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Cardiovascular Mediation of Clonidine-Induced Ultrasound Production in Infant Rats

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In infant rats, administration of the α_2 adrenoceptor agonist clonidine simultaneously evokes ultrasound production and bradycardia. In this study the authors examined in 8-day-old rats whether these 2 responses to clonidine are causally related. In Experiment 1 pups were pretreated with saline or prenalterol (0.1 or 1.0 mg/kg), a β_1 adrenoceptor agonist that increases cardiac rate, followed by administration of clonidine (1.0 mg/kg). Prenalterol pretreatment suppressed clonidine-induced ultrasound production at both doses. Prenalterol also increased skin temperature, however, suggesting that suppression of ultrasound was modulated in part by increased body temperature. Consistent with this suggestion, in Experiment 2 mild hyperthermia significantly inhibited clonidine-induced ultrasound production. Finally, in Experiment 3 the authors found that the pretreatments used in Experiments 1 and 2 prevent or dampen the effects of clonidine on cardiac rate. These results suggest that clonidine's effect on ultrasound production is mediated by its effects on the cardiovascular system.

It has been known for many years that isolating infant rats and exposing them to cold evokes emission of an ultrasonic vocalization (Allin & Banks, 1971; Okon, 1971). This vocalization has received considerable attention over the years and has typically been interpreted as a distress call designed to elicit maternal retrieval to the warmth and comfort of the nest (Noirot, 1972). In addition to cold exposure, administration of the α_2 adrenoceptor agonist clonidine evokes high rates of ultrasound production, even at warm air temperatures (Hård, Engel, & Lindh, 1988; Kehoe & Harris, 1989). Week-old rats treated with clonidine also exhibit intense locomotor activity, including crawling and wall climbing (Reinstein & Isaacson, 1977). These vocal and locomotor responses to clonidine, which stand in marked contrast to the anxiolytic and sedative effects reported in adults (Kalin & Shelton, 1988; Leibowitz, Fyer, McGrath, & Klein, 1981; Reinstein & Isaacson, 1977), have supported the interpretation of ultrasonic vocalizations as cries indicative of an emotional state of distress or anxiety (Hård et al., 1988; Miczek, Weerts, Vivian, & Barros, 1995; Winslow & Insel, 1991). On the other hand, the inability of stimuli associated with the dam and nest to inhibit clonidine-induced ultrasound production has left the door open for alternative hypotheses (Hansen, 1993; Kehoe & Harris, 1989).

As described above, cold exposure has long been considered a sufficient stimulus for ultrasound production. Recent studies, however, have shown that elicitation of ultrasound production depends on the magnitude of the thermal challenge. Specifically, during moderate cold exposure, defined as the range of air temperatures in which heat production by brown adipose tissue (BAT) increases progressively (i.e., from about 25 °C to about 34 °C in week-old rats), pups do not vocalize (Blumberg & Sokoloff, 1998; Blumberg & Stolba, 1996). In contrast, at extreme air temperatures (i.e., < 25 °C in week-old rats), BAT thermogenesis increases no further, resulting in accelerated cooling and high rates of ultrasound production. In addition, whereas cardiac rate is maintained throughout the range of moderate air temperatures, exposure to extreme air temperatures results in substantial hypothermia. In turn, hypothermia results in bradycardia and increased blood viscosity, both of which conspire to decrease venous return to the heart (Blumberg, Sokoloff, & Kent, 1999; Blumberg, Sokoloff, & Kirby, 1997; Kirby & Blumberg, 1998; Sokoloff, Kirby, & Blumberg, 1998).

Similar to cold exposure, clonidine has profound effects on cardiovascular function. When administered to adults, clonidine acts on central and peripheral α_2 adrenoceptors, resulting in sympathoinhibition and bradycardia (Gillis, Gatti, & Quest, 1985; van Zweiten, 1996). Furthermore, because sympathetic outflow influences both cardiac rate and stroke volume (Guyton & Hall, 1996), it is likely that clonidine administration also causes a significant reduction in cardiac output. Given that clonidine administration in infant rats rapidly and simultaneously evokes bradycardia and ultrasound production (Sokoloff, Blumberg, Mendella, & Brown, 1997), it is possible that the effects of extreme cold exposure and clonidine administration on ultrasound production are due not to their common activation of a putative anxiety center but rather to their common effects on cardiovascular function.

If one hypothesizes that clonidine-induced ultrasound production is mediated by a state of anxiety, then one might test this hypothesis using known or suspected anxiolytics to try to block clonidine's effects. This research strategy has been recommended by those who view the infant's vocalization (Winslow & Insel, 1991) and rodent vocalizations in general (Miczek et al., 1995) as

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a tool for exploring affective states in animals. In contrast, if one hypothesizes that the infant's vocalization is an acoustic by-product of a physiological maneuver that promotes cardiovascular function (Blumberg, Sokoloff, Kirby, & Kent, 2000; Kirby & Blumberg, 1998), then one might administer an agent that stimulates cardiac rate through a peripheral action to try to block clonidine's effects. We used this latter approach in the present study. Therefore, in Experiment 1 prenalterol, a selective β_1 adrenoceptor agonist, was administered at one of two doses as a clonidine pretreatment to stimulate β_1 adrenoceptors on cardiac muscle and increase cardiac rate as well as stroke volume (Jennings, Bobik, Oddie, Hargreaves, & Korner, 1983). Because it acts peripherally, prenalterol was also expected to dampen or prevent clonidine-induced bradycardia. We hypothesized that inhibition of clonidine-induced bradycardia would also inhibit clonidine-induced ultrasound production.

Because β_1 adrenoceptors are not unique to cardiac tissue, it is possible that any effect that prenalterol pretreatment might have on clonidine-induced ultrasound production is mediated through stimulation of another system. For example, although β_3 adrenoceptors are predominantly responsible for heat production by BAT, β_1 receptors are also present in BAT and could play a role in heat production (Cannon, Jacobsson, Rehnmark, & Nedergaard, 1996). Therefore, in Experiment 1 we also measured skin temperature above the interscapular area (T_{is}), a region that overlies a major BAT deposit (Blumberg & Stolba, 1996; Smith & Horwitz, 1969).

Experiment 1: Does Prenalterol Pretreatment Inhibit Clonidine-Induced Ultrasound Production?

Method

Subjects. Twenty-four 8-day-old Harlan Sprague-Dawley male (n = 12) and female (n = 12) rat pups from eight litters were used. Weights ranged from 11.6 to 22.9 g. All pups were born to females in the animal colony at the University of Iowa. The pups were raised in litters culled to 8 pups within 3 days after birth (day of birth = Day 0). Mothers and their litters were housed in standard laboratory cages (48 × 20 × 26 cm), in which food and water were available ad libitum. All rats were maintained on a 12-hr light–dark schedule with lights on at 6 a.m.

Test environment. Individual pups were tested inside an incubator that was maintained at a thermoneutral air temperature (i.e., 34.5–36.0 °C) and a relative humidity greater than 40%. Pups were tested within a Plexiglas cylinder (i.d. = 11.5 cm, height = 7 cm).

Ultrasonic vocalizations. Ultrasonic vocalizations were detected with an ultrasonic microphone attached to a detector (Model SM100 QMC, London, UK) tuned to 42 ± 5 kHz. The microphone was placed inside the incubator near the pup. An observer, unaware of the experimental condition, scored ultrasound production by pressing the key of an event recorder every time an ultrasonic pulse was detected.

Temperature. Physiological temperature was measured using a digital thermometer (Omega, Stamford, CT). A chromel-constantan thermocouple (Omega) was held in place against the skin in the interscapular region to obtain a measure of T_{is}. The thermocouple was held in place until a stable reading was obtained. All thermocouples were calibrated before the experiment by using a mercury thermometer with an accuracy of 0.1 °C.

Procedure. On the day of testing, a pup with a visible milk band was removed from the nest and weighed. The pup was placed inside the incubator and allowed to acclimate for 60 min. Five minutes before the end of the acclimation period, T_{is} was measured. After the 60-min acclimation period, the pup was injected subcutaneously with saline or prenalterol (0.1 or 1.0 mg/kg; Hassle, Mölndal, Sweden) in a volume of 1 μl/g body weight. Thirty minutes after the pretreatment injection, T_{is} was again measured, followed immediately by a subcutaneous injection of clonidine hydrochloride (1.0 mg/kg; Sigma Chemical, St. Louis, MO); this dose is comparable to those used in other studies (Hård et al., 1988; Kehoe & Harris, 1989; Reinstein & Isaacson, 1977). Ultrasonic vocalizations were scored for another 30 min after the clonidine injection. At the end of the 60-min test, T_{is} was measured for the last time and the pup was returned to the nest. Two same-sex littermates were then tested in succession in the other experimental conditions, and test order was balanced across litters.

Data analysis. Data were imported into StatView 4.5 for the Macintosh for statistical analysis. The number of ultrasonic vocalizations emitted during the 30-min periods before and after clonidine injection was determined. Because ultrasonic vocalizations do not distribute normally, nonparametric tests were used (Blumberg & Stolba, 1996). Specifically, the Wilcoxon matched-pairs signed-ranks test was used to test for differences in ultrasound production between the saline and prenalterol pretreatment groups. Although both pre- and postclonidine administration data are presented, statistical analyses were conducted only for the postclonidine administration data. Alpha was set at .05 and was adjusted for multiple comparisons using the Bonferroni procedure.

T_{is} data obtained 30 min after pretreatment with prenalterol and 30 min after clonidine administration were compared with the baseline value of T_{is} to generate delta scores (ΔT_{is}). A single-factor analysis of variance (ANOVA) was used to determine whether there was an effect of pretreatment on T_{is}. Differences between treatment groups were tested using a Fisher's protected least significant difference (PLSD) post hoc test. Alpha was set at .05.

Results and Discussion

The upper plot of Figure 1 presents ultrasound production data for each of the 30-min periods before and after clonidine administration for pups pretreated with saline, 0.1 mg/kg prenalterol, or 1.0 mg/kg prenalterol. (All means are presented with their standard errors.) Before clonidine administration, ultrasound was infrequent in all three groups. After clonidine administration, ultrasound production increased in all three groups, but pups pretreated with saline vocalized significantly more than pups pretreated with either dose of prenalterol, $z = 2.5, p < .05$. Ultrasound production was not significantly different between the two prenalterol pretreatment groups.

The lower plot of Figure 1 presents the change in T_{is} from baseline (ΔT_{is}) immediately before and 30 min after clonidine administration for pups pretreated with saline or prenalterol. A single-factor ANOVA indicated a significant effect of pretreatment, $F(2, 21) = 22.6, p < .0001$, and post hoc analyses revealed that all three pretreatment groups were significantly different from each other. In addition, the effect of prenalterol on ΔT_{is} was dose dependent; specifically, pups pretreated with the low dose of prenalterol exhibited a 1.2 °C increase in T_{is} by the end of the 60-min test, and pups pretreated with the high dose of prenalterol exhibited a 2.7 °C increase.

To assess whether the increase in T_{is} after prenalterol pretreatment was because of activation of BAT thermogenesis, we used infrared thermography to image the dorsal skin surface of 2 additional week-old rats after administration of 1.0 mg/kg prenalterol. We found that although prenalterol administration produced increased skin temperatures, there was little indication that the heat production was isolated to the interscapular region, as is normally

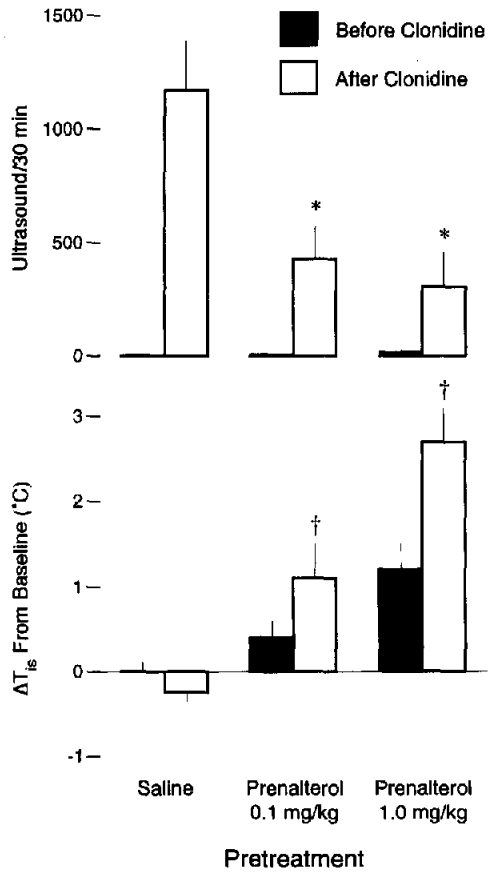

Figure 1. Mean (± *SEM*) ultrasound production and change in interscapular skin temperature (T_{is}) from baseline (ΔT_{is}) immediately before and 30 min after clonidine injection (1.0 mg/kg) for three pretreatment groups in Experiment 1: saline, low dose of prenalterol (0.1 mg/kg), and high dose of prenalterol (1.0 mg/kg). n = 8 per group. Statistical analyses were performed only for the 30-min period after clonidine injection. * Significantly different from the saline pretreatment group. † Significantly different from the other two pretreatment groups.

the case with increased BAT thermogenesis (Blumberg & Sokoloff, 1998). Therefore, because week-old rats cannot produce heat by shivering (Taylor, 1960), it is reasonable to conclude that prenalterol caused hyperthermia through a generalized increase in metabolic rate.

Although prenalterol's effect on T_{is} was dose dependent, its effect on ultrasound production was not. One might conclude from this that prenalterol's effects on heat production and ultrasound production are independent. Alternatively, it is possible that there exists a threshold below which ultrasonic vocalizations can be suppressed no further. Therefore, the present experiment does not allow us to rule out the possibility that temperature is an intervening variable that contributes to prenalterol's suppression of clonidine-induced ultrasound production. If temperature is an intervening variable, then merely exposing pups to a warm environment that increases body temperature should be sufficient to suppress ultrasound production. We examined this possibility in the next experiment.

Experiment 2: Does Mild Hyperthermia Inhibit Clonidine-Induced Ultrasound Production?

In Experiment 1, prenalterol pretreatment suppressed clonidine-induced ultrasound production but also increased T_{is}, thus suggesting that a raised body temperature contributed to prenalterol's effect on ultrasound production. To examine this possibility, we next exposed pups to either a 34.5 °C or 38 °C environment before and after clonidine administration. We chose 38 °C because pilot experiments revealed that this temperature would produce a 2 °C increase in T_{is}, similar to the increases produced by prenalterol pretreatment in Experiment 1.

Method

Subjects. Twelve 8-day-old Harlan Sprague-Dawley male (n = 6) and female (n = 6) rat pups from six litters were used. Weights ranged from 14.8 to 20.4 g. The pups were housed and raised as in Experiment 1.

Test environment. Individual pups were tested inside a circular, double-walled glass chamber, as previously described (Blumberg & Stolba, 1996): Water was circulated through the walls of the chamber, and by controlling the temperature of the water, air temperature (T_a) inside the chamber was also controlled. Compressed, humidified air passed through the chamber at a rate of 300 ml/min. Small access holes in the top and side of the chamber allowed for the passage of thermocouples. A round platform constructed of polyethylene mesh was fitted inside the chamber. When placed on the platform, a pup could move freely on the platform's surface.

Temperature. Chromel-constantan thermocouples were used to measure physiological and air temperatures. For measurement of T_{is}, a thermocouple was attached to the skin in the interscapular region using collodion as an adhesive. A thermocouple placed 4 cm below the polyethylene platform measured T_a within the metabolic chamber.

Ultrasonic vocalizations. Ultrasonic vocalizations were detected using a microphone that was sealed inside the lid of the metabolic chamber and attached to a bat detector. Ultrasound production was scored as in Experiment 1.

Procedure. On the day of testing, a pup with a visible milk band was removed from its cage, weighed, and placed inside the metabolic chamber maintained at an air temperature of 34.5 °C. After a 45-min acclimation period, scoring of ultrasonic vocalizations began and thermal data were acquired by computer using a customized data acquisition system (National Instruments, Austin, TX). For this first 30-min period, T_a either remained unchanged or was increased to 38.0 °C. Then, after this initial 30-min period, the animal was injected with 1.0 mg/kg clonidine in a volume of 1 μl/g body weight, and scoring of ultrasound production continued for another 30 min. After the 60-min test, the thermocouples were removed and the pup was returned to the nest. Finally, a same-sex littermate was tested in the other experimental condition, and test order was counterbalanced across litters.

Data analysis. Ultrasonic data were analyzed as in Experiment 1. ΔT_{is} data were derived as in Experiment 2, and the data were analyzed using a paired t test. Alpha was set at .05.

Results and Discussion

The upper plot of Figure 2 presents ultrasound production for pups that were exposed to the 34.5 °C or 38.0 °C environments during the 30-min periods before and after clonidine administration. (All means are presented with their standard errors.) Before clonidine administration, ultrasonic vocalizations were rare for pups in both thermal environments. After clonidine administration, however, ultrasound production increased in both groups, but the

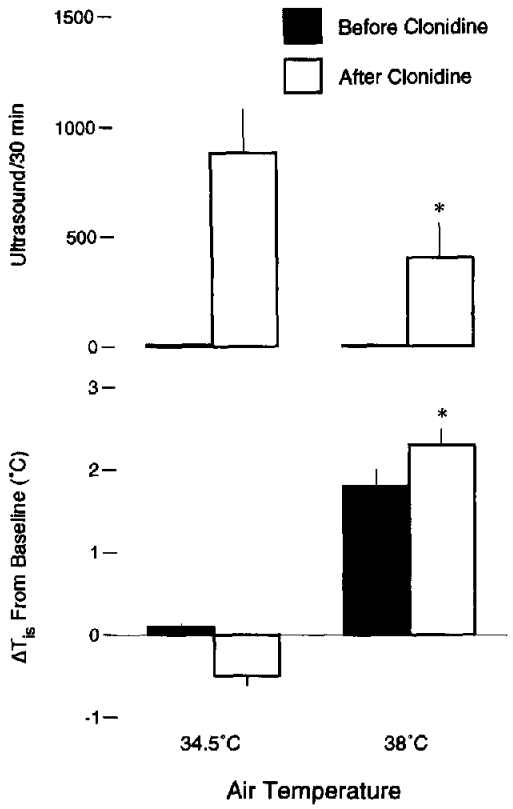

Figure 2. Mean (± *SEM*) ultrasound production and change in interscapular skin temperature (T_{is}) from baseline (ΔT_{is}) immediately before and 30 min after clonidine injection (1.0 mg/kg) for the two air temperature conditions in Experiment 2. Air temperature was either maintained at 34.5 °C throughout the 60-min test or increased to 38.0 °C at the beginning of the test. $n = 6$ per group. Statistical analyses were performed only for the 30-min period after clonidine injection. * Significantly different from the 34.5 °C condition.

pups in the 38 °C environment vocalized significantly less, $z = 2.0$, $p < .05$.

The lower plot of Figure 2 presents ΔT_{is} immediately before and 30 min after injection of clonidine for the pups in the two thermal environments. By the end of the test, pups in the 38.0 °C condition exhibited an increase in T_{is} of 2.3 °C, whereas pups in the 34.5 °C condition exhibited a small decrease. A paired t test indicated a significant effect of condition on ΔT_{is}, $t(5) = 11.1$, $p < .0001$.

The present results suggest that mild hyperthermia is sufficient to inhibit clonidine-induced ultrasound production, thus raising the possibility that prenalterol's inhibitory effects on ultrasound production in Experiments 1 and 2 were mediated in part by that drug's ability to raise body temperature. On the other hand, an increase in body temperature, whether produced by stimulation of endogenous heat production or by external heating, is sufficient to increase cardiac rate (Sokoloff et al., 1998). Thus, it is possible that both prenalterol pretreatment and hyperthermia suppressed clonidine-induced ultrasound production through their effects on cardiac rate or a related variable. To examine this possibility, in Experiment 3 we repeated the manipulations of the two previous experiments and monitored cardiac rate and T_{is} continuously.

Experiment 3: Cardiovascular and Thermoregulatory Effects of Clonidine Administration: Effect of Hyperthermia and Pretreatment with Prenalterol

We began this study by choosing prenalterol as a pretreatment for clonidine on the basis of its known ability to increase cardiac rate through peripheral action (Jennings et al., 1983) and its hypothesized ability to dampen the bradycardia produced by clonidine administration. Having demonstrated in Experiment 1 that prenalterol suppresses clonidine-induced ultrasound production, and with the additional finding of Experiment 2 that hyperthermia can do the same, we conducted the present experiment to determine the relative effects of prenalterol pretreatment and hyperthermia on cardiac rate both before and after clonidine administration.

Method

Subjects. Sixteen 8-day-old Harlan Sprague-Dawley male ($n = 8$) and female ($n = 8$) rat pups from nine litters were used. Weights ranged from 15.4 to 21.0 g. All pups were housed and raised as in Experiment 1.

Test environment. Individual pups were tested in a double-walled glass chamber as described in Experiment 2.

Temperature. T_a and T_{is} were measured as in Experiment 2.

Electrocardiogram (ECG). Three electrodes were used to obtain ECG data. Two electrodes were positioned transcutaneously on either side of the thoracic cavity, and a ground wire was attached near the base of the tail. All electrodes were secured with collodion. The ECG signals from the pup passed through the chamber lid to an impedance pneumograph (UFI, Morro Bay, CA) that was connected to a computerized data acquisition system.

Procedure. On the day of testing, a pup with a visible milk band was removed from the nest and weighed. The pup was then anesthetized with ether and placed on a heating pad. After ECG leads and the thermocouple were attached, the pup was placed inside the metabolic chamber maintained at 34.5 °C. The pup was given 45 min to recover from the anesthesia and to acclimate to the chamber.

After the 45-min acclimation period, data collection began and the pup experienced one of four pretreatment conditions: a subcutaneous injection of saline; a 0.1 or 1.0 mg/kg subcutaneous injection of prenalterol; or a change in temperature of the chamber from 34.5 °C to 38.0 °C (in the other three conditions, air temperature was maintained at 34.5 °C throughout the test). After 30 min, the pup was injected subcutaneously with 1.0 mg/kg clonidine. After the 60-min test ended, the pup was removed from the chamber, the ECG leads and thermocouples were removed, and the pup was returned to the nest.

Data acquisition. Throughout the test, thermal data were acquired by computer every 15 s as in Experiment 2. In addition, a second data acquisition system was used to acquire the ECG data. Specifically, the ECG signal was digitized at a rate of 1,000 samples/s, and interbeat intervals (IBIs) were determined on-line using a customized software program that uses a peak threshold detector (this method provides identical results to direct measurement of intervals between successive R-waves; Sokoloff et al., 1998). IBI data were acquired by the computer at a rate of 30 samples/min.

Data analysis. T_{is} and IBI data were imported into StatView 4.5 for the Macintosh for statistical analysis. T_{is} data were obtained from the minute before pretreatment, the minute preceding clonidine injection, and the 30th minute after clonidine injection. These data were compared with baseline data to generate ΔT_{is} scores for the four groups. A single-factor ANOVA was conducted to determine whether there was an effect of treatment on T_{is}, and a Fisher's PLSD post hoc test was used to determine which pretreatment groups differed from each other.

IBI data were obtained from the first minute before pretreatment, the minute preceding the clonidine injection, and the 30th minute after the

clonidine injection. IBI data were transformed to cardiac rate (beats per min) before statistical analysis. Cardiac rate data were compared with baseline to generate delta scores for the four groups (Δcardiac rate). A single-factor ANOVA was conducted to determine whether there was an effect of pretreatment on cardiac rate, and a Fisher's PLSD post hoc test was used to determine which pretreatment groups differed from each other. Alpha was set at .05 for all statistical tests.

Results and Discussion

The upper plot of Figure 3 presents ΔT_{is} immediately before and 30 min after administration of clonidine for pups in the four pretreatment groups: saline, 38.0 °C environment, low-dose prenalterol (0.1 mg/kg), and high-dose prenalterol (1.0 mg/kg). A single-factor ANOVA indicated a significant effect of experimental condition on ΔT_{is} at the end of the 60-min test, $F(3, 11) = 9.8$, $p < .005$. Post hoc tests revealed that the pups in the 38.0 °C and high-dose prenalterol groups differed significantly from the pups in the saline and low-dose prenalterol groups.

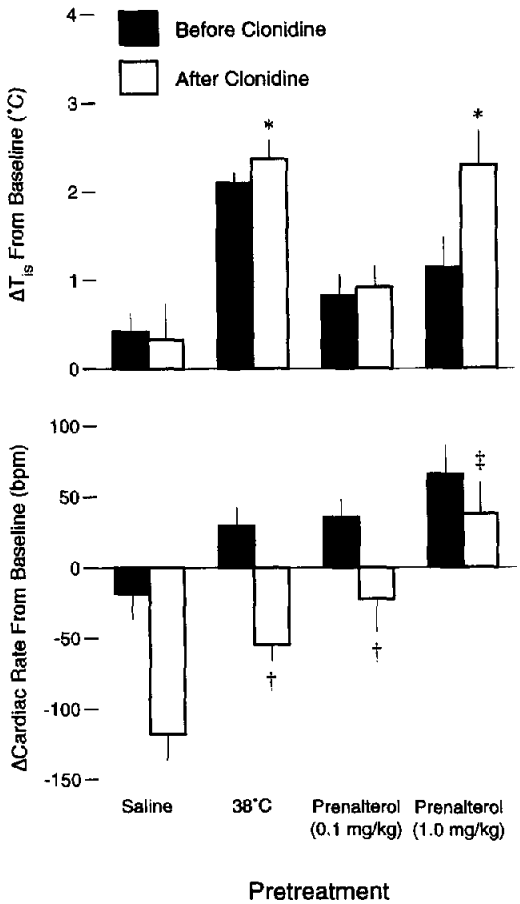

Figure 3. Mean (± *SEM*) change in interscapular skin temperature (T_{is}) from baseline (ΔT_{is}) and change in cardiac rate (Δcardiac rate) from baseline before and after clonidine injection (1.0 mg/kg) for the four pretreatment groups in Experiment 3: saline, testing at 38.0 °C, low-dose prenalterol (0.1 mg/kg), and high-dose prenalterol (1.0 mg/kg). $n = 4$ per group. Statistical analyses were performed only for the 30-min period following clonidine injection. Ultrasonic vocalizations were not scored in this experiment. bpm = beats per minute. * Significantly different from the saline and low-dose prenalterol pretreatment groups. † Significantly different from the saline and low-dose prenalterol pretreatment groups. †† Significantly different from the three other pretreatment groups.

The lower plot of Figure 3 presents Δcardiac rate immediately before and 30 min after administration of clonidine. As previously found (Sokoloff et al., 1997), clonidine administration produces a substantial decrease in cardiac rate in saline-pretreated pups. Consistent with our hypothesis, pretreatment with prenalterol and mild hyperthermia were sufficient to prevent or dampen clonidine-induced bradycardia. A single-factor ANOVA indicated a significant effect of experimental condition on Δcardiac rate at the end of the 60-min test, $F(3, 12) = 11.3$, $p < .001$. Post hoc tests revealed that the pups in the high-dose prenalterol group differed significantly from the other three groups and that pups in the 38.0 °C and low-dose prenalterol groups differed significantly from the pups in the saline group.

It is clear that prenalterol's effects on cardiac rate were not merely due to an indirect effect of increased body temperature. First, as shown in Figure 3, 1.0 mg/kg prenalterol and mild hyperthermia produced similar increases in T_{is}, and yet Δcardiac rate was significantly greater in the former condition at the end of the test. Second, 0.1 mg/kg prenalterol produced a significantly smaller increase in T_{is} than did the 38.0 °C condition, and yet Δcardiac rate was similar in the two conditions at the end of the test. On the other hand, it is clear that the mild hyperthermia alone was sufficient to dampen the effect of clonidine on cardiac rate. Therefore, it appears that prenalterol's impact on cardiac rate results from a combination of direct action on β_1 adrenoceptors on heart muscle and indirect action through its effect on the temperature of heart muscle.

General Discussion

The present series of experiments has explored the hypothesis that clonidine's stimulatory effect on ultrasound production is mediated by its effects on the cardiovascular system. This hypothesis was tested in Experiment 1 by pretreating infant rats with prenalterol, a β_1 adrenoceptor agonist that, as shown in Experiment 3, increases cardiac rate and dampens the bradycardic effects of clonidine. Pretreatment with prenalterol was also able to suppress the expression of clonidine-induced ultrasound production. In addition, however, to prenalterol's effects on cardiac rate, prenalterol increased body temperature, a variable that, as shown in Experiment 2, may mediate some of prenalterol's effects on ultrasound production. Interestingly, as shown in Experiment 3, hyperthermia also dampened the bradycardia produced by clonidine. Therefore, just as bradycardia—whether induced by clonidine administration or extreme cold—is associated with increased ultrasound production, prevention of clonidine-induced bradycardia—using either prenalterol or hyperthermia—is associated with reduced ultrasound production.

The focus on clonidine's cardiovascular effects in the present experiments derives from the hypothesis (Kirby & Blumberg, 1998) that ultrasonic vocalizations are acoustic by-products of the abdominal compression reaction (ACR), a maneuver in which contraction of the abdominal muscles at the end of expiration helps

to propel venous blood back to the heart when such return has been compromised (Youmans et al., 1963; Youmans, Tjioe, & Tong, 1974). It is likely that clonidine, by withdrawing sympathetic tone to the vasculature and heart, results in venodilation and decreased cardiac output and, consequently, decreased venous return (Goslinga, 1984; Guyton & Hall, 1996). The ACR hypothesis has been supported by two recent findings: First, pronounced intraabdominal pressure pulses occur in synchrony with ultrasound production in week-old rats during cold exposure (Kirby & Blumberg, 1998); and second, ultrasonic vocalizations emitted by 15-day-old rats injected with clonidine are associated with pulsatile increases in venous pressure, indicative of increased venous return (Blumberg et al., 2000).

Despite the support for the ACR hypothesis provided by the present and previous experiments, we have not demonstrated directly that venous return decreases as a result of clonidine administration or that prenalterol pretreatment prevents a decrease in venous return. In the present experiments, and for practical reasons, we have used cardiac rate as a "bioassay" of the cardiovascular changes with the understanding that both clonidine and prenalterol influence other aspects of cardiovascular function. Therefore, it is important that future experiments explicitly address the relationship between cardiac rate and venous return in infant rats.

It has been argued that one test of a drug's anxiolytic activity is its ability to reverse a pharmacologically or behaviorally induced anxiogenic state (Miczek et al., 1995). For example, if diazepam (a benzodiazepine agonist) suppresses ultrasound production in isolated infant rats, one might conclude that diazepam is anxiolytic in infant rats (Gardner, 1985). Using similar logic, because prenalterol suppresses clonidine-induced ultrasound production, one might conclude that prenalterol is anxiolytic in infant rats. The implausibility of such a conclusion, however, is apparent: Just as sympathoinhibitory agents such as clonidine are not typically considered to be anxiogenic, sympathomimetic agents such as prenalterol are not typically considered to be anxiolytic (Berkow, 1987).

Given the above considerations, the present results provide additional support for the hypothesis that extreme cold exposure and clonidine administration evoke ultrasound production through a common cardiovascular mechanism, that is, decreased venous return to the heart (Blumberg et al., 1999; Blumberg et al., 2000; Kirby & Blumberg, 1998). Clearly, further support for this hypothesis must come from the demonstration that any manipulation that decreases venous return is sufficient to trigger ultrasound production.

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