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Anxiolytic effects of the $GABA_A$ receptor partial agonist, L-838,417: Impact of age, test context familiarity, and stress ☆

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ABSTRACT

The partial α2,3,5 $GABA_A$ receptor agonist, L-838,417 has been reported to have anxiolytic effects in adult rodents. Although maturational differences exist for the $GABA_A$ receptor subunits, the anxiolytic effects of L-838,417 have not been tested in younger animals. The goal of the present experiments was to determine whether L-838,417 reverses anxiety-like behavior induced by either an unfamiliar environment (Experiment 1) or repeated restraint stress (Experiment 2) differentially in adolescent and adult, male and female Sprague–Dawley rats using a modified social interaction test. In Experiment 1, rats were injected with 0, 0.5, 1.0, 2.0, or 4.0 mg/kg L-838,417, i.p. and tested 30 min later in an unfamiliar test context for 10 min. In Experiment 2, rats were exposed to restraint stress (90 min daily for 5 days). Immediately after the last restraint session, animals were injected with L-838,417 and placed alone for 30 min in the test apparatus to familiarize them to this context prior to the 10 min social interaction test. In Experiment 1, L-838,417 produced anxiolytic effects in adults at 1.0 mg/kg, as indexed by a transformation of social avoidance into preference and an increase in social investigation. In adolescents, a dose of 2.0 mg/kg eliminated social avoidance, but had no anxiolytic effects on social investigation. Testing under familiar circumstances (Experiment 2) after repeated restraint stress eliminated age differences in sensitivity to L-838,417, with 0.5 mg/kg reversing the anxiogenic effects of prior stress regardless of age, but with doses ≥ 1 mg/kg decreasing social investigation, an effect possibly due in part to locomotor-impairing effects of this compound. Although locomotor activity was suppressed in both experiments, higher doses of L-838,417 were necessary to suppress locomotor activity in Experiment 1. Thus, anxiolytic effects of L-838,417 were found to be context-, age-, and stress-dependent.

1. Introduction

Adolescence is a developmental period associated with high levels of peer-directed social interactions that are observed in humans (Berndt, 1982; Csikszentmihalyi et al., 1977) as well as in rodent models (Varlinskaya and Spear, 2002, 2004, 2009). The increases in social interactions observed during this time are believed to be beneficial for the organism's successful transition into adulthood (Spear, 2000). For example, among human adolescents, social interactions have been reported to positively influence their self-esteem as well as to help them practice social skills for the future (Berndt, 1982; Connolly et al., 1987), whereas peer-directed social interactions among adolescent rodents have been suggested to help develop and improve their communication signals (Trezza et al., 2010; Vanderschuren et al., 1997). Not only is adolescence a period of increased social interactions, but adolescents also appear to find the opportunity to interact with peers more rewarding than do adults (Douglas et al., 2004). Given evidence that many of the hormonal, behavioral, and neural changes of adolescence has been conserved across a variety of mammalian species (Spear, 2000, 2010), experiments using laboratory animals have proved useful to model certain basic aspects of adolescence. For example, Sprague–Dawley rats are particularly social animals, and this strain of rats has been used extensively for investigation of socially facilitating, socially inhibiting, and anxiolytic drug effects (Morales et al., 2011; Varlinskaya and Spear, 2002, 2006, 2010).

The social interaction test has been widely used as a model for testing anxiety-like behavior in animals (File, 1980; File and Hyde, 1978). For example, the social behavior of animals is suppressed under anxiogenic circumstances (e.g., under bright light or in an unfamiliar test environment), an effect that can be reversed with anxiolytic compounds (File and Seth, 2003). Although total time spent in all forms of social activity has traditionally been measured in the social interaction test (File and Hyde, 1978; Sanders and Shekhar, 1995), in prior work we have found specific forms of social behavior to be especially sensitive to anxiogenic manipulations and anxiolytic compounds (Doremus-Fitzwater et al. (2009) Varlinskaya et al., 2010; Varlinskaya and Spear, 2012). These measures include social investigation (e.g., sniffing of the social partner) and a measure of social preference/avoidance (indexed via a coefficient reflecting approaches toward versus away from the non-manipulated test partner)

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(Varlinskaya et al., 1999). For example, both social investigation and the preference/avoidance coefficient were reduced by testing rats in an unfamiliar environment (Varlinskaya and Spear, 2002) or following repeated restraint stress (Doremus-Fitzwater et al., 2009; Varlinskaya et al., 2010), with these anxiogenic effects reversed by acute ethanol administration under both circumstances (Varlinskaya et al., 2010; Varlinskaya and Spear, 2002). Ethanol-related reductions in anxiety-like behaviors following anxiogenic manipulations in the social interaction test have been observed in both adolescent and adult rats, although when tested in an unfamiliar environment adolescents tend to be less sensitive than adults to ethanol's anxiolytic effects (Varlinskaya and Spear, 2002).

Anxiety assessed in the modified social interaction test may be modulated in part by $GABA_A$ receptors. The $GABA_A$ system has been implicated in anxiolytic properties of ethanol Eckardt et al. (1998) and GABA agonists reduce anxiety in animals tested under anxiogenic circumstances (File and Hyde, 1978; Gardner and Guy, 1984; Pellow and File, 1984). Indeed, benzodiazepines enhance GABA function and have been among the more commonly prescribed drugs for treatment of anxiety disorders, often despite undesired sedative effects (Griffiths and Weerts, 1997; Licata et al., 2010) thought to be mediated by the α1 subunit (McKernan et al., 2000). In the pursuit for non-sedative anxiolytics, a variety of GABA-related compounds have been developed. Of particular interest is L-838,417, a compound thought to act as an antagonist at the α1 subunit and as a partial agonist at α2,3,5 subunits (Atack, 2003) and to produce anxiolytic effects without compromising motor activity (McKernan et al., 2000). Mice with a point mutation for the α1 subunit that rendered the subunit insensitive to benzodiazepines demonstrated a resistance to the sedative effects of benzodiazepines, whereas the anxiolytic properties were left intact (Rudolph et al., 1999). In contrast, in another study involving selective mutations of the α2 and α3 subunits, benzodiazepines were found to be ineffective as anxiolytics in mice with disrupted α2 but not the α3 subunit, suggesting a role for the α2 subunit in mediating the anxiolytic effects of benzodiazepines (Low et al., 2000).

Despite the fact that the median age of onset for anxiety disorders is around the age of 11 (Kessler et al., 2005) and that childhood anxiety disorders put an individual at risk for psychiatric disorders in adulthood (Ramsawh et al., 2010), most studies of pre-clinical models of anxiety have not included younger populations. Examining younger animals is important because they may respond differently than adults to drugs that target the GABA receptor, given that GABAergic innervation is a prolonged developmental process. For example, in the rat primary visual cortex, peak GABA innervation occurs during mid-adolescence plateauing thereafter (Morales et al., 2002). Notable maturational differences are also evident in terms of subunit expression within the neurons of the developing rat cortex and thalamus, and to a lesser extent, the cerebellum (Henschel et al., 2008; Laurie et al., 1992; Liu and Wong-Riley, 2005). For example, expression of α1 mRNA is only observed postnatally and increases with age, peaking to adult levels by postnatal day 30 (Yu et al., 2006) whereas α3 mRNA is abundantly expressed in the embryo and subsequently declines to reach the low levels typically seen in adults by P12 (Laurie et al., 1992). Given that expression of mRNA for the GABA subunits differs notably between developing and adult rats, the slowly maturing GABA system may result in differences in sensitivity to drugs that affect this system.

Due to the lack of research examining the anxiolytic effects of compounds in younger animals despite evidence that $GABA_A$ receptor subunit expression changes developmentally (Yu et al., 2006), the present series of experiments examined the effectiveness of L-838,417 as an anxiolytic compound in adolescent and adult male and female Sprague–Dawley rats. Female rats were included in the current experiments because females appear to be at greater risk for most anxiety disorders (McLean and Anderson, 2009). Rats in Experiment 1 were tested in an unfamiliar environment whereas animals in Experiment 2 were tested in a familiar environment following 5 days of restraint stress. These two test circumstances may reflect different forms of anxiety, given that adolescents typically express a resistance to the anxiolytic effects of ethanol compared to adults when tested in an unfamiliar environment (Varlinskaya and Spear, 2002) but not when tested in a familiar environment after repeated (Varlinskaya et al., 2010) or acute restraint stress (Varlinskaya and Spear, 2012). Indeed, the anxiety induced by external cues associated with testing in an unfamiliar and uncertain environment has been suggested to provide an index of generalized anxiety (File and Hyde, 1978) whereas the social suppression seen following repeated stressors may reflect anxiety resulting from internal cues associated with the prior stressor and may perhaps provide a model of social anxiety (Varlinskaya and Spear, 2012). The goal of the current experiments was to explore the possibility that age-related differences in the anxiolytic properties of ethanol observed in these tests of anxiety are related to $GABA_A$ subunit expression by investigating whether the partial α2,3,5 $GABA_A$ receptor agonist might induce a similar pattern of anxiolytic effects.

2. General methods

2.1. Subjects

A total of 460 male and female adolescent and adult Sprague–Dawley rats derived from our breeding facility served as experimental subjects for the current experiments. An equal number of animals served as partners. On postnatal day (P) 1, all litters were culled to 10 pups (5 males, 5 females) and were group-housed with their same-sex littermates at weaning (P21). Rats were given ad libitum access to food (Purina Lab chow, Lowell, MA) and water, and were maintained in a temperature-controlled (22 °C) vivarium with a 12:12 h light-dark cycle (lights on at 0700 h). At all times animals were treated in accordance with guidelines for animal care established by the National Institutes of Health under protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2. Drug administration

The selective $GABA_A$ α2,3,5 partial agonist, L-838,417 (Tocris) was dissolved in a (2-Hydroxypropyl)-β-cyclodextrin solution and administered intraperitoneally (i.p.) at a volume of 2 ml/kg.

2.3. Experimental design

The purpose of Experiment 1 was to assess the anxiolytic effects of L-838,417 when animals were tested in an unfamiliar social environment. Thus, the design for Experiment 1 was a 2 age (adolescent: P35, adult: P70) × 2 sex (male, female) × 5 L-838,417 dose (0, 0.5, 1.0, 4.0 mg/kg) factorial, with 7 animals placed into each of the 20 groups. Experiment 2 examined the potential anxiolytic effects of L-838,417 following repeated restraint stress, and used a 2 age (adolescent, adult) × 2 sex (male, female) × 2 stress (non-stressed [NS], repeated restraint stress [RS]) × 5 L-838,417 dose (0, 0.5, 1.0, 2.0, 4.0 mg/kg) factorial design, with 8 animals placed into each of the 40 groups. No more than one subject/sex from a given litter was placed into an experimental group, reducing the possibility of litter effects (Holson and Pearce, 1992).

2.4. Experimental procedure

In Experiment 1, experimental animals were taken from their home cage and injected i.p. with one of the five doses of L-838,417 (0, 0.5, 1.0, 2.0, 4.0 mg/kg). Immediately after injection, each experimental animal was marked with a vertical black line across the back to differentiate it from its test partner, and placed alone in a novel holding cage for 30 min to increase levels of social behavior. At