Increasing illumination in the OF did not alter these results. The non-benzodiazepine anxiolytic, buspirone (BUSP) also failed to increase center time in the OF while the anxiogenic meta-chlorophenylpiperazine (mCPP) increased center time. Additional inbred mouse strains, BALB/cJ (BALB) and DBA/2J (D2) did not show any change in center time in response to CDP. Moreover, evaluation of CDP in B6 mice in the elevated plus maze (EPM), elevated zero maze (EZM) and light dark assay (LD) did not reveal changes in anxiety-like behavior while stress-induced hyperthermia (SIH) was decreased by DZ. Pharmacokinetic (PK) studies suggest that adequate CDP is present to induce anxiolysis. We conclude that the measure of center time in the OF does not show predictive validity for anxiolysis in these inbred mouse strains.

1. Introduction

Anxiety disorders continue to plague society and the healthcare system as nearly one in five individuals worldwide suffer each year and only about one third receive treatment (Demyttenaere et al., 2004; Kessler et al., 2009). Moreover, the most commonly prescribed psychoactive drugs for anxiety, benzodiazepines and selective serotonin reuptake inhibitors, are flawed. Fast-acting benzodiazepines sedate patients and are prone to result in physical and psychological dependence (Woods et al., 1992). In light of these features, selective serotonin reuptake inhibitors have risen in prominence for treating anxiety but take weeks of consistent therapy to achieve equivalent anxiolytic effects and may exacerbate symptoms in the interim (Altamura et al., 2013; Lader, 1987). Research into more effective treatments with a better side effect profile is necessary to advance treatment of these debilitating disorders.

Animal models of anxiety-like behavior have been useful for identifying compounds with anxiolytic properties. The open field (OF) assay, developed by Hall in 1932, is by far the most commonly reported rodent anxiety assay (Hall, 1934; Hall and Ballechey, 1932). The OF takes advantage of the tendency of rodents to avoid brightly lit open spaces that might expose them to predation (Grossen and Kelley, 1972; Post et al., 2011). Rodents in the wild tend to stay in contact with objects or perimeters in the environment (Barnett, 1963) and in the OF, rodents also spend much of their time near the walls and corners of the arena. This behavior, called thigmotaxis, is reduced in rats upon the administration of commonly used anxiolytics such as diazepam (DZ) and chlordiazepoxide (CDP) (Bruhwyler, 1990; Gentsch et al., 1987; Nichols and Schreur, 1987). Thus, thigmotaxis in the OF as a model of anxiety in rats has been shown to have predictive validity in many, but not all cases, (for a review see Carola et al., 2002; Prut and Belzung, 2003) and the amount of time a rodent spends in the center of the OF arena is often cited as the primary measure of anxiety-like behavior.

A review of the literature describing validation studies of anxiolytics in the OF, however, reveals a significant dearth of evidence for the validity of center time as a measure of anxiety in mice (Prut and Belzung,

Abbreviations: CDP, chlordiazepoxide; norCDP, norchlordiazepoxide; DZ, diazepam; norDZ, nordiazepam; mCPP, meta-chlorophenylpiperazine; BUSP, buspirone; LC, liquid chromatography; MS, mass spectrometry; LD, light–dark; B6, C57BL/6J; OF, open field; EZM, elevated zero maze; EPM, elevated plus maze; SIH, stress-induced hyperthermia; BALB, BALB/cJ; D2, DBA/2J; IP, intraperitoneal; AUC, area under the curve; Cmax, maximum concentration; Tmax, time to maximum concentration.

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2003). Studies of classical anxiolytics in mice in the OF typically report drug-induced locomotor and rearing effects but often do not report or observe an increase in time in the center of the arena (Birkett et al., 2011; Crabbe et al., 1998; Crawley, 1981; Fahey et al., 1999; Heredia et al., 2013a; Lalonde and Strazielle, 2010; Lopez et al., 1988; Novas et al., 1988; Seredenin et al., 1990). The lack of evidence for changes in center time in response to anxiolytics suggests that this measure may not be a valid gauge of anxiety in mice. In the current study, we attempted to validate center time as a measure of the anxiolytic activity of mice in the OF. We tested the anti-thigmotactic effects of standard benzodiazepines, CDP and DZ, a serotonin 5-HT_{1A} receptor partial agonist, buspirone (BUSP), and an anxiogenic, meta-chlorophenylpiperazine (mCPP) in the OF. We also assessed the behavioral effects of CDP in two additional inbred strains, BALB/cJ (BALB) and DBA/2J (D2). Moreover, we assessed anxiolytic properties of CDP in other commonly used assays of anxiety-like behavior, the elevated plus maze (EPM), elevated zero maze (EZM) and the light/dark assay (LD). Our results highlight the importance of careful behavioral analysis of animal models of anxiety and the need for cross-validation of such models across species.

2. Materials and methods

2.1. Testing locations

Most experiments were performed at the Genomics Institute of the Novartis Research Foundation (GNF) in San Diego California. Behavioral testing in a brightly lit OF and pharmacokinetic (PK) experiments were performed at the University of North Carolina at Chapel Hill (UNC). Data collected at GNF and UNC were analyzed separately. A complete list of behavioral experiments, mouse strains, drugs and doses tested are presented in Suppl Table 1.

2.2. Drugs

CDP (Sigma-Aldrich, St. Louis, MO and Grace Davison Discovery Sciences, Columbia, MD), mCPP (Sigma-Aldrich), and BUSP (Sigma-Aldrich) were dissolved in 0.9% saline. Mice were injected intraperitoneally (IP) with 0.01 mL solution:g of body weight of testing solution 30 min prior to behavioral and physiologic testing. Mice were returned to their home cages between injection and testing. Control solution and solvent was 0.9% saline for all CDP, BUSP, and mCPP tests. DZ (Sigma-Aldrich) was dissolved in propylene glycol and ethanol for OF testing and suspended in a solution of sterile water and 0.05% Tween-20 for stress-induced hyperthermia (SIH). DZ solvents and controls were 0.8% propylene glycol with 0.2% ethanol vehicle, 1.6% propylene glycol with 0.4% ethanol vehicle, for 1 and 2 mg/kg, respectively (Choleris et al., 2001). For SIH, DZ vehicle control, 3, 6, 9, or 12 mg/kg was administered by oral gavage (Olivier et al., 2002). Subjects were randomized as to dose of drug received.

2.3. Inbred mouse strains

Male mice were used for all studies. For studies conducted at UNC, C57BL/6J (B6) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). B6, BALB and D2 mice tested at the Genomics Institute of the Novartis Research Foundation (GNF) were bred in an in-house colony and stocks were refreshed regularly from the Jackson Laboratory to avoid genetic drift. At both locations, mice were maintained in AAALAC-accredited specific pathogen-free colonies in ventilated cages (Thoren Caging Systems, Hazelton, PA [GNF] or Tecniplast, Italy [UNC]) on a 12-h light–dark cycle (lights on at 6:00 am [GNF] or 7:00 am [UNC]). Mice were group-housed in cages containing bedding (Bed-o-cob) and a cotton nestlet (GNF) or a cotton nestlet and PVC tube (UNC). Irradiated food (GNF: Purina Pico rodent chow 20, Purina, St. Louis, MO and UNC: Purina RMH 3000, Purina, St. Louis, MO, USA) and water were provided ad libitum. A minimum of eight mice were tested per dose for each behavioral assay (range n = 8 to n = 40).

2.4. General methods

Most behavioral assays were automated and thus non-subjective. Therefore, the investigator was not blinded as to dose or strain during behavioral testing. Mice were 63.7 days of age (±4.7 days) at the start of behavioral testing. All testing was conducted between 8 am and 12 pm during the light part of the light/dark cycle. At GNF, mice were moved to a quiet anteroom adjacent to the testing room 1–2 h prior to testing. At UNC, mice were transported from the colony to the testing room immediately prior to testing. All behavioral testing equipment was cleaned between each animal with a dilute (0.1%) bleach solution. For all tests, mice were naïve to both drug and behavioral testing.

2.5. Behavioral testing procedures and equipment

2.5.1. Open field

The OF (ENV-515-16, Med Associates, St. Albans, VT) was a 43.2 × 43.2 × 30.5 cm Plexiglas arena with a white floor and clear walls surrounded by infrared beams at 2.54 cm intervals on the x-, y- and z-axes that tracked the animals' position and activity throughout the experiment session. At GNF, the apparatus was isolated within a 73.5 × 59 × 59 cm testing chamber and illuminated by two 28 V lamps (14 lx at testing floor). At UNC, the OF was enclosed in the same chamber but illuminated with two 28 V lamps and overhead fluorescent light (280 lx at testing floor). Mice were placed in a front corner of the OF at the start of testing. Behaviors in the OF were recorded during a single 10-minute testing period and scored in post-session analyses using Activity Monitor 5.83 or 6.02 (Med Associates). Behaviors included distance traveled (in cm), ambulatory episodes (number of times the animal breaks 3 beams before coming to rest), percent time resting, average velocity (in cm per second) and number of rearings. Time spent in a center zone of 3 sizes ranging from 316.1 to 780.6 cm^2 and corners of the arena were also assessed. Boli were counted in the brightly lit OF following CDP administration and in the dimly lit OF following BUSP, DZ and mCPP administration.

2.5.2. Elevated plus maze (EPM)

The EPM (7001-0336; San Diego Instruments, San Diego, CA) was beige in color and consisted of two open arms and two closed arms [67.3 × 6.4 cm] that directly opposed each other. The walls of the enclosed arms completely surrounded the end of the runway and were 15.2 cm high. The top of the enclosed arms was open to the testing room and illuminated by room fluorescent lights (441 lx). The entire apparatus was elevated 38.1 cm above the floor. A video camera above the maze captured animals' location in the maze. The animals were placed in the center of the apparatus and allowed to investigate the maze for 5 min. Regions were defined as closed arms, open arms and the central square at the intersection of all four arms. Experiments were video tracked (Big Brother Recorder Window, Actimetrics, Wilmette, IL, USA) and analyzed (Big Brother Analysis Program) to obtain total distance, time spent in and number of entries into each arm. Experiments were also hand scored in real time to obtain measures of rearing, stretch-attend postures and head dips over the edge of the open arms.

2.5.3. Elevated zero maze (EZM)

The EZM (San Diego Instruments, San Diego, CA) was a white ABS plastic circular runway with an outside diameter of 61 cm and an inside diameter of 51 cm. Two opposing quarters of the circular runway were enclosed by 15.2 cm high walls and the entire apparatus was elevated 51 cm above the floor by 3 metal legs. The tops of the enclosed quadrants were open to the fluorescent room lighting (441 lx). Animals were placed on the EZM in a closed quadrant facing an open quadrant and tested for 5 min. Behaviors were hand scored in real time and