Pharmacology, Biochemistry and Behavior 102 (2012) 58–63

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Decision time and perseveration of adolescent rats in the T-maze are affected differentially by buspirone and independent of 5-HT-1A expression

Dennis E. Rhoads *, Nicole Grimes, Sunaina Kaushal, Janine Mallari, Krystal Orlando

Department of Biology, Monmouth University, W. Long Branch NJ 07764, United States

ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form 19 March 2012
Accepted 25 March 2012
Available online 1 April 2012

Keywords:
Adolescence
Buspirone
Long-Evans rats
Serotonin
Serotonin receptor
Repetitive behavior

ABSTRACT

Disruption of spontaneous alternation behavior (SAB) by the serotonin 1A (5-HT-1A) receptor agonist, 8-hydroxy-dipropylaminotetraline (8-OH-DPAT), results in repetitive behaviors that have been used to model the perseveration and indecisiveness of human obsessive-compulsive disorder (OCD). In the present study, we compared the effects of buspirone to those of 8-OH-DPAT in two strains of adolescent rats and analyzed repetitive choices of arms of the maze and prolonged apparent decision time due to induction of vicarious trial and error (VTE) behavior. In adolescent Sprague–Dawley (SD) rats, 8-OH-DPAT induced repetitive choices of arms of the maze (perseveration) and increased the apparent decision time. Buspirone induced VTE behavior and increased apparent decision time without perseveration. This distinct effect of buspirone was seen in SD adolescents but not in Long-Evans (LE) adolescents which appeared to be insensitive to buspirone. Lack of responsiveness to buspirone was dependent on the developmental stage because buspirone induced VTE behavior and prolonged decision time in LE adults. Western blotting of brain 5-HT-1A receptors showed expression of receptor protein in adolescent LE brain was comparable to that of adolescent SD and adult LE. The 5-HT-1A antagonist WAY 100365 blocked the effect 8-OH-DPAT on repetitive choice of arms but not the effect of buspirone on VTE behavior. We conclude that the adolescent LE rat has normal levels of 5-HT-1A receptor and that the effect of buspirone on VTE behavior is not mediated by the 5-HT-1A receptor. The LE strain may provide a useful system for further study of the adolescent brain and potential genetic differences in induction of repetitive behaviors.

1. Introduction

Given a choice between two arms of a T- or Y-maze, rodents tend to alternate in the arm entered, a phenomenon known as spontaneous alternation behavior (reviewed by Lalonde, 2002). Spontaneous alternation behavior (SAB) can be disrupted by treatment of rats with the serotonin analog 8-hydroxy-dipropylaminotetraline (8-OH-DPAT) (Fernandez-Guasti et al., 2003; Seibell et al., 2003; Yadin et al., 1991) with the resulting repetition of choices thought to model the perseveration and indecision of human obsessive-compulsive disorder (OCD) (for recent review see Albelda and Joel, 2011). The association of OCD with serotonin has long been bolstered by the relative efficacy of selective serotonin reuptake inhibitors (SSRI) in treating symptoms of OCD (El Mansari and Blier, 2006; Greist et al., 1995; Ordacgi et al., 2009; Pigott et al., 1990). Chronic pretreatment of rats with an SSRI abolished the induction of repetitive behaviors by 8-OH-DPAT (Yadin et al., 1991). Chronic pretreatment with clomipramine (Fernandez-Guasti et al., 2003) or allopregnanolone (Umathe et al., 2009) also blocked the effects of 8-OH-DPAT on SAB. The modulatory influences of SSRI and clomipramine and those of neurosteroids are important in comparison with human OCD because they underscore the pharmacological predictive validity of the system (reviewed in Albelda and Joel, 2011).

Adolescence has emerged as an important period wherein the brain is changing structurally and functionally, with parallel changes in responses to a number of drugs (reviewed by Spear, 2000; Sturman and Moghaddam, 2011). Studies of peri-adolescent Sprague–Dawley (SD) (Seibell et al., 2003) and Wistar (Fernandez-Guasti et al., 2003) rats showed that 8-OH-DPAT induced repetitive choices of arms in the T-maze and that these were comparable to adult responses. However, for juvenile Wistar rats, 8-OH-DPAT induced repetitive choices in males but not in females (Ulloa et al., 2004a), an important demonstration in this model because of sex differences in the prevalence of childhood OCD. Another important developmental stage-dependent difference in the disruption of SAB was seen with clomipramine. Chronic pretreatment with clomipramine blocked the effects of 8-OH-DPAT on SAB in adult but not in adolescent rats (Fernandez-Guasti et al., 2003).

Adolescent SD rats responded to a second serotonin agonist, buspirone, with prolonged decision time due to pronounced vicarious trial and error (VTE) behavior (Seibell et al., 2003). The VTE behavior persisted in these adolescent rats to the time limit of the trials

* Corresponding author. Tel.: +1 732 571 3429; fax: +1 732 263 5243.
E-mail address: drhoads@monmouth.edu (D.E. Rhoads).

0091-3057/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2012.03.024

(5 min) and precluded determining whether buspirone also induced repetitive choices of an arm of the T-maze. The different, albeit still repetitive, response to the two drugs was of interest because both are agonists at the serotonin 1A (5-HT-1A) receptor. Long considered a selective 5-HT-1A agonist, 8-OH-DPAT is now known to act at the more recently characterized 5-HT-7 receptor in addition to 5-HT-1A (Hedlund, 2009; Hedlund and Sutcliffe, 2007). Buspirone acts as both a partial agonist of the 5-HT-1A receptor and an antagonist of the dopamine D2 receptor (Riblet et al., 1982). Thus, both 8-OH-DPAT and buspirone have at least one unique target in addition to the one that they share.

The present study extends the characterization of this system in three important ways. First, we modified the procedure to measure repetitive choices as well as VTE behavior with buspirone, and have thereby further characterized the distinct actions of buspirone and 8-OH-DPAT. Second, we present differences in VTE responses between adolescents of the SD rat strain and the Long-Evans (LE) strain and between adolescent LE rats and the corresponding adults. Third, we have used quantitative Western blotting to estimate the levels of 5-HT-1A receptor in the two rat strains and combined this with additional pharmacological tests using a 5-HT-1A antagonist as evidence that induction of the repetitive VTE behavior by buspirone is not occurring via the 5-HT-1A receptor.

2. Methods

2.1. Animals

Male LE and SD rats were obtained from Charles River Laboratories (Raleigh NC) and used after a minimum of 7 days of acclimation to our animal facility. Rats were maintained in a controlled temperature and humidity environment with a light cycle from 0700 to 1900. Rats included in the periadolescent range (reviewed by Spear, 2000) were an average postnatal (P) age of P45 at the time of testing while those included as adults averaged P85 at the time of testing. Using doses previously shown to give maximal responses (Seibell et al., 2003), rats were injected with 8-OH-DPAT (2 mg/kg, ip), buspirone (4 mg/kg, ip) or saline vehicle (0.5 ml) as described previously. Behavior was tested 15–20 min after injection. In trials with the 5HT-1A antagonist WAY 100635, rats were injected with WAY 100635 (1 mg/kg, ip) 15 min prior to injection with 8-OH-DPAT or buspirone. All protocols involving rats were reviewed and approved by the Institutional Animal Care and Use Committee of Monmouth University as prescribed in the Public Health Service Guide for Care and Use of Laboratory Animals.

2.2. SAB and VTE behaviors

Disruption of SAB and induction of VTE behaviors were as described previously (Seibell et al., 2003) except that the arms of the T-maze were baited with equal amounts of peanut butter instead of chocolate milk. An SAB score of 1 reflected perfect alternation in choices of arms of the maze (left–right or right–left) while a score greater than one reflected the number of repetitions (perseveration) in choice of either the left or right arm of the maze. Also as described previously (Seibell et al., 2003), the duration of VTE behavior was recorded from the time the rat entered the decision area of the maze until the rat exited the decision area down one of the arms of the maze. Behaviors qualified as VTE because of indications both arms of the maze were being considered: stopping at the decision point with repetitive head movements back and forth toward each arm of the maze and/or starting down an arm of the maze only to stop and reverse direction before exiting the decision area. Drug-induced excessive serotonin efflux triggers a distinct behavioral syndrome in rats with important parallels to the serotonin-toxicity syndrome in humans (Zhang et al., 2009). For rats, it should be noted that VTE behavior as measured in the present study is distinct from behaviors of the serotonin syndrome including head shakes, forepaw treading and hindleg abduction (Lucki and Wieland, 1990). In this regard, our results are consistent with previous work showing that buspirone is very weak in eliciting the serotonin syndrome (Lucki and Wieland, 1990).

2.3. Fractionation of rat brain

Drug-naïve rats were sacrificed by rapid decapitation. Crude synaptosomal/mitochondrial fractions were prepared from brain homogenates by differential centrifugation using a modification of the original method of Gray and Whittaker (1962) as described previously (Wang et al., 2009). Synaptosomes were purified from the crude fraction by Ficoll density gradient centrifugation (Booth and Clark, 1978). In addition, the post-mitochondrial supernatant was subjected to centrifugation at 105,000 $\times g$ for 1 h to isolate the microsomal fraction. Protein concentrations in subcellular fractions were determined by the Bradford method (Quick Start Bradford Assay Kit, Bio-Rad Laboratories, Hercules CA) with bovine serum albumin as the protein standard.

2.4. Western blotting

Proteins were solubilized in Laemmli sample preparation buffer (Bio-Rad). Polyacrylamide gels (10%, Bio-Rad Ready gels) were loaded with 30 μg protein per lane. Following separation by electrophoresis, proteins were transferred to nitrocellulose and probed with antibody against the 5-HT-1A receptor (EMD Millipore, Billerica MA; 1:1000 dilution). The antibody was detected by chemiluminescence (Bio-Rad) using goat anti-rabbit secondary antibody conjugated with horseradish peroxidase (EMD Millipore, 1:10,000 dilution) and Pico chemiluminescent substrate (Pierce Thermo Scientific, Rockford IL). As an additional loading control, blots were stripped and reprobed with primary antibody against actin (Sigma-Aldrich, St. Louis MO). Initial analysis confirmed that 30 μg protein was within the linear range for quantitative detection of actin and density of 5-HT-1A was expressed relative to that for actin in each lane.

2.5. Data analysis

Results are expressed as mean ± SEM. Analysis of SAB score and time spent in VTE behavior were each analyzed by two-factor ANOVA (2 strains × 3 treatment groups [control, DPAT, buspirone] with repetition) using ProStat (Poly Software International, Pearl River NY). Where a significant difference was found, Bonferroni post hoc testing was used for multiple comparisons. Drug responses with and without prior exposure to WAY 100635, relative protein densities from Western blot analyses for LE vs. SD, and adult DPAT and buspirone responses vs. control, were each compared by two-tailed paired student t tests (GraphPad Instat, La Jolla CA). In all cases, significance was set at $p<0.05$.

3. Results

SAB scores were determined for two strains (SD and LE) and three treatment groups (control, 8-OH-DPAT and buspirone) (Fig. 1). Control (saline vehicle) groups of both strains of adolescents had SAB scores reasonably close to one. Average control SAB score was 1.6 for SD and 1.8 for LE. Average SAB score following injection with 8-OH-DPAT was 3.8 for SD and 3.4 for LE. In contrast, average SAB score following injection with buspirone was 1.7 for SD and 1.6 for LE. Two factor ANOVA indicated a significant effect of treatment group [$F(2,68)=20.433$, $p<0.001$] with no significant effect of strain [$F(1, 68)=0.137$, $p=0.712$] and no significant interaction effect