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European Journal of Pharmacology 517 (2005) 200 – 207

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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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Received 17 January 2005; received in revised form 20 May 2005; accepted 24 May 2005
Available online 27 June 2005

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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doi:10.1016/j.ejphar.2005.05.021

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

individually taken to the experimental room and placed in the testing chamber. Ultrasonic vocalizations were measured during a 20-min testing session, with rectal temperature being recorded immediately afterwards. As testing on each rat was completed, it was anesthetized using sodium pentobarbital and returned to the home cage. This procedure has the advantage of maintaining litter size, while eliminating the ultrasonic vocalizations of the returning rat (see Carden et al., 1993; Nazarian et al., 2001).

2.4.2. Experiment 2

The purpose of the second experiment was to determine whether central administration of clonidine or ST-91 into the third ventricle would increase the ultrasonic vocalizations of preweanling rats. To that end, nine litters of 11-day-old rats ($N=72$) were used. Individual rats from each litter were injected with distilled water, vehicle (a 0.25% crystal violet dye solution), clonidine (1, 3, or 10 μg, i.c.v.), or ST-91 (5, 15, or 50 μg, i.c.v.). [Clonidine and ST-91 were administered at doses similar to those used in studies involving adult rats (Scriabine et al., 1977; Weiss et al., 1986).] A distilled water group was included to determine whether the crystal violet vehicle affected behavior. After i.c.v. injections, rats were placed in a temperature-controlled Plexiglas holding cage ($19\times16\times20$ cm; 34 °C) rather than being returned to their home cage as was done in Experiment 1. After 10 min, rats were removed from the heated holding cage and placed in the testing chamber where ultrasonic vocalizations were measured during a 20-min testing session. Rectal temperatures were then recorded.

Drugs were administered via freehand injection using a 30-gauge disposable needle attached to a 25-μl Hamilton syringe (see Carden et al., 1991; McPhie and Barr, 2000). A guard was placed over the needle so that the tip would extend 1.1 cm into the brain (i.e., to the level of the third ventricle). The needle was inserted through the foramen magnum and the drug was injected at a volume of 4 μl over a 10-s period. Entry to the third ventricle via the foramen magnum is possible in preweanling rats because the foramen magnum–third ventricle plane is straight, whereas this plane bends with additional maturation (Vilmann and Moss, 1979). The needle was left in place for an additional 10 s and then slowly removed. This freehand injection procedure causes minimal distress to rat pups.

Immediately after testing, each rat was deeply anesthetized with sodium pentobarbital and the brain was removed to verify injection site. Brains were carefully dissected to determine whether the needle track led to the third ventricle and if the ventricle contained dye (see Carden et al., 1991). Rats with incorrect needle placement were removed from the experiment and replaced by a littermate. As pups were taken from the home cage for testing, non-littermates were added to the nest to maintain litter size (Kehoe and Boylan, 1994; Nazarian et al., 1999).

2.4.3. Experiment 3

The purpose of the third experiment was to determine whether peripheral administration of the α_2-adrenoceptor antagonist yohimbine would block the ultrasonic vocalization production of preweanling rats receiving microinjections of clonidine or ST-91 into the third ventricle. To that end, eight litters of 11-day-old rats ($N=72$) were used. Individual rats from each litter were injected with yohimbine (0.0, 0.5, or 1 mg/kg, i.p.) and returned to their home cage for 15 min. [These doses of yohimbine fully attenuate the ultrasonic vocalizations induced by peripheral administration of clonidine (Nazarian et al., 2001).] Rats were then given freehand i.c.v. injections of vehicle, 1 μg clonidine, or 15 μg ST-91 and placed in a temperature-controlled Plexiglas holding cage. Dosages were based on the results of Experiment 2 because rats given 1 μg clonidine or 15 μg ST-91 emitted the most ultrasonic vocalizations. After 10 min, rats were removed from the heated holding cage and placed in the testing chamber where ultrasonic vocalizations were measured during a 20-min testing session. Rectal temperatures were then recorded. Injection site verification was conducted as described above.

2.5. Statistics

Analyses of variance (ANOVAs) were used for statistical analysis of ultrasonic vocalization and rectal temperature data. For these analyses, litter effects were controlled by using within-litter statistical procedures (i.e., a within analysis using one value/condition/litter) (Zorrilla, 1997). In Experiment 1, ultrasonic vocalization data were analyzed using a 5×4 (drug × time block) ANOVA, while rectal temperature data were analyzed using a one-way (drug) ANOVA. In Experiment 2, separate 5×4 (drug × time block) ANOVAs for each drug were used to analyze ultrasonic vocalization data, with rectal temperature data being analyzed using separate one-way (drug) ANOVAs. In Experiment 3, ultrasonic vocalization data were analyzed using a $3\times3\times4$ (pretreatment × drug × time block) ANOVA and rectal temperature data were analyzed using a 3×3 (pretreatment × drug) ANOVA. Preliminary analyses indicated that ultrasonic vocalizations did not differ according to sex (see also Meyer and Yacht, 1993; Dastur et al., 1999; Nazarian et al., 1999, 2001), so this variable was not included in subsequent analyses. Significant higher-order interactions were further analyzed using one- or two-way ANOVAs. Post hoc analysis of behavioral data was made using Tukey tests ($P<0.05$).

3. Results

3.1. Effects of peripheral administration of clonidine or ST-91 on ultrasonic vocalization production

Overall, peripheral administration of ST-91 (0.125, 0.25, or 0.5 mg/kg, i.p.) did not alter the ultrasonic vocalizations of 11-day-old

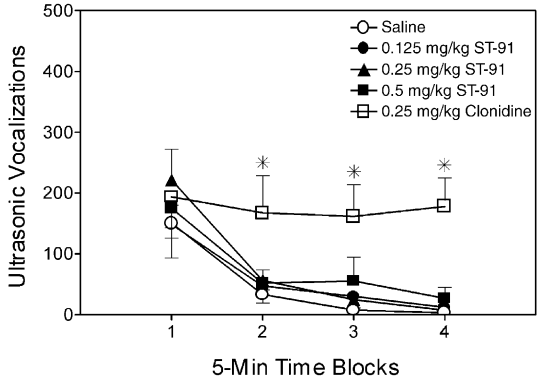

Fig. 1. Mean (±S.E.M.) number of ultrasonic vocalizations of 11-day-old rats ($n=8$ per group) injected with saline, clonidine (0.25 mg/kg, i.p.), or ST-91 (0.125, 0.25, or 0.5 mg/kg, i.p.) 15 min prior to being placed in the testing apparatus. Ultrasonic vocalizations were assessed for 20 min (divided into four 5-min time blocks). *Significantly different from rats given saline, $P<0.05$.

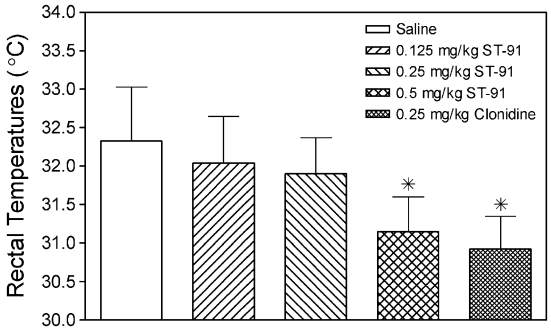

Fig. 2. Mean (±S.E.M.) rectal temperatures of 11-day-old rats ($n=8$ per group) injected with saline, clonidine (0.25 mg/kg, i.p.), or ST-91 (0.125, 0.25, or 0.5 mg/kg, i.p.) 15 min prior to being placed in the testing apparatus. Ultrasonic vocalizations were assessed for 20 min, with rectal temperatures being measured immediately afterwards. *Significantly different from rats given saline, $P<0.05$.

rats, while clonidine (0.25 mg/kg, i.p.) significantly increased the production of ultrasonic vocalizations (Fig. 1), drug main effect, $F(4,28)=3.23$, $P<0.05$. Clonidine-treated rats emitted more ultrasonic vocalizations than saline controls on time blocks 2–4, drug × time block interaction, $F(12,84)=2.18$, $P<0.05$. Rectal temperatures were affected by both α_2-adrenoceptor agonists because rats injected with clonidine (0.25 mg/kg) and the higher dose of ST-91 (0.5 mg/kg) had significantly lower rectal temperatures than saline-treated rats (Fig. 2), drug effect, $F(4,28)=6.49$, $P<0.01$.

3.2. Effects of central administration of clonidine or ST-91 on ultrasonic vocalization production

Administering clonidine (1, 3, or 10 μg, i.c.v.) into the third ventricle caused a significant enhancement in ultrasonic vocalizations when compared to control rats given either distilled water or vehicle (Fig. 3), drug main effect, $F(4,32)=25.78$, $P<0.001$. Clonidine's effects differed according to dose because rats injected with 1 μg clonidine had more ultrasonic vocalization emissions than rats injected with 3 or 10 μg clonidine, Tukey tests ($P<0.05$). Importantly, administering distilled water or vehicle (crystal violet solution) into the third ventricle did not differentially affect ultrasonic vocalization production, thus indicating that the drug, and not the vehicle, was responsible for changes in ultrasonic vocalizations. Rectal temperatures of the 11-day-old rats were not affected by any dose of clonidine (1, 3, or 10 μg, i.c.v.) (Table 1).

Table 1
Mean (±S.E.M.) rectal temperatures (°C) of preweanling rats ($n=9$ per group) injected with distilled water, vehicle, clonidine, or ST-91 into third ventricle

Treatment	Rectal temperatures
Distilled water	35.77 (±0.13)
Vehicle	35.85 (±0.17)
1 μg clonidine	35.57 (±0.24)
3 μg clonidine	35.31 (±0.26)
10 μg clonidine	35.32 (±0.31)
5 μg ST-91	35.96 (±0.34)
15 μg ST-91	34.81 (±0.44)
50 μg ST-91	35.44 (±0.49)

When administered centrally, ST-91 (5, 15, or 50 μg, i.c.v.) increased ultrasonic vocalization production relative to the two control groups (Fig. 4), drug main effect, $F(4,32)=8.83$, $P<0.001$. Ultrasonic vocalization emissions differed depending on the dose of ST-91 administered, since rats injected with 5 or 15 μg ST-91 exhibited significantly more ultrasonic vocalizations than rats given 50 μg ST-91, Tukey tests ($P<0.05$). These drug effects did not differ according to time block. Administering ST-91 into the third ventricle did not significantly alter rectal temperatures (Table 1), although rats injected with 15 μg ST-91 exhibited a non-significant decline in rectal temperatures ($P<0.07$).

Some baseline behavioral and physiological differences were apparent between the initial two experiments. Specifically, ultrasonic vocalization production of control rats was lower and rectal temperatures were generally higher in Experiment 2 (when compared to Experiment 1). These effects are likely due to differences in experimental methodology (i.e., rats were returned to their home cage after i.p. injections, while rats were placed in a heated holding cage after i.c.v. injections). It is apparent that the heated holding cage better maintained body temperature, thus resulting in higher rectal temperatures. Higher rectal temperatures, in turn, may have reduced basal rates of ultrasonic vocalization production.

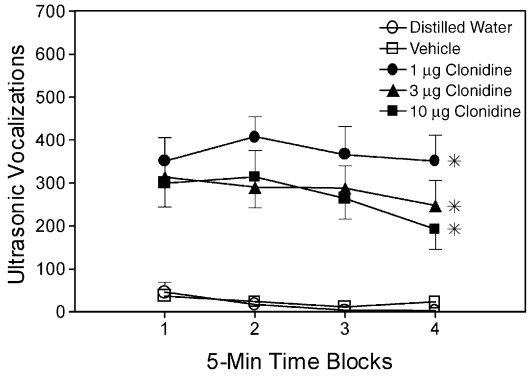

Fig. 3. Mean (±S.E.M.) number of ultrasonic vocalizations of 11-day-old rats ($n=9$ per group) injected with distilled water, vehicle, or clonidine (1, 3, or 10 μg, i.c.v.) into the third ventricle 10 min prior to being placed in the testing apparatus. Ultrasonic vocalizations were assessed for 20 min (divided into four 5-min time blocks). *Significantly different from rats given distilled water or vehicle, $P<0.05$.

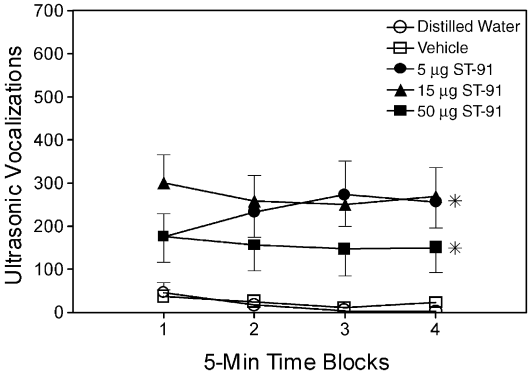

Fig. 4. Mean (±S.E.M.) number of ultrasonic vocalizations of 11-day-old rats ($n=9$ per group) injected with distilled water, vehicle, or ST-91 (5, 15, or 50 μg, i.c.v.) into the third ventricle 10 min prior to being placed in the testing apparatus. Ultrasonic vocalizations were assessed for 20 min (divided into four 5-min time blocks). *Significantly different from rats given distilled water or vehicle, $P<0.05$.

3.3. Effects of yohimbine on the ultrasonic vocalization production of rats administered clonidine or ST-91 into the third ventricle

Once again, microinjecting clonidine (1 μg, i.c.v.) or ST-91 (15 μg, i.c.v.) into the third ventricle increased the ultrasonic vocalizations of 11-day-old rats (Fig. 5), drug main effect, $F(2,14)=17.17$, $P<0.001$. Peripheral administration of the α_2-adrenoceptor antagonist yohimbine caused an overall reduction in ultrasonic vocalization production; however, yohimbine's effectiveness varied according to which agonist was being tested, pretreatment main effect, $F(2,14)=27.19$, $P<0.001$; pretreatment × drug interaction, $F(4,28)=4.59$, $P<0.01$. More specifically, both doses of yohimbine (0.5 and 1 mg/kg, i.p.) fully attenuated the ultrasonic vocalizations of clonidine-treated rats, Tukey tests ($P<0.05$) (middle graph, Fig. 5). In contrast, yohimbine significantly reduced the ultrasonic vocalizations of ST-91-treated rats, but only to about 43% of control values, Tukey tests ($P<0.05$) (lower graph, Fig. 5). Curiously, the larger dose (1 mg/kg) of yohimbine was not more efficacious than the smaller dose (0.5 mg/kg) at attenuating ST-91-induced ultrasonic vocalizations. Yohimbine did not affect the ultrasonic vocalizations of saline-treated rats (upper graph, Fig. 5). None of the treatment effects varied according to time block.

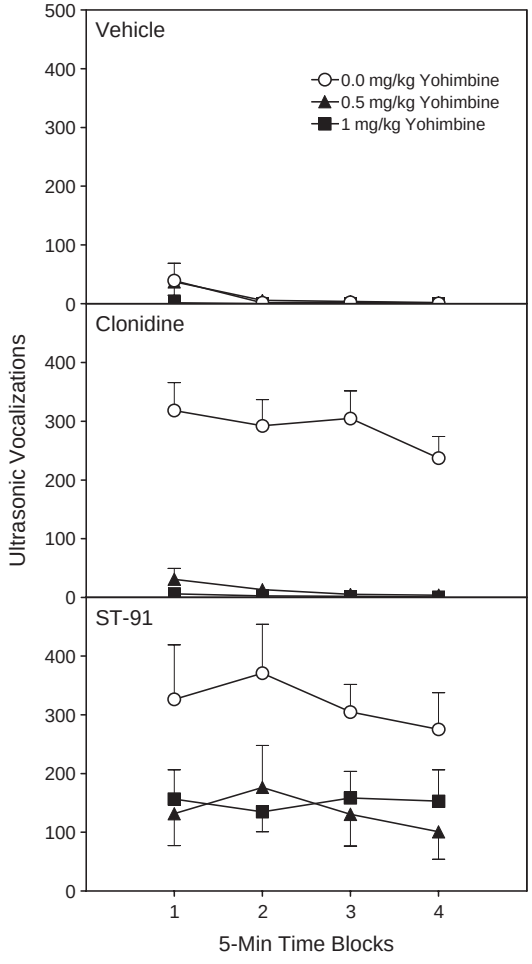

Fig. 5. Mean (±S.E.M.) number of ultrasonic vocalizations of 11-day-old rats ($n=8$ per group) injected with yohimbine (0, 0.5, or 1.0 mg/kg, i.p.) 25 min prior to behavioral testing. The same rats were injected with vehicle, clonidine (1 μg, i.c.v.), or ST-91 (15 μg, i.c.v.) into the third ventricle 10 min prior to being placed in the testing apparatus. Ultrasonic vocalizations were assessed for 20 min (divided into four 5-min time blocks). *Significantly different from similarly treated rats given 0.0 mg/kg yohimbine, $P<0.05$.

Table 2
Mean (±S.E.M.) rectal temperatures (°C) of preweanling rats ($n=8$ per group) pretreated with yohimbine and then injected with vehicle, clonidine, or ST-91 into the third ventricle

Treatment	Dose of yohimbine (mg/kg)		
	0.0	0.5	1
Vehicle	34.65 (±0.73)	34.92 (±0.31)	34.34 (±0.30)
Clonidine (1 μg)	34.76 (±0.23)	34.79 (±0.25)	34.48 (±0.30)
ST-91 (15 μg)	33.96 (±0.44)[a]	33.49 (±0.57)[a]	34.00 (±0.27)[a]

[a] Significantly different from vehicle-treated rats ($P<0.05$).

Rectal temperatures of 11-day-old rats were not affected by central administration of clonidine (1 μg, i.c.v.), however ST-91 (15 μg, i.c.v.) caused a significant reduction in rectal temperatures, drug main effect, $F(2,14)=17.94$, $P<0.001$ (Table 2). Yohimbine did not alter basal rectal temperatures nor did it influence the ST-91-induced reductions in rectal temperatures.

4. Discussion

Stimulation of α_2-adrenoceptors has long been known to increase the ultrasonic vocalization production of preweanling rats; however, it had not been determined whether these effects are mediated peripherally or centrally. In the present study, peripheral administration of the lipophilic α_2-adrenoceptor agonist clonidine (0.25 mg/kg, i.p.), which easily crosses the blood–brain barrier, increased ultrasonic vocalization emissions. Conversely, peripheral administration of the hydrophilic α_2-adrenoceptor agonist ST-91 (0.125, 0.25, or 0.5 mg/kg, i.p.), which does not readily cross the blood–brain barrier, did not affect ultrasonic vocalizations. Both clonidine (1–10 μg, i.c.v.) and ST-91 (5–50 μg, i.c.v.) increased ultrasonic vocalization production when administered directly into the third ventricle. The effects of these α_2-adrenoceptor agonists varied according to dose because lower doses of clonidine (1 μg, i.c.v.) and ST-91 (5 and 15 μg, i.c.v.) caused the most robust increase in ultrasonic vocalizations. When the results of these experiments are considered together, it is apparent that ultrasonic vocalization production is modulated by α_2-adrenoceptors located in the brain.

Peripheral administration of the α_2-adrenoceptor antagonist yohimbine (0.5 or 1 mg/kg, i.p.) fully attenuated the ultrasonic vocalizations produced by central administration of clonidine, while only partially attenuating ST-91-induced ultrasonic vocalizations. An unlikely explanation for these drug effects involves the relative affinities of clonidine, ST-91, and yohimbine for the various α_2-adrenoceptor subtypes. Specifically, clonidine has greater affinity at α_{2A}- and α_{2B}-adrenoceptors than does ST-91, whereas ST-91 has a relatively greater affinity at α_{2C}-adrenoceptors (Jasper et al.,

1998; Graham et al., 2000; Millan et al., 2000a). Yohimbine also has a greater affinity for the α_{2C}-adrenoceptor than the other α_2-adrenoceptor subtypes (Uhlen et al., 1992, 1998; Millan et al., 2000b), therefore yohimbine should have been more effective at attenuating ST-91-induced, rather than clonidine-induced, ultrasonic vocalizations. Alternatively, clonidine and ST-91 stimulate imidazoline binding sites as well as α_2-adrenoceptors (for a review, see Dardonville and Rozas, 2004). Imidazoline binding sites are typically found in tissues containing α_2-adrenoceptors, including the brain, heart, and other organs (Ernsberger et al., 1995; King et al., 1995; Ruggiero et al., 1998; Molderings and Göthert, 1999). In heart tissue, ST-91, when compared to clonidine, preferentially activates imidazoline binding sites (Mukaddam-Daher et al., 1997). Although comparable studies have not been done using brain tissue, it is possible that yohimbine (a selective α_2-adrenoceptor antagonist) did not fully attenuate ST-91-induced vocalizations because some of ST-91's effects were mediated by centrally located imidazoline receptors. The accuracy of this hypothesis will need to be tested using selective imidazoline agonists and antagonists.

Interestingly, peripheral administration of clonidine (0.25 mg/kg, i.p.) or ST-91 (0.5 mg/kg, i.p.) reduced the rectal temperatures of 11-day-old rats. The latter finding suggests that there is a population of peripherally located α_2-adrenoceptors that affects core body temperature (but see McLennan, 1981). This interpretation should be viewed cautiously, however, because control rats had body temperatures that were below thermoneutrality (see Fig. 2) and a number of studies using adult rats and mice indicate that the α_2-adrenoceptors responsible for hypothermia are located in the central nervous system (Drew et al., 1979; Bugajski et al., 1980; O'Donnell et al., 1996). Curiously, centrally administered ST-91 (15 μg, i.c.v.) reduced rectal temperatures, while clonidine (1–10 μg, i.c.v.) was without effect. It is possible that a higher dose of clonidine would have decreased rectal temperatures; however the results are also consistent with a hypothesis that ST-91 reduced core body temperature by preferentially stimulating α_{2C}-adrenoceptors or imidazoline receptors. In any event, the present results show that central administration of clonidine substantially increases ultrasonic vocalization production without causing a concomitant reduction in body temperature. This suggests that clonidine's actions on body temperature and ultrasonic vocalizations are dissociable (see also Nazarian et al., 2001).

Although the present study shows that the α_2-adrenoceptors mediating ultrasonic vocalizations are located in the central nervous system, the neural mechanisms responsible for ultrasonic vocalization production remain poorly understood. There is evidence that α_2-adrenoceptor and κ-opioid receptor systems interact to mediate vocalizations, as yohimbine attenuates the ultrasonic vocalization emissions of preweanling rats treated with either the κ-opioid receptor agonist *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonate (U50,488) or clonidine (Nazarian et al., 2001). Thus, the α_2-adrenoceptor system must be functional for the occurrence of κ-opioid-mediated ultrasonic vocalizations. These critical κ-opioid receptors may be located in the dorsal midbrain because ultrasonic vocalization emissions increase when U50,488 is microinjected into the periaqueductal gray (Goodwin and Barr, 1997), a brain area implicated in both defense responses and vocalizations (Jürgens, 1994; Wiedenmayer et al., 2000). Although speculative, available evidence suggests that α_2-adrenoceptor and κ-opioid receptor systems are interconnected components of a neural system, which may include the periaqueductal gray, that mediates ultrasonic vocalization production. Alternatively, stimulation of α_2-adrenoceptors and κ-opioid receptors in the hypothalamus and medulla decreases heart rate and blood pressure of adult rats (Feuerstein and Faden, 1982; Hassen et al., 1984; Ebihara et al., 1993; Yamazato et al., 2001); thus, it remains possible that stimulation of central α_2-adrenoceptors increases ultrasonic vocalization emissions through the process of abdominal compression reactions (Blumberg et al., 2000a,b, 2002). Additional research is needed to delineate more fully those neural mechanisms that underlie ultrasonic vocalization production.

Acknowledgements

This research was partially supported by an ASI research grant (CSUSB) to Catherine M. Krall. We thank Cynthia A. Crawford for her technical expertise.

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