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Cardiovascular Concomitants of Ultrasound Production During Cold Exposure in Infant Rats

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Two experiments explored the cardiovascular consequences of extreme cold exposure and their relationship with ultrasound production in infant rats. Experiment 1 addressed the thermoregulatory and cardiovascular concomitants of ultrasound production during cold exposure in rats pretreated with saline or the ganglionic blocker chlorisondamine (5 mg/kg). For both groups, emission of ultrasound was associated with hypothermia and bradycardia. Experiment 2 explored whether the hypothermia experienced by pups in Experiment 1 is associated with increased blood viscosity, which is an important factor affecting venous return to the heart. Blood viscosity increased significantly as temperature decreased from 38 °C to 22 °C. These experiments suggest that, during extreme cold exposure, decreased cardiac output and increased blood viscosity combine to diminish venous return. The authors have hypothesized that pups respond to decreased return by recruiting the abdominal compression reaction, a physiological maneuver that propels blood back to the heart, resulting in emission of ultrasound as an acoustic by-product.

Infant rats emit ultrasonic vocalizations when separated from the nest (Hofer & Shair, 1978; Noirot, 1972). It has been known for many years that cold exposure, one of the consequences of nest separation, is perhaps the most effective stimulus for eliciting ultrasound production (Allin & Banks, 1971; Okon, 1971). In this context, exposure to cold has been conceptualized as a *token* or *sign stimulus,* providing information to the pup that it is no longer residing in the comfort of the nest. In addition, the vocalization itself has been interpreted as an indicator of an emotional state of anxiety or distress (Winslow & Insel, 1991). Thus, emission of the vocalization is viewed both as an outward manifestation of an emotional state as well as a communicatory behavior by which the pup informs the dam of its predicament and its location, resulting in maternal retrieval to the nest (Allin & Banks, 1972). The significance of this vocalization as a behavioral response to cold has been amplified by the view, prevalent for the past 50 years, that infant rats are physically and physiologically incapable of effective thermoregulation. Thus, because pups "can neither prevent progressive loss of body heat nor find their way back to the nest or to the mother" (Allin & Banks, 1971, p. 155), a distress call that elicits maternal retrieval would be an adaptive response.

Contrary to the conventional view of infant rats as inadequate thermoregulators, they in fact exhibit many signs of thermoregulatory success when tested under appropriate conditions (Blumberg & Sokoloff, 1998). For example, recent work has shown that isolated infant rats respond to moderate cold exposure (i.e., air temperatures of 25–35 °C for 1-week-olds) by producing heat using brown adipose tissue (BAT) and, by doing so, maintain cardiac rate and remain asleep; in addition, moderately cooled pups rarely emit ultrasonic vocalizations. In contrast, pups respond to extreme cold exposure (i.e., air temperatures below 25 °C for 1-week-olds) with bradycardia, behavioral arousal, and ultrasound production. On the basis of these and other results, we have hypothesized that cold exposure is not merely a token stimulus for the infant rat but rather initiates a cascade of events that culminates in recruitment of a physiological maneuver, called the abdominal compression reaction (ACR), that helps to propel venous blood back to the heart when such return has been compromised (Blumberg, Sokoloff, Kirby, & Kent, in press; Kirby & Blumberg, 1998). In addition, because this maneuver entails increased intraabdominal pressure and laryngeal constriction, ultrasound is produced as a by-product. It is important to stress, however, that this mechanistic perspective does not negate the potential communicatory benefits of ultrasound production for the infant (Blumberg & Alberts, 1997).

Although we have examined the thermal, cardiovascular, and ultrasonic responses of infant rats to cold exposure in separate experiments, we have not yet examined all three variables simultaneously. Therefore, in Experiment 1, these responses were monitored in week-old rats during moderate and extreme cooling. In addition, a second group of pups was cold-challenged after ganglionic blockade to assess the relations between cardiac rate and ultrasound production during cold exposure in the absence of BAT thermogenesis (Blumberg, Sokoloff, & Kirby, 1997; Sokoloff & Blumberg, 1998). The results of this first experiment provide evidence

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that both body temperature and cardiac rate contribute to the expression of ultrasound production during cold exposure.

Experiment 1: Relations Between Cardiac Rate and Ultrasound Production in Week-Old Rats During Cold Exposure

Method

Subjects. Fourteen 7-day-old Harlan Sprague-Dawley male rat pups from 14 litters were used. At the time of testing, pups weighed 15.2–18.9 g. All pups were born to females in the animal colony at the University of Iowa. The pups were raised in litters that were culled to 8 pups within 3 days after birth (day of birth = Day 0). Litters and mothers were raised 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 a double-walled glass chamber, as described in detail by Blumberg and Stolba (1996). Water passed through the walls of the chamber, and, by controlling the temperature of the water with a water circulator, air temperature (T_a) inside the chamber could also be controlled. Compressed, humidified air passed through the chamber at the rate of 300 ml/min. A round platform constructed of polyethylene mesh was fitted inside the chamber. When placed on the platform, the pup could move freely on the platform's surface.

Electrocardiogram (ECG). ECG data were acquired using three electrodes (Blumberg et al., 1997). Two electrodes were attached on either side of the thoracic cavity, and a ground electrode was attached near the base of the tail. Collodion was used to secure the electrodes and to improve the electrical connection. The ECG leads from the pup were connected to an impedance pneumograph (UFI, Morro Bay, CA) before being acquired by the computer.

Temperature. Physiological and air temperatures were measured with chromel–constantan thermocouples (Blumberg & Stolba, 1996). T_a within the metabolic chamber was measured with a thermocouple located 4 cm beneath the platform. The two physiological temperatures were attained by attaching thermocouples to the skin surface, using collodion as an adhesive. One thermocouple was attached in the interscapular region above the brown fat pad, thus providing a measure of interscapular temperature (T_{is}). The other thermocouple was attached in the lumbar region, thus providing a measure of back temperature (T_{back}).

Oxygen consumption ($\dot{V}O_2$). $\dot{V}O_2$ measurements were made as described elsewhere (Blumberg & Stolba, 1996). Briefly, a two-channel electrochemical oxygen analyzer was used to compare the oxygen concentration of the airstream passing to the chamber with the exhaust airstream passing from the chamber. The percentage difference in oxygen concentration between these two airstreams was computed to 0.001% and input to the data-acquisition system that computed $\dot{V}O_2$ in ml of O_2 per kilogram body weight per min.

Ultrasonic vocalizations. Ultrasonic vocalizations were detected using a microphone sealed inside the lid of the metabolic chamber. The microphone was connected to a bat detector (QMC, Ltd., London, UK, Model SM100) tuned to a ±5 kHz range centered on 42 kHz.

Procedure. On the day of testing, a pup with a visible milk band was removed from its cage, lightly anesthetized with ether, and placed on a heating pad. After ECG leads were attached, the pup was then placed inside an incubator maintained at 35–36 °C and injected with either saline or chlorisondamine hydrochloride (5 mg/kg; Ciba Geigy) in a volume of 1 µl/g body weight. After injection, the thermocouples were attached. Next, the pup was placed inside the metabolic chamber maintained at approximately 35 °C. The pup was given at least 45 min to recover further from the anesthesia and acclimate to the chamber.

The test began with a 15-min period of baseline data acquisition at 35 °C. Then, the test consisted of a series of air temperature drops to 29 °C and 23 °C with 60 min between each successive decrease; in addition, the saline-treated pups were cooled further to 17 °C for an additional 60 min. Throughout the test, each pup was videorecorded through the Plexiglas lid of the chamber by a microcamera connected to a VHS videorecorder; the stopwatch on a time–date generator was reset after each air temperature drop. After the test, the pup was removed from the chamber, ECG leads and thermocouples were removed, and the pup was returned to its home cage. The oxygen consumption system was allowed to rezero to confirm minimal drift over the course of the test.

We should note that the use of long test durations in which air temperature is decreased in succession raises questions regarding the effect of time on behavioral and physiological responding in the cold. The test durations used here, however, are well within the time limits for the expression of normal responses (see Sokoloff & Blumberg, 1998).

Data acquisition. Throughout the test, thermal and metabolic data were recorded every 15 s by a customized data-acquisition system (National Instruments, Austin, TX). The ECG analog signal was digitized at a rate of 1,000 samples/s, and interbeat intervals (IBI) were determined on-line with a customized software program using a peak threshold detector (this method provides identical results to direct measurement of intervals between successive R-waves; Sokoloff, Kirby, & Blumberg, 1998). IBI data were acquired by the computer at a rate of 30 samples/min. In addition to on-line data acquisition, the ECG signal was recorded to one of the videotape's audio channels; ultrasonic vocalizations were recorded to the second audio channel.

Ultrasonic vocalization data were scored after the test by an experienced observer. To do this, the observer played back the videotape and, using an event recorder written in HyperCard for the Macintosh, pressed a computer key each time an ultrasonic pulse was detected. Each key press recorded the time at which the ultrasonic pulse occurred, synchronized to the stopwatch impressed on the video record.

Statistical analysis. IBI and ultrasound data were imported into StatView 4.5 for the Macintosh (SAS Institute, 1998). For some analyses, IBI data were converted to cardiac rate data in beats per min (bpm). Values at the end of the baseline period and at the end of each 60-min period of cooling were compared. A repeated-measures analysis of variance (ANOVA) was used to compare the T_{is}, $T_{is} - T_{back}$, $\dot{V}O_2$, and cardiac rate data at baseline and after the first two air temperature drops. Post hoc two-tailed tests consisted of unpaired *t* tests for between-group analyses and paired *t* tests for within-group analyses. For all tests, alpha was set at .05 and was adjusted for multiple comparisons using the Bonferroni procedure.

Cumulative amounts of ultrasound production were calculated for the baseline period (15 min) and for each of the subsequent cooling periods (60 min each); baseline levels of ultrasound production were multiplied by a factor of 4 to compensate for the shorter time period. The Wilcoxon matched-pairs signed-ranks test was used to test for between- and within-group differences in ultrasound production. All of these tests were one-tailed, and alpha was set at .05 and was adjusted for multiple comparisons using the Bonferroni procedure.

Loss of oxygen consumption data sometimes occurred when the chamber lid was opened during the test (e.g., to untangle thermocouples) and there was insufficient time for the chamber to restabilize before the next reading was taken. In addition, for one

pup injected with chlorisondamine, it was apparent that the ganglionic blockade was no longer complete for the final reading at 23 °C; in this case, all affected data were discounted.

All means are presented with their standard errors.

Results and Discussion

Physiological data from the end of each period of the test are presented in Figure 1. These data are similar to those of previous experiments performed using similar procedures (Blumberg et al., 1997; Blumberg & Stolba, 1996; Sokoloff & Blumberg, 1998). Specifically, saline-injected pups responded to a decrease in T_a to 29 °C by increasing BAT thermogenesis, as evidenced by increasing values of $\dot{V}O_2$ and $T_{is} - T_{back}$, as well as maintenance of cardiac rate. As T_a was decreased further to 23 °C and 17 °C, however, and as BAT thermogenesis was maximized, the pups exhibited large decreases in T_{is}, $\dot{V}O_2$, and cardiac rate. In contrast, pretreatment with chlorisondamine resulted in progressive decreases in these variables with each drop in T_a. All main effects and interactions as tested by repeated-measures ANOVA were significant, T_{is}, $T_{is} - T_{back}$, and cardiac rate: main effect of condition, Fs(1, 11) > 67.4, $p < .0001$; main

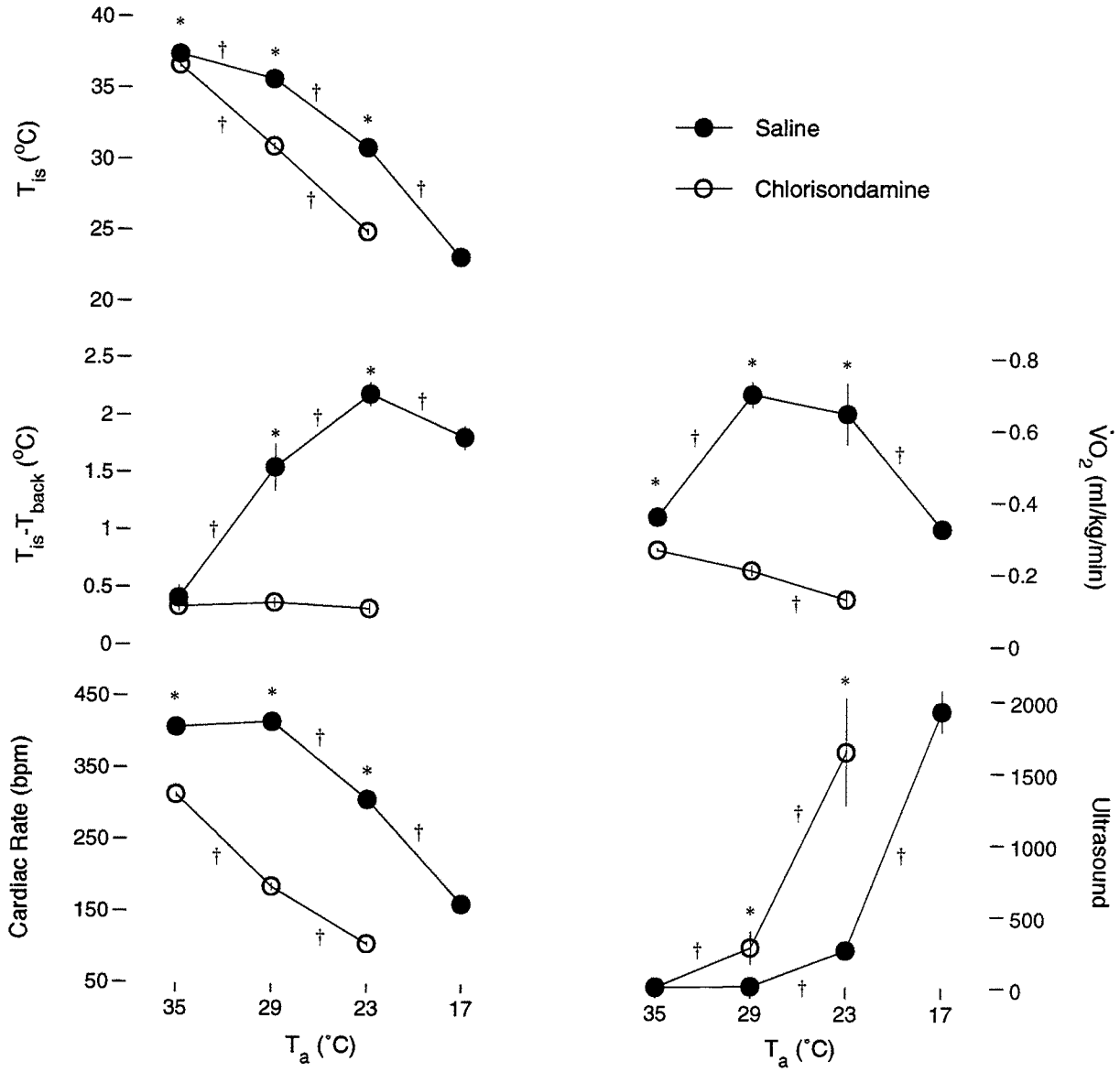

Figure 1. Interscapular temperature (T_{is}), interscapular temperature minus back temperature ($T_{is} - T_{back}$), cardiac rate (beats per min; bpm), oxygen consumption ($\dot{V}O_2$), and ultrasound production at each air temperature (T_a) for the week-old rats in Experiment 1. Pups were injected after the baseline period with either saline or a ganglionic blocker (chlorisondamine, 5 mg/kg) before testing. n = 6–7 per group. Values are mean ± *SE*. Asterisks denote significant differences between groups, $p < .05$. Daggers denote significant differences between adjacent points, $p < .05$.

effect of air temperature, $Fs(2, 22) > 79.3$, $p < .0001$; interaction, $Fs(2, 22) > 47.7$, $p < .0001$; oxygen consumption: main effect of condition, $F(1, 8) = 125.6$, $p < .0001$; main effect of air temperature, $F(2, 16) = 11.4$, $p < .001$; interaction, $F(2, 16) = 33.8$, $p < .0001$. Finally, the physiological responses of pups pretreated with chlorisondamine indicate that ganglionic blockade was maintained throughout the experiment, consistent with earlier experiments that used even longer test durations (Sokoloff & Blumberg, 1998).

Cumulative ultrasound production during the 15-min period at thermoneutral and during each of the 60-min periods of cooling is also presented in Figure 1. In unblocked pups, ultrasound production increased substantially across the transition from moderate to extreme cold exposure, as we have shown previously (Sokoloff & Blumberg, 1997). The most dramatic increase in ultrasound production occurred as T_a decreased from 23 °C to 17 °C when both $\dot{V}O_2$ and cardiac rate fell considerably. Figure 1 also presents ultrasound production for the ganglionically blocked pups. Although the form of the ultrasound response is similar to that for the saline-injected pups, the curve has been shifted to the left such that the T_a of 23 °C now elicited very high rates of ultrasound production.

Figure 2 presents polynomial regressions for IBI and ultrasound production versus T_{is} for the pups pretreated with saline and chlorisondamine. T_{is} accounts for 98% or more of the variance in IBI for both groups, consistent with previous results (Blumberg et al., 1997). In addition, T_{is} accounts for 92% of the variance in ultrasound production for the unblocked pups and 69% of the variance for the ganglionically blocked pups. These regressions should be viewed with

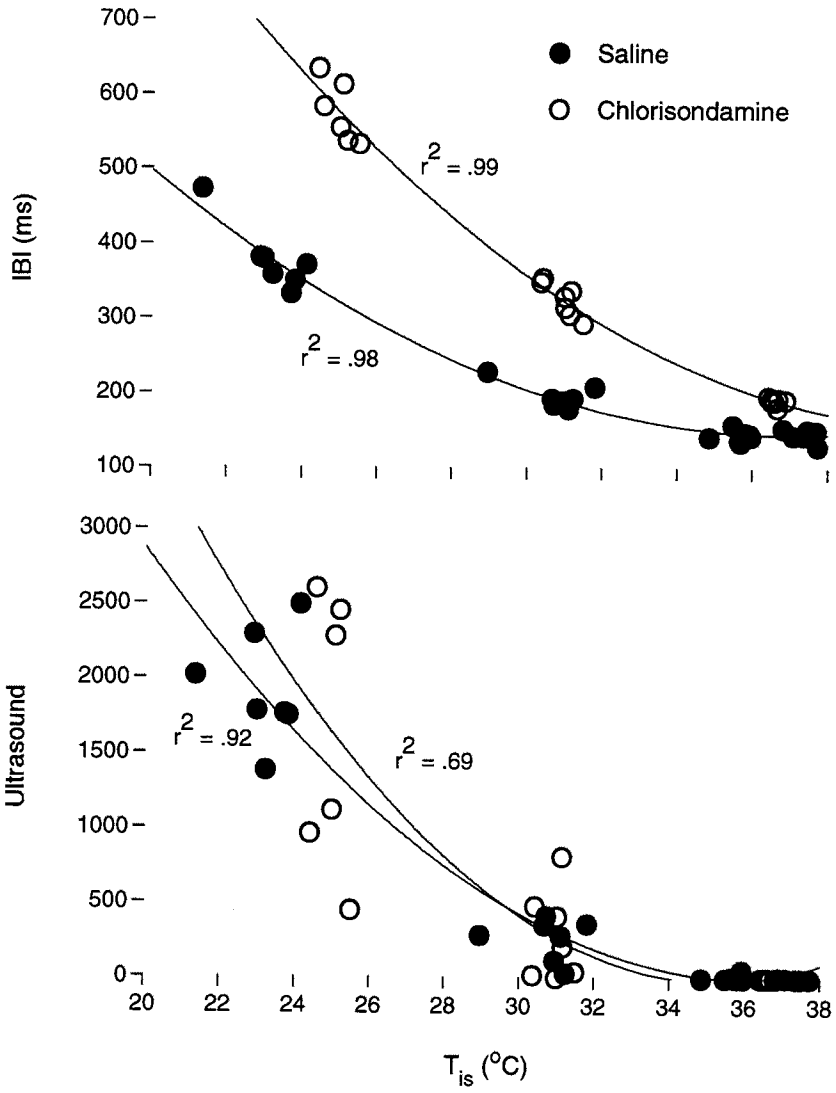

Figure 2. Polynomial regressions for interbeat interval (IBI) versus interscapular temperature (T_{is}) and ultrasound production versus T_{is} for the week-old rats in Experiment 1. Pups were injected with either saline or a ganglionic blocker (chlorisondamine, 5 mg/kg) before testing. Multiple data points from each pup are used in these regressions.

caution given that they are based on the use of multiple data points from each pup (see Blumberg et al., 1997) and that ultrasound data are not ideally analyzed using parametric statistics. Nonetheless, these analyses clearly suggest similar nonlinear effects of decreasing body temperature on IBI and ultrasound production.

Selective antagonists of BAT thermogenesis are still not available, and surgical removal of the highly vascularized interscapular BAT is too invasive. Therefore, we used ganglionic blockade to inhibit BAT thermogenesis in the present experiment, and in previous experiments (Blumberg et al., 1997; Sokoloff & Blumberg, 1998; Sokoloff et al., 1998). Ganglionic blockers like chlorisondamine, however, affect autonomic control of multiple organs, including the heart. To address this issue of nonselectivity, we have previously used multiple techniques in ganglionically blocked infants, including selective activation of BAT thermogenesis with a β_3 agonist and manipulation of interscapular temperature with a thermode (Sokoloff & Blumberg, 1998; Sokoloff et al., 1998). Those experiments have provided strong evidence that cold-induced changes in cardiac rate and active sleep behavior in ganglionically blocked pups are primarily attributable to the absence of BAT thermogenesis. The orderly vocal responses of blocked pups in the cold seen in the present experiment are consistent with that interpretation.

Although the present results suggest that changes in cardiovascular function modulate ultrasound production, it is clear that cardiac rate alone is not a strong predictor. For example, as shown in Figure 1, bradycardia induced by ganglionic blockade at 35 °C was not sufficient to evoke ultrasound production. In addition, although blocked pups at an air temperature of 29 °C and unblocked pups at 17 °C exhibited similar cardiac rates, their ultrasound production was very different (280 vs. 1932 pulses, respectively). When one considers the corresponding values of T_{is}, however, a possible explanation presents itself: For example, even though cardiac rates are similar for blocked and unblocked pups at air temperatures of 29 °C and 17 °C, respectively, T_{is} is substantially higher in the blocked pups (i.e., 30.9 °C vs. 23.1 °C). Therefore, it is possible that thermal and cardiovascular variables act together to modulate the expression of ultrasound production.

As described above, we hypothesized that ultrasound production is the acoustic by-product of the ACR, a maneuver that increases venous return to the heart (Kirby & Blumberg, 1998). It follows from this hypothesis that decreased venous return, or a related variable, is the initiating stimulus for the ACR and, therefore, ultrasound production; indeed, this hypothesis received considerable support from Youmans and his colleagues in their exhaustive studies of the ACR in adult dogs (Youmans et al., 1963). Interestingly, the two major factors that affect venous return are associated with cardiac rate and body (i.e., blood) temperature. To clarify this point, some background is necessary.

Cardiac output (i.e., the volume of blood pumped by the heart per unit time) is the product of cardiac rate and stroke volume. In infant mammals, however, all available evidence indicates that stroke volume cannot be increased above resting levels. Because of this limitation in stroke volume, cardiac output in infants is largely dependent on cardiac rate (Shaddy, Tyndall, Teitel, Li, & Rudolph, 1988; Teitel et al., 1985); when cardiac rate decreases, cardiac output decreases as well. In turn, when cardiac output decreases, backpressure within the heart leads to increased right atrial pressure and subsequent retardation of venous return (Guyton & Hall, 1996).

Another major factor that influences venous return is resistance in the peripheral circulation, and one of the major factors influencing this resistance is blood viscosity (Goslinga, 1984). This factor is particularly relevant for this discussion because blood temperature is a major determinant of its viscosity (Guard & Murrish, 1975; Maclean, 1981): As temperature decreases, viscosity increases, and venous return is compromised.

Therefore, in light of the ACR hypothesis and based on considerations of venous return and the factors that influence it, the apparent mutual contributions of cardiac rate and body temperature to ultrasound production begin to make sense. It is not known, however, whether blood viscosity increases in infant rats at body temperatures corresponding to those measured in the present experiment. This question is addressed in Experiment 2.

Experiment 2: Effect of Temperature on Blood Viscosity in Week-Old Rats

The effect of temperature on blood viscosity has been examined in a number of mammalian and nonmammalian species, including flounder, hibernating and nonhibernating rodents, and humans (Graham & Fletcher, 1983; Maclean, 1981; Rand, Lacombe, Hunt, & Austin, 1964; Snyder, 1971). To our knowledge, however, the effect of temperature on blood viscosity in infant mammals has not been investigated. Therefore, in the present experiment, the blood viscosity of week-old rats is measured at three temperatures: 38 °C, 30 °C, and 22 °C. These three temperatures were chosen to be evenly spaced and to correspond roughly with the interscapular temperatures measured in Experiment 1 (see Figure 1); for the present purposes, interscapular temperature provides a reasonable estimate of deep body temperature (Spiers & Adair, 1986). The aim of the present experiment was to determine whether the decreases in body temperature during cold exposure found in Experiment 1 were sufficient to significantly increase blood viscosity and thereby influence venous return and trigger recruitment of the ACR and emission of ultrasound.

Method

Subjects. Seventeen 8-day-old Harlan Sprague-Dawley male and female rats from 8 litters were used. At the time of testing, pups weighed 9.3–18.6 g. All pups were born to females in the animal colony at the University of Iowa. The pups were raised in litters that were culled to 8 pups within 3 days after birth (day of birth = Day 0). Litters and mothers were raised in standard laboratory cages (48 × 20 × 26 cm) in which food (Purina rat chow) and water

were available ad libitum. All rats were maintained on a 12-hr light–dark schedule with lights on at 6 a.m.

Blood viscosity. Blood viscosity measurements were made with a cone-plate viscometer with a cone angle of 0.8° (Model DV-II+, Brookfield Engineering, Stoughton, MA). The viscometer was preprogrammed to step through a series of speeds for the determination of blood viscosity at multiple shear rates (shear rate within a blood vessel is proportional to flow velocity and inversely proportional to vessel radius; Graham & Fletcher, 1983). The sample cup, which holds the blood to be tested, is double walled to allow the circulation of temperature-controlled water; a temperature probe is embedded in the cup for continuous measurement of sample temperature. Blood sample volumes were always equal to 0.6 ml.

Procedure. On the day of testing, a pup with a visible milk band was removed from its cage and injected with 0.1 ml of Nembutal (sodium pentobarbitol). After the pup was anesthetized, the thoracic cavity was opened and the heart was exposed. A 27-g needle attached to a heparinized syringe was used to withdraw as much blood as possible from the right ventricle. In all but 1 case, blood from littermates was pooled to achieve a sample volume greater than 0.6 ml; pooling of blood from 2 littermates occurred in five cases, and pooling from 3 littermates occurred in two cases. A small portion of the withdrawn blood was transferred to a capillary tube for measurement of hematocrit and plasma protein concentrations.

The 0.6-ml volume of blood was quickly transferred to the sample cup, which was then attached to the viscometer. At the start of the experiment, the sample temperature was set at either 38 °C or 22 °C. After 5 min, the test began by turning on the viscometer motor to the lowest speed and recording the displayed viscosity. The motor was then turned off, the viscometer was set to the next highest speed, the motor was turned on again, and viscosity was measured. This procedure was repeated for all eight speeds, each of which corresponds to shear rates ranging from 7.5 s^{-1} to 113 s^{-1}, and then the entire procedure was repeated twice. Therefore, three viscosity measurements were made at each shear rate. Finally, this procedure was repeated at each of the other two sample temperatures in ascending (22°–30°–38°) or descending (38°–30°–22°) order. The test was completed in less than 60 min. After the test, blood samples were inspected for clotting; in no case was clotting observed.

Statistical analysis. Viscosity data were imported into StatView 4.5 for the Macintosh. First, average viscosity measures were computed for each shear rate at each sample temperature. Next, a repeated-measures ANOVA was used to examine the effects of shear rate (repeated measure) and sample temperature (single factor) on viscosity. Post hoc single-factor ANOVAs were performed on the data at a low, intermediate, and high shear rate (i.e., 7.5, 37.5, and 113.0 s^{-1}), and Fisher's protected least significant difference was used to test for differences between sample temperatures at each shear rate. For all tests, alpha was set at .05. Means are presented with their standard errors.

For one blood sample at one temperature, blood viscosity measurements were more than 2 standard deviations from the mean. Therefore, all data from this sample were excluded from the analyses below.

Results and Discussion

For the 8 blood samples, mean hematocrit and plasma protein concentrations were 36 ± 1% and 4.2 ± 0.1 g/100 ml.

Figure 3 presents viscosity as a function of shear rate for the three sample temperatures used. First, it can be seen that viscosity increases dramatically at the two lowest shear rates tested, as is typically found (Maclean, 1981). Second, the 22 °C curve is substantially higher than the 30 °C and 38 °C curves at all shear rates. A repeated measures ANOVA indicated a significant effect of temperature, $F(1, 18) = 18.0$, $p < .0001$, and shear rate, $F(7, 126) = 123.8$, $p < .0001$, as well as a significant Temperature × Shear Rate interaction, $F(14, 126) = 4.3$, $p < .0001$.

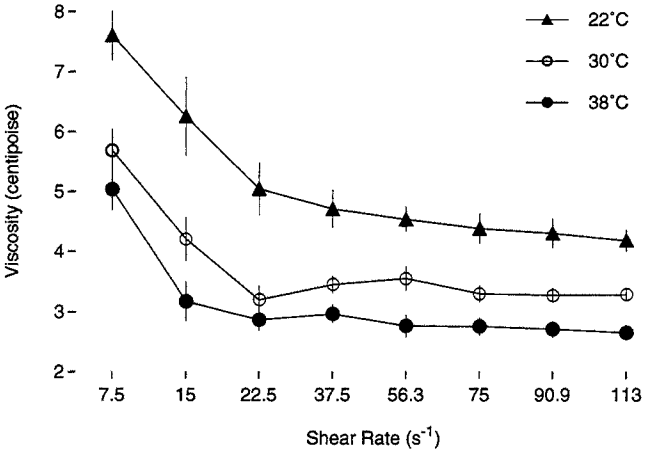

Figure 3. Mean (±*SE*) viscosity (centipoise) as a function of shear rate (s^{-1}) for the blood samples of week-old rats. Each sample was tested at three temperatures, 22 °C, 30 °C, and 38 °C. $n = 7$ per group.

Figure 4 presents viscosity as a function of temperature at a low, intermediate, and high shear rate. The plots clearly indicate that viscosity increases progressively with decreasing temperature at each shear rate, $Fs(2, 18) > 11.2$, $p < .001$. Moreover, these increases in viscosity are nonlinear, especially at the lower shear rates. A nonlinear increase in viscosity at the lower shear rates is particularly significant because lower shear rates are associated with venous flow and higher shear rates are associated with arterial flow (Goslinga, 1984; Graham & Fletcher, 1983), thus suggesting that extreme cold exposure has a disproportionate impact on venous flow and, therefore, venous return. Unfortunately, there are no data that allow us to relate particular shear rates with blood flow in particular blood vessels (e.g., capillaries, arteries, venules, large veins) in infant rats under varying conditions. Regardless, the present experiment makes it clear that decreasing temperature has a dramatic impact on blood viscosity, a finding that has important consequences for blood flow on the venous side of the circulation because of its marked sensitivity to blood viscosity (Goslinga, 1984).

Perhaps the most striking aspect of the plots in Figure 4 is their correspondence with those presented in Figure 2. Specifically, blood viscosity (at low shear rates), IBI, and ultrasound production all exhibit similar, nonlinear increases with decreasing temperature. Therefore, the present results, in conjunction with those in Experiment 1, provide converging evidence that extreme cold exposure, through its effects on cardiac rate and blood viscosity, has a significant impact

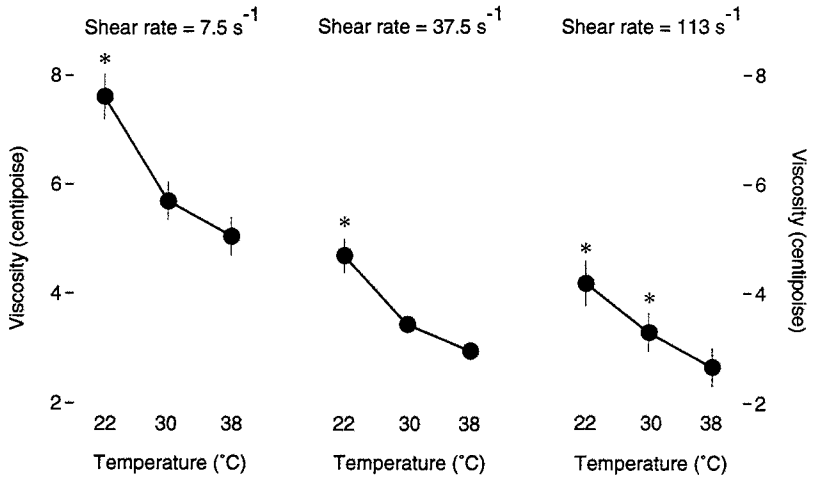

Figure 4. Mean (±*SE*) viscosity (centipoise) as a function of sample temperature at low (7.5 s^{-1}), intermediate (37.5 s^{-1}), and high (113 s^{-1}) shear rates. $n = 7$ per group. Asterisks denote significant difference from the other two temperatures, $p < .05$.

on venous return to the heart, thus providing ample justification for recruitment of the ACR.

General Discussion

By utilizing cold exposure to evoke ultrasound production, Experiment 1 demonstrated a systematic relationship between BAT thermogenesis, cardiac rate, and ultrasound production in week-old rats, regardless of whether or not pups were ganglionically blocked. This experiment also suggested that bradycardia is a contributing factor to ultrasound production. A second factor, hypothermia, was also implicated in that experiment. These two responses—bradycardia and hypothermia—have important consequences for venous return. First, because infant mammals have a limited capacity to increase stroke volume (Shaddy et al., 1988), bradycardia implies decreased cardiac output and, therefore, decreased venous return (Guyton & Hall, 1996). Second, as shown in Experiment 2, hypothermia, even in the range of body temperatures examined in Experiment 1, results in dramatic increases in blood viscosity; increased viscosity has important implications for venous flow and, ultimately, venous return (Goslinga, 1984). Finally, the present finding of increased blood viscosity during cold exposure adds to the list of physiological and behavioral responses that are altered substantially across the transition from moderate to extreme cold exposure (Blumberg & Sokoloff, 1998).

Viscosity of whole blood, such as that tested in Experiment 2, is determined by plasma viscosity, cell concentration (i.e., hematocrit), red cell deformability, and cell aggregation (Chien, 1975), and the two primary determinants of plasma viscosity are temperature and plasma protein concentration (Lowe, 1987). Furthermore, whole blood is a non-Newtonian fluid; that is, its effective viscosity depends on its shear rate (Goslinga, 1984); it is the presence of deformable red cells, whose behavior depends to a large degree on flow rate, that is responsible for the shear rate dependence of whole blood seen in Figure 3. Therefore, the effective viscosity of whole blood is highest at low temperatures and at low shear rates, as shown in Figure 4. Finally, because shear rates on the venous side of the circulation are lower than those on the arterial side, resistance to venous flow is particularly sensitive to changes in blood viscosity (Allen & Patterson, 1995; Goslinga, 1984; Graham & Fletcher, 1983). In their entirety, the present results indicate that during extreme cold exposure, decreased cardiac output, decreased shear rates in blood vessels, and increased blood viscosity all conspire to impede venous return.

Faced with decreased venous return, the infant rat must respond appropriately, and we have hypothesized that one such response is recruitment of the ACR (Kirby & Blumberg, 1998). The ACR was studied intensively by Youmans and his colleagues over 25 years ago but has received little attention in the intervening period (Youmans et al., 1963; Youmans, Tjioe, & Tong, 1974). The ACR is characterized by contraction of the abdominal muscles during or after expiration, resulting in the propulsion of blood back to the heart. As in adults, contraction of the abdominal muscles in week-old rats results in intraabdominal pressure pulses that are synchronized with ultrasound production (Kirby & Blumberg, 1998). Furthermore, laryngeal constriction during expiration, by braking expiratory flow, would amplify these increases in intraabdominal pressure as well as produce sound as a by-product (Blumberg & Alberts, 1990; Kirby & Blumberg, 1998; Roberts, 1972; Symonds et al., 1995). Finally, the ACR hypothesis was given its strongest support by the recent finding that emission of ultrasonic vocalizations corresponds with pulsatile increases in venous pressure, indicative of increased venous return (Blumberg et al., in press). Therefore, the available evidence indicates a correspondence between the physiological cause of ultrasound production (i.e., decreased venous return) and the physiological consequence of ultrasound production (i.e., increased venous return).

Figure 5 is a schematic representation of the hypothesized cascade of physiological and behavioral consequences of extreme cold exposure in unmanipulated infant rats. This cascade begins with the direct effects of cooling on the functional properties of heart muscle and blood, that is, decreased cardiac rate and increased blood viscosity, respectively. Consequently, venous return is compromised. Recruitment of the ACR improves venous return and, in conjunction with increased peripheral resistance, contributes to the maintenance of arterial pressure. Initiation of the ACR also results in the production of ultrasound as a by-product. If, as a consequence of ultrasound production, the mother retrieves the pup to the nest, the pup's exposure to extreme cold terminates and the stresses on the cardiovascular system are alleviated.

All together, the present results reinforce the notion that the thermoregulatory and cardiovascular systems are functionally intertwined, especially in infants that are more susceptible to hypothermia during extreme thermal challenge. The effects of hypothermia on cardiac rate (Blumberg et al., 1997; Lyman & Blinks, 1959; Sokoloff et al., 1998) and blood viscosity (Maclean, 1981) are well established.

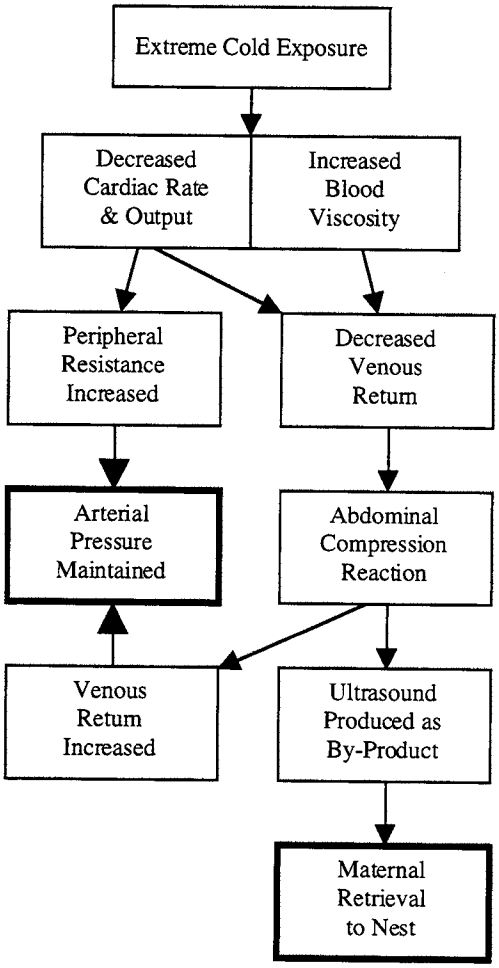

Figure 5. Cascade of events hypothesized to result from extreme cold exposure in infant rats.

The present results, however, go further by revealing in the infant rat how corresponding changes in cardiac rate and blood viscosity at the transition from moderate to extreme cold exposure can collaborate to impede venous return and thereby trigger recruitment of the ACR and initiation of ultrasound production.

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