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Ultrasonic vocalization production of preweanling rats: Effects of central and peripheral administration of α_2-adrenoceptor agonists

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Abstract

Stimulation of α_2-adrenoceptors increases the ultrasonic vocalization production of preweanling rats, however it is not known whether these critical α_2-adrenoceptors are located peripherally or centrally. In a series of three experiments, ultrasonic vocalizations were measured after 11-day-old rats had been administered clonidine or 2-[2,6-diethylphenylamino]-2-imidazole (ST-91) either systemically (i.p.) or into the third ventricle (i.c.v.). These particular α_2-adrenoceptor agonists were chosen because clonidine is lipophilic and enters the central nervous system, while ST-91 is hydrophilic and does not readily cross the blood–brain barrier. In the third experiment, clonidine- (1 μg, i.c.v.) and ST-91-induced (15 μg, i.c.v.) ultrasonic vocalizations were measured after systemic injection of the α_2-adrenoceptor antagonist yohimbine (0.5 or 1 mg/kg, i.p.). Results showed that central administration of both clonidine and ST-91 increased the ultrasonic vocalization production of 11-day-old rats, whereas peripheral administration of only clonidine, and not ST-91, increased ultrasonic vocalizations. These results indicate that the α_2-adrenoceptors mediating ultrasonic vocalization production are located in the central nervous system. Yohimbine fully attenuated clonidine-induced ultrasonic vocalizations but only partially attenuated ST-91-induced vocalizations. This pattern of results may have been due to the differential selectivity of clonidine and ST-91 for α_2-adrenoceptor subtypes (α_{2A}, α_{2B}, and α_{2C}) or imidazoline receptors. When combined with past research, the present results are consistent with the hypothesis that centrally located α_2-adrenoceptors are a component of a neural system that mediates ultrasonic vocalization production.

Keywords: Ultrasonic vocalization; α_2-adrenoceptor; Clonidine; ST-91 (2-[2,6-diethylphenylamino]-2-imidazole)

1. Introduction

Young rats typically emit ultrasonic vocalizations when they are under cold stress or separated from their dam and littermates (Hofer and Shair, 1978; Blumberg et al., 1999). A single vocalization may sweep across many frequencies (2–130 kHz), with the average peak frequency being beyond the range of human hearing (Brudzynski et al., 1999). The traditional interpretation of ultrasonic vocalizations is that they are distress or anxiety responses that elicit maternal behaviors from the dam (Noirot, 1972; Brunelli et al., 1994; Hofer, 1996). Blumberg and colleagues have challenged this traditional view and argue that ultrasonic vocalizations are a byproduct of a cardiovascular process called the abdominal compression reaction (for a review, see Blumberg and Sokoloff, 2001). According to this hypothesis, ultrasonic vocalizations are involuntarily produced when a rat experiencing isolation-induced cold stress engages in abdominal compression reactions to combat decreased venous return.

Pharmacological manipulation of various neurotransmitter systems (e.g., dopamine, μ- and κ-opioid, γ-aminobutyric acid, noradrenergic, etc.) has been shown to alter ultrasonic vocalization production (for reviews, see Kehoe, 1989; Hård and Engel, 1991). The noradrenergic system is

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perhaps the most studied because drugs that stimulate α_2-adrenoceptors (e.g., clonidine) dramatically increase the ultrasonic vocalization emissions of preweanling rodents (Thiessen and Upchurch, 1981; Hård et al., 1988; Kehoe and Harris, 1989; Hansen, 1993; Blumberg et al., 2000a,b; Nazarian et al., 2001). At present, it is not known whether α_2-adrenoceptor agonists affect ultrasonic vocalization production by acting in the central or peripheral nervous system. For example, stimulation of α_2-adrenoceptors located either centrally in the medulla and hypothalamus or peripherally in the heart causes hypotension and bradycardia (Pitts et al., 1986; Szemeredi et al., 1988; McAuley et al., 1989; Ebihara et al., 1993; Yamazato et al., 2001; Akers et al., 2004). Thus, centrally or peripherally mediated changes in cardiovascular functioning could potentially increase ultrasonic vocalization production through the process of abdominal compression reactions (see Blumberg et al., 2000a,b). Conversely, α_2-adrenoceptors are located diffusely throughout the forebrain (Boyajian et al., 1987; Scheinin et al., 1994; Holmberg et al., 2003), including regions (e.g., amygdala and periaqueductal gray) that have been implicated in anxiety and threat responses (Goldstein et al., 1996; Caldji et al., 1998; Wiedenmayer et al., 2000; Khoshbouei et al., 2002). Thus, α_2-adrenoceptor agonists may increase ultrasonic vocalization production by affecting neural mechanisms that mediate anxiety and distress.

To characterize more fully the relationship between α_2-adrenoceptors and ultrasonic vocalization production, we attempted to determine whether the relevant α_2-adrenoceptors were located peripherally or centrally. In Experiment 1, ultrasonic vocalizations of 11-day-old rats were assessed after peripheral administration of the lipophilic α_2-adrenoceptor agonist clonidine or the hydrophilic α_2-adrenoceptor agonist 2-[2,6-diethylphenylamino]-2-imidazole (ST-91). ST-91 is a polar analog of clonidine that does not readily cross the blood–brain barrier of adult animals (Kobinger and Pichler, 1975). It was hypothesized that the peripherally acting ST-91 would not affect ultrasonic vocalizations, while the lipophilic clonidine would increase ultrasonic vocalization production. In Experiment 2, ultrasonic vocalizations were measured after clonidine or ST-91 was directly administered into the third ventricle of 11-day-old rats. It was hypothesized that both α_2-adrenoceptor agonists would increase ultrasonic vocalization production when administered centrally. The ability of peripherally administered yohimbine (an α_2-adrenoceptor antagonist) to attenuate clonidine- and ST-91-induced ultrasonic vocalization production was examined in Experiment 3. Because both clonidine and ST-91 are putative α_2-adrenoceptor agonists, it was predicted that yohimbine would attenuate clonidine- and ST-91-induced ultrasonic vocalizations. If these various hypotheses were supported by the data, it would indicate that the α_2-adrenoceptors modulating ultrasonic vocalization production are located in the central nervous system.

2. Materials and methods

2.1. Subjects

The subjects were 184 rat pups of Sprague–Dawley descent, born and raised at California State University, San Bernardino. Litters were culled to 10 rat pups at postnatal day 4 (day 0=parturition). One rat from each litter was randomly assigned to each treatment group. There were approximately equal numbers of male and female rats per group. The colony room was maintained at 22–24 °C and kept under a 12:12 light:dark cycle. Testing was done in a separate experimental room and was conducted during the light phase of the cycle. Subjects were treated according to the National Institute of Health's guidelines, "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996), under a research protocol approved by the Institutional Animal Care and Use Committee of California State University, San Bernardino.

2.2. Apparatus

Ultrasonic vocalizations were assessed in a clear Plexiglas testing chamber (20×20×20 cm) housed inside a heated incubator maintained at 34 °C (±1 °C). A Mini-3 ultrasonic detector (Ultrasound Advice, London, UK) was tuned to 42 kHz and suspended 8 cm above the floor of the behavioral testing apparatus. Ultrasonic vocalizations were measured using both UltraVox data acquisition software (Noldus, Sterling, VA, USA) and by observers blind to drug treatment conditions. These data provided essentially the same results, so only data provided by blind human observers are presented. Rectal temperatures were assessed using a BAT-12 microprobe thermometer (Physitemp Instruments, Piscataway, NJ, USA).

2.3. Drugs

Clonidine hydrochloride and yohimbine hydrochloride were purchased from Sigma (St. Louis, MO, USA), while ST-91 was generously provided by Boehringer Ingelheim (Ridgefield, CT, USA). For peripheral administration, drugs were dissolved in saline and injected intraperitoneally (i.p.) at a volume of 5 ml/kg. For central administration, drugs were dissolved in vehicle (distilled water containing 0.25% crystal violet dye) and injected into the third ventricle (i.c.v.) at a volume of 4 μl. Drugs were dissolved in crystal violet vehicle to ascertain injection site accuracy (see Carden et al., 1991).

2.4. Procedure

2.4.1. Experiment 1

The purpose of the first experiment was to determine whether peripheral administration of clonidine or ST-91 would increase the ultrasonic vocalizations of preweanling rats. To that end, eight litters of 11-day-old rats (N=40) were used. Individual rats from each litter were injected with saline, clonidine (0.25 mg/kg, i.p.), or ST-91 (0.125, 0.25, or 0.5 mg/kg, i.p.) and returned to their home cage for 15 min. [Similar doses of ST-91 (0.1–0.5 mg/kg) have been used in behavioral studies involving adult rats (Clark et al., 1987; Durant et al., 1988; Mohammad et al., 1993), while 0.25 mg/kg clonidine reliably induces ultrasonic vocalizations in preweanling rats (Nazarian et al., 2001).] After 15 min, each rat was