Neural and hormonal control of arterial pressure during cold exposure in unanesthetized week-old rats

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Blumberg, Mark S., Tricia G. Knoot, and Robert F. Kirby. Neural and hormonal control of arterial pressure during cold exposure in unanesthetized week-old rats. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R1514–R1521, 2001.—Infant rats respond to cold exposure with increased heat production by brown adipose tissue (BAT). BAT thermogenesis increases steadily with increasing cold exposure, but a point occurs at which thermogenesis can increase no further, resulting in cold-induced bradycardia. Previous work has shown that mean arterial pressure (MAP) is maintained even when cardiac rate decreases as much as 50% from baseline values. We examined the neural and hormonal contributions to peripheral resistance during cold exposure after pups were injected subcutaneously with vehicle, an α_1-adrenoceptor antagonist (prazosin; 0.5 mg/kg), an ANG II receptor antagonist (losartan; 1 mg/kg), a vasopressin receptor antagonist (Manning compound; 0.5 mg/kg), or simultaneous administration of all three antagonists (triple block). Interscapular temperature, oxygen consumption, cardiac rate, and arterial pressure were monitored as air temperature was sequentially decreased from thermoneutral (i.e., 35°C) to 29, 23, and 17°C. Only pups in the triple block condition exhibited significant decreases in MAP with cooling, even though all pups exhibited substantial decreases in cardiac rate. A followup study suggested that blockade of all three systems was more effective than blockade of any two systems. Finally, at 17°C, ultrasonic vocalizations were accompanied by significant increases in MAP, replicating a previous finding and supporting the hypothesis that the vocalization is the acoustic by-product of the abdominal compression reaction, a maneuver that helps to maintain venous return during cardiovascular challenge.

sympathetic nervous system; vasopressin; angiotensin; cardiac rate; ultrasonic vocalization; thermoregulation; blood viscosity

CONTRARY TO A ONCE PREVALENT VIEW, infant rats are capable thermoregulators (2). When sequentially exposed to decreasing air temperatures, pups incrementally increase nonshivering thermogenesis using brown adipose tissue (BAT) and also increase oxygen consumption and respiratory rate. During this moderate cooling, the temperature of the interscapular BAT deposit, the largest such deposit in infant rats, is maintained, as is cardiac rate (5, 26). Behaviorally, these pups remain asleep (25). In contrast, when the thermal challenge intensifies and becomes extreme, BAT thermogenesis can no longer compensate for heat loss, resulting in substantial bodily cooling and bradycardia as well as behavioral arousal and emission of an ultrasonic vocalization.

The transition from moderate to extreme cooling affects all of the physiological and behavioral variables studied thus far, except arterial pressure. To demonstrate this, we developed a method for the continuous monitoring of blood pressure in unanesthetized rat pups at ages younger than had heretofore been studied (17). With the use of this method, when 7- to 9-day-old rat pups were thermally challenged, and even as cardiac rate decreased by nearly 50%, mean arterial pressure (MAP) was maintained. Because arterial pressure is primarily a function of cardiac output and peripheral resistance, and because there may be limited contractile reserve in altricial infant rats as there is in precocial newborn lambs (22), it was concluded that infant rats must be maintaining arterial pressure primarily by increasing peripheral resistance.

What neural or hormonal mechanisms mediate increases in peripheral resistance during cold exposure? Studies in adult animals have demonstrated that, during cold exposure, the sympathetic nervous system (SNS) is activated and results in constriction of peripheral vessels via α_1-adrenoceptors (9, 34). In addition, the renin-angiotensin system (RAS) and vasopressin (VP) system are activated during thermal challenges (11, 29); ANG II and vasopressin are both potent vasoconstrictors. All three systems are functional in infant rats (16, 18, 30, 31), but their role in cardiovascular regulation during cold exposure is not known. Therefore, one of the aims of the present study is to assess the contributions of the SNS, RAS, and VP system to the maintenance of arterial pressure during cold exposure through selective blockade of these systems using prazosin (a selective α_1-adrenoceptor antagonist), losartan (a selective AT$_1$ receptor antagonist), and Manning compound (a selective V$_1$ receptor antagonist). Furthermore, simultaneous blockade of all three sys-

tems allows us to determine whether bradycardia implies decreased cardiac output in infant rats during extreme cooling: if MAP decreases in lock-step with cardiac rate, then this would support the notion that infant rats have a limited capacity to increase stroke volume during thermal challenge.

Another aim of the present study concerns the emission of ultrasonic vocalizations during extreme cooling. These vocalizations, although long interpreted as communicatory signals of emotional distress, have been reinterpreted as acoustic by-products of a maneuver that plays a role in maintaining cardiovascular function (3). This maneuver, called the abdominal compression reaction (ACR), is characterized by abdominal contractions near the end of expiration and helps to increase venous return (32, 33). In support of this ACR hypothesis, ultrasonic vocalizations by infant rats occur in contexts associated with decreased venous return (3), and each ultrasonic pulse is associated with increased intra-abdominal and arterial pressure (17) and increased central venous pressure (6). Therefore, because ultrasound production can influence arterial pressure, it was also monitored here to allow for comparisons of arterial pressure during periods of high and low rates of vocalization for each of the experimental groups.

METHODS

The methods used here for the collection of cardiovascular, thermal, and metabolic data are adapted from those used previously (5, 17).

Subjects. Thirty 8-day-old rat pups from 30 litters were used. At the time of surgery, the pups weighed 15.6–24.5 g. They were born to Harlan Sprague-Dawley females in the animal colony at the University of Iowa and were raised in litters that were culled to eight pups within 3 days after birth (day of birth = *day 0*). Litters and mothers were housed in standard laboratory cages ($48 \times 20 \times 26$ cm) in which food and water were available ad libitum. All animals were maintained on a 12:12-h light-dark schedule with lights on at 6:00 A.M.

In a followup study, six 8-day-old pups from six litters were used. At the time of surgery, these pups weighed 16.4–19.0 g. They were housed and raised as described above.

Surgery. For direct recording of arterial pressure, the femoral artery was catheterized on the day of testing. The catheter was constructed from Micro-Renathane tubing (MRE-040; Braintree Scientific, Braintree, MA) with a 1-cm tip hand-drawn under a hot air stream; the catheter was filled with heparinized (50 IU/ml) isotonic saline. For catheter implantation, pups were anesthetized with isoflurane, and the left femoral artery was exposed under a Wild dissecting microscope. The catheter was introduced into the artery through a small hole made with a 30-gauge needle and advanced 0.4–0.8 cm into the artery. This distance placed the tip at the level of the branching of the femoral artery from the abdominal aorta. After checking for adequate blood flow, the catheter was sutured in place and stabilized with a drop of cyanoacrylate adhesive. The incisions were then sutured closed, and a thermocouple was attached to the skin in the interscapular region. The animal was then allowed to recover in an incubator at an air temperature of 35–36°C.

Test environment. Pups were tested inside a double-walled glass chamber. Air temperature (T_a) within the chamber was controlled by pumping temperature-controlled water through the chamber walls using a water circulator (Neslab, Portsmouth, NH). Pups were allowed to move freely inside the chamber on a platform constructed of polyethylene mesh.

Blood pressure measurements. Polyethylene tubing was used to connect the arterial catheter to a pressure transducer (Argon, Athens, TX). The entire length of tubing from pup to transducer was filled with heparinized saline. The output of the transducer passed through an analog-to-digital converter, whose signal was then fed into a computerized data-acquisition system. Before each pup was tested, the system was calibrated using a sphygmomanometer with a resolution of 1 mmHg.

Temperature measurements. All temperatures were measured using chromel-constantan thermocouples. T_a within the metabolic chamber was monitored using a thermocouple located beneath the platform. Skin temperature was obtained by attaching a thermocouple to the skin surface using collodion as an adhesive. This thermocouple was attached on the midline in the interscapular region above a major deposit of BAT, thus providing a measure of interscapular temperature (T_{is}). Based on previous experiments in week-old rats (4, 7), we have found that measures of T_{is} and oxygen consumption together provide a reliable indicator of thermoregulatory competence.

Oxygen consumption measurements. Compressed air passed through a two-stage regulator and was split into two lines, one of which was circulated through the metabolic chamber. After passing through the chamber, the exhaust air was drawn through one of two channels of an electrochemical oxygen analyzer (model S-3A/II, AEI Technologies, Pittsburgh, PA). The second line of air traveled directly from the air cylinder to the second channel of the oxygen analyzer. The percent difference in oxygen between the chamber's effluent air stream and the nonrespired air stream was used to determine the amount of oxygen consumed by the pup. This system is sensitive to 0.0001% oxygen differences between the two lines with an accuracy of 0.01% oxygen. All oxygen consumption values are presented as milliliters O_2 per 100 g per minute.

Ultrasonic vocalizations. During extreme cold exposure, week-old rats emit ultrasonic vocalizations at a frequency of 40–45 kHz. These vocalizations are accompanied by significant increases in arterial pressure (17). Therefore, to provide consistency in the measurement of blood pressure, ultrasonic vocalizations were recorded simultaneously. These vocalizations were detected using a microphone sealed inside the lid of the metabolic chamber and connected to a detector (Anabat, Titley Electronics, Ballina, Australia). The output of the detector was fed to the same data-acquisition computer that was used for acquiring blood pressure data.

Drugs. There were five drug conditions in this experiment. First, the α_1-adrenoceptor antagonist, prazosin (RBI, Natick, MA), was dissolved in DMSO and administered at a dose of 0.5 mg/kg. Second, the ANG II AT_1 receptor antagonist, losartan (Merck, Rahway, NJ), was dissolved in isotonic saline and administered at a dose of 1 mg/kg. Third, the vasopressin V_1 receptor antagonist, Manning compound (Sigma, St. Louis, MO), was dissolved in isotonic saline and administered at a dose of 0.5 mg/kg. Fourth, prazosin, losartan, and Manning compound were prepared as described above and administered in three separate injections (hereafter referred to as the triple block condition). Finally, one-half of the vehicle pups were injected once with isotonic saline; the remaining vehicle pups were injected twice with saline and once with DMSO to control for the triple block injection procedure.

In a followup study to probe the effect of double blockade on arterial pressure, two pups were each assigned to one of three double block conditions: Manning compound plus losartan, Manning compound plus prazosin, and losartan plus prazosin. All drugs were prepared and injected as described above.

All drugs were injected subcutaneously in a volume of 1 μl/g.

Procedure. As noted above, the pup recovered from surgery in an incubator maintained at a thermoneutral air temperature. This period of recovery varied depending on the status of the animal; this period was always more than 40 min and, in all but one case, less than 85 min. While recovering in the incubator and to ensure that the pup was in a postabsorptive state at the time of testing, each pup was intubated with commercial half-and-half at a volume of 1.4–3.0% body wt depending on the size of the pup's milk band, which is visible through the abdominal skin. At the appropriate time, the pup was transferred from the incubator to the metabolic chamber maintained at 35.25°C. Again, depending on the pup's status as well as its behavior in the chamber, this period of acclimation varied from 17 to 111 min. Therefore, the total period of postsurgical recovery in the incubator and chamber acclimation varied from 77 to 157 min.

When the experiment began, T_a, T_{is}, and oxygen consumption data were acquired every 15 s using a customized data-acquisition system that employs LabView software (National Instruments, Austin, TX) for the Macintosh computer. In addition, acquisition of blood pressure (and ultrasonic vocalization) data commenced for 5 min using a second data-acquisition system; these data were acquired at the rate of 200 samples·s^{-1}·channel^{-1}. Next, the chamber lid was removed and the pup was injected according to its random assignment to one of the five experimental conditions. The chamber lid was then replaced, and, 30–55 min later (78 and 95 min for 2 subjects), a second 5-min acquisition period of blood pressure data began. Then, three periods of cooling began in which air temperature within the chamber was decreased in succession to 29, 23, and 17°C. At each temperature, pups were given at least 40 min to stabilize, after which a 5-min period of blood pressure data was acquired before air temperature was decreased again. After the final data-acquisition period at 17°C, the pup was removed from the chamber, and the oxygen consumption system was allowed to rezero to verify minimal drift in the system over the course of the test. The pup was then overdosed with Nembutal and autopsied to determine the location of the catheter. Finally, in the followup double block study, all procedures were similar to those described above.

The use of long test durations in which air temperature is decreased in succession may raise concerns regarding the effect of time on physiological responding in the cold. First, it is standard practice to conduct tests in the warm-to-cold direction only (27); the reason for this is that counterbalancing the order of exposure to each air temperature may address one confounding variable but introduces a number of new ones, including test duration and intensity of challenge. Second, the test durations used here are well within the limits for maintaining adequate energy stores for normal thermoregulatory responding (1). Indeed, despite the surgery, control pups in the present experiment exhibited thermoregulatory responses that are similar to those observed in unmanipulated pups.

Data analysis. Thermal and metabolic measures were imported into StatView 4.5 for the Macintosh. The values of T_{is} and oxygen consumption at each phase of the experiment were determined. Blood pressure and ultrasound data were imported into DataDesk 6.0 for the Macintosh. A scatterplot of the data was produced for each session, and each point in the scatterplot was linked to a row number that represented the passage of 1/200 s. The times at which maximum systolic and minimum diastolic pressures occurred were determined, and from these data, interbeat interval (IBI) was determined (resolution ± 2.5 ms). MAP was calculated as $P_d + (1/3)(P_s - P_d)$, where P_d and P_s are diastolic and systolic pressure, respectively. For each of the five phases of the experiment, 11 pressure waves were identified to generate a mean IBI and MAP for that phase. Toward the end of the experiment when pups were likely to be vocalizing, pressure waves were selected in which the pups were silent. Finally, IBIs were converted to cardiac rate (CR) in beats per minute before analysis.

Body weight and baseline data for T_{is}, oxygen consumption, CR, and MAP were analyzed using single-factor ANOVA. ANOVA was also used to analyze the differences between pre- and postinjection values for all four variables. Repeated-measures ANOVA was used to examine changes in the four variables at the four air temperatures after drug or vehicle administration. When significant main effects or interactions were found, single-factor ANOVAs were performed at each air temperature; the post hoc test used was Fisher's protected least significant difference. Finally, second-order polynomial regressions were used to assess the interrelations between T_{is}, CR, and MAP. For all tests, α was set at 0.05. Means are presented with their SEs.

RESULTS

There were no significant differences in body weight between pups in any of the experimental conditions ($F_{4,25} = 0.4$).

Figure 1 presents mean values of T_{is}, oxygen consumption, CR, and MAP at each phase of the experiment and for each of the five experimental conditions. At an air temperature of 35°C and before the pups were injected, there were no significant differences between any of the experimental conditions for T_{is} ($F_{4,23} = 0.4$), oxygen consumption ($F_{4,23} = 0.9$), CR ($F_{4,23} = 2.5$), or MAP ($F_{4,23} = 0.9$). In addition, only CR changed significantly from its preinjection baseline values (T_{is}: $F_{4,24} = 2.0$; oxygen consumption: $F_{4,22} = 0.1$; CR: $F_{4,24} = 5.4$, $P < 0.005$; MAP: $F_{4,24} = 0.8$). Post hoc tests revealed that the change in CR was due primarily to increases in the prazosin and triple block conditions (see Fig. 1*C*); specifically, CR increased 47.3 ± 14.9 and 35.8 ± 10.3 beats/min in those two groups, respectively. Although the change in MAP was not significant by the time these postinjection measures were recorded, the prazosin and triple block groups did exhibit the largest decreases in MAP (see Fig. 1*D*). This suggests that the increases in CR resulted from baroreflex-mediated tachycardias.

In Fig. 1*A*, it can be seen that, as air temperature decreased, T_{is} decreased during cooling in all five groups. For the four air temperatures after drug or vehicle administration, repeated-measures ANOVA indicated significant main effects of condition ($F_{4,23} = 3.5$, $P < 0.05$) and air temperature ($F_{3,69} = 775.6$, $P < 0.0001$) and a significant condition × air temperature interaction ($F_{12,69} = 3.0$, $P < .005$). Post hoc analyses revealed that, toward the end of the test, pups in the
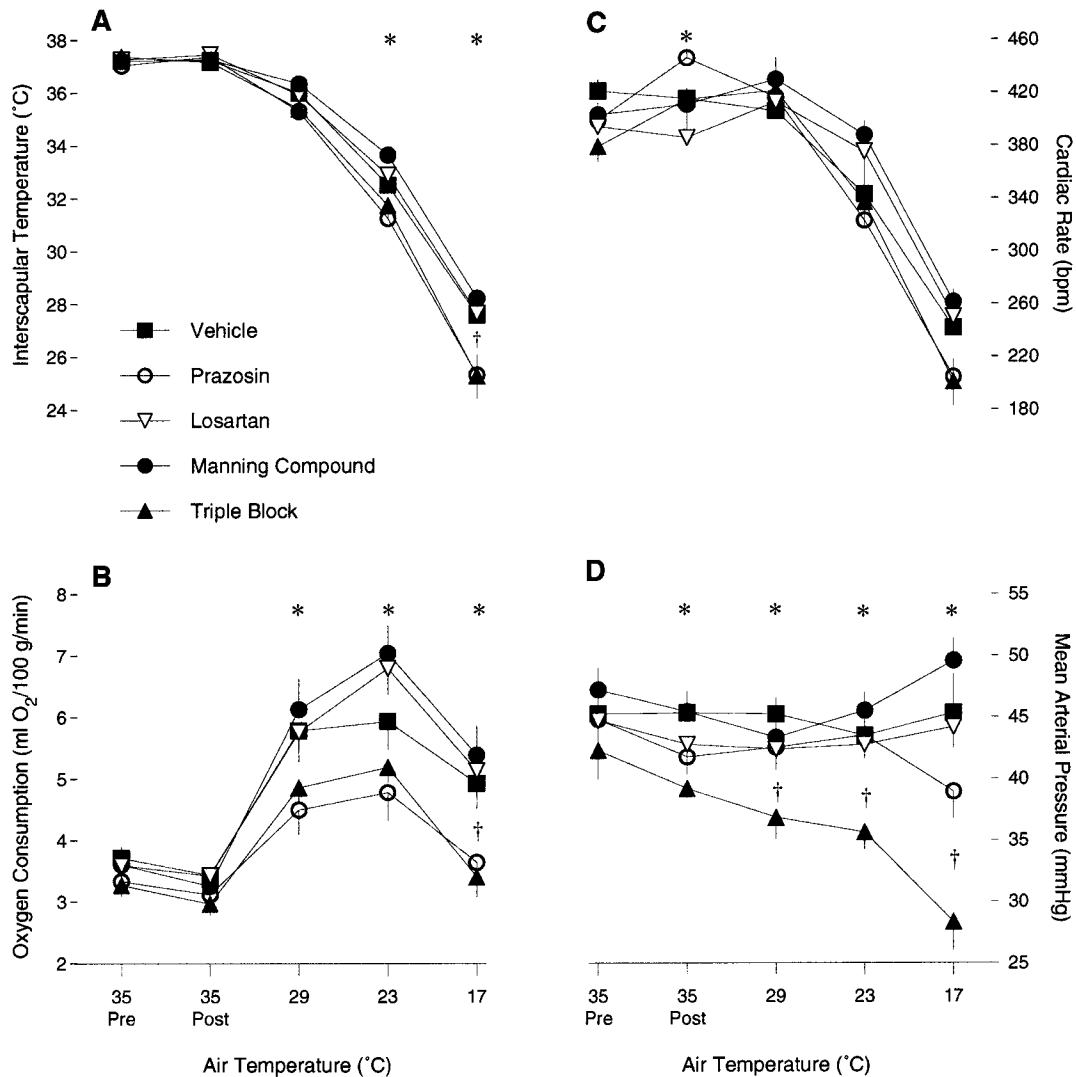
Fig. 1. Interscapular temperature (*A*), oxygen consumption (*B*), cardiac rate (*C*), and mean arterial pressure (*D*) during moderate and extreme cooling in 8-day-old rats. Data are presented from pups before (Pre) and after (Post) subcutaneous administration of vehicle, a selective α_1-adrenoceptor antagonist (prazosin; 0.5 mg/kg), an ANG II receptor antagonist (losartan; 1 mg/kg), a vasopressin receptor antagonist (Manning compound; 0.5 mg/kg), or simultaneous administration of all 3 antagonists (triple block). Values are means ± SE; n = 5–6 pups/condition. *Significant difference between conditions at that air temperature. †Significant difference between groups separated by the symbol.

prazosin and triple block conditions cooled further than the pups in the other three conditions.

In conjunction with the T_{is} data just described, the oxygen consumption data presented in Fig. 1*B* are consistent with the notion that BAT thermogenesis was suppressed in pups in the prazosin and triple block conditions. Again, repeated-measures ANOVA indicated significant main effects of condition ($F_{4,22} = 5.2$, $P < 0.005$) and air temperature ($F_{3,66} = 96.6$, $P < 0.0001$) and a significant condition $\times$ air temperature interaction ($F_{12,66} = 2.6$, $P < 0.01$). These effects were driven primarily by diminished oxygen consumption in the prazosin and triple block groups at temperatures below thermoneutrality coupled with increases in oxygen consumption in the Manning compound and losartan groups at the moderate air temperatures.

As shown in Fig. 1*C*, CR decreased as cooling progressed. Repeated-measures ANOVA indicated a significant main effect of air temperature ($F_{3,75} = 330.2$, $P < 0.0001$) and a significant condition $\times$ air temperature interaction ($F_{12,75} = 4.5$, $P < 0.0001$), but the main effect of condition was not significant ($F_{4,25} = 0.9$). Although the prazosin and triple block groups tended to exhibit greater decreases in CR, these decreases were not significant.

The MAP data presented in Fig. 1*D* present a clear picture of the effects of pharmacological blockade on MAP. Repeated-measures ANOVA indicated significant main effects of condition ($F_{4,25} = 10.9$, $P < 0.0001$) and a significant condition $\times$ air temperature interaction ($F_{12,75} = 5.0$, $P < 0.0001$), but the main effect of air temperature was not significant

($F_{3,75}$ = 1.4). Post hoc tests revealed that MAP was significantly lower in the triple block group than in the vehicle and Manning compound groups after injection but before air temperature was decreased. With cooling below 35°C, the differential between the triple block group and all the other groups increased significantly. There also was a trend toward lower pressure in the prazosin group at 17°C, although this trend only reached significance in relation to the Manning compound group, a group that exhibited a slight elevation in MAP at 17°C. Given that the prazosin and triple block groups exhibited similar decreases in T_{is}, oxygen consumption, and CR, it is important to stress that only the triple block group exhibited a pronounced decrease in MAP. This indicates that inhibition of heat production and increased bradycardia are not sufficient to produce decreased blood pressure in these pups. These re-

sults also indicate that blockade of at least two of the three systems examined here is necessary to drive blood pressure downward during cold challenge.

Figure 2 presents a series of scatterplots for the five conditions across all air temperatures after drug or vehicle administration. Figure 2, *left*, presents scatterplots for CR vs. T_{is}, and it can be seen that as T_{is} decreased throughout the test from highs of 38°C to lows of 23°C, cardiac rate decreased from highs of 480 beats/min to lows of 140 beats/min. Polynomial second-order regressions, performed separately for each condition, yielded r^2 values of 0.87–0.98, similar to values obtained previously in both unmanipulated and ganglionically blocked rat pups during cold challenge (5). Therefore, it is again apparent that, regardless of treatment condition, heat produced by BAT plays a predominant role in determining the temperature of heart muscle and, in turn, CR (20, 26).
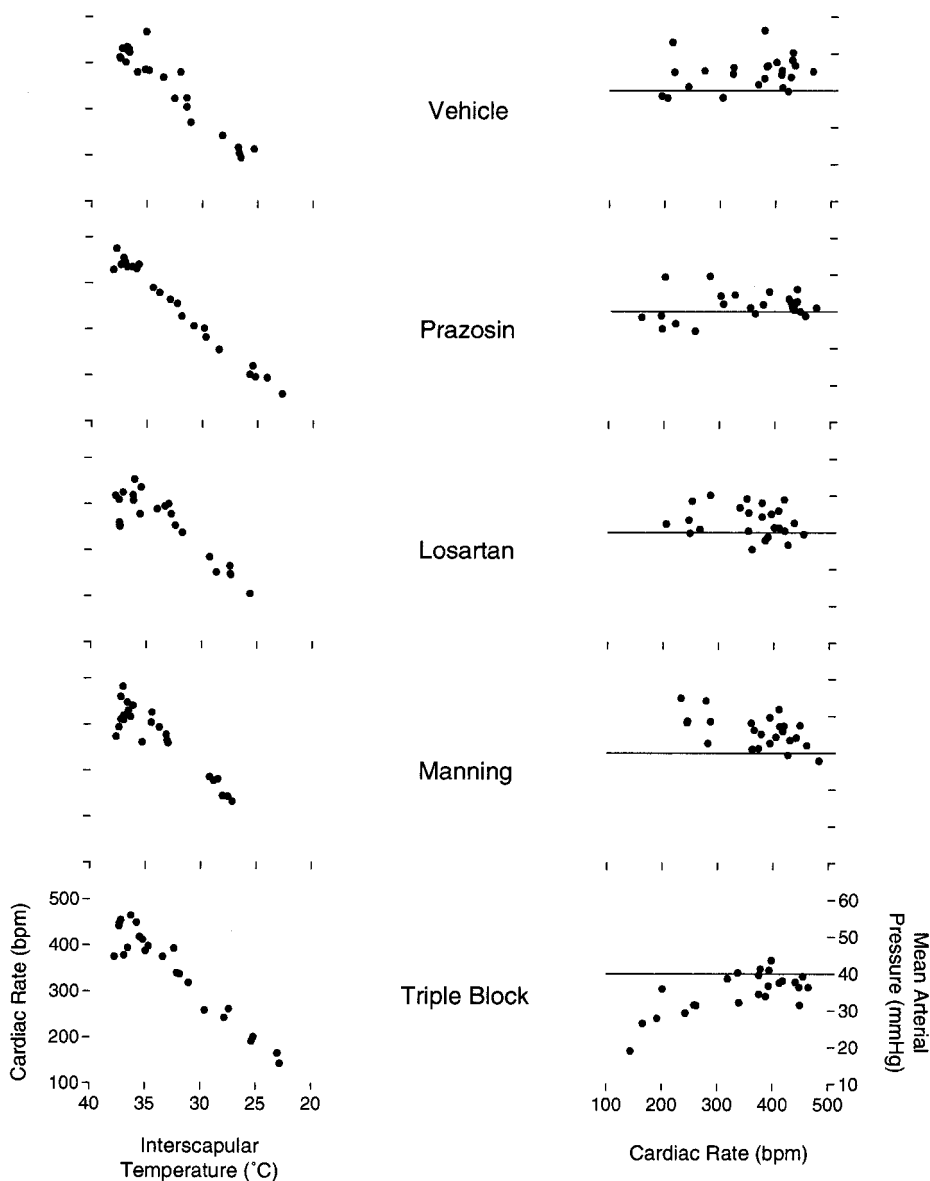
Fig. 2. Scatterplots of cardiac rate vs. interscapular temperature (*left*) and mean arterial pressure vs. cardiac rate (*right*) during moderate and extreme cooling in 8-day-old rats. Only data from pups after drug or vehicle administration are presented (see legend of Fig. 1 for description of drugs and dosages). Horizontal line at mean arterial pressure value of 40 mmHg is for comparison purposes only; n = 5–6 pups/condition.

Figure 2, *right*, presents MAP vs. CR for the five conditions across all air temperatures after drug or vehicle administration. For all but the triple block group, MAP either increased or did not change as CR decreased, and polynomial regressions yielded r^2 values of 0.001–0.24. In contrast, for the triple block group, MAP decreased substantially when CR decreased below ~300 beats/min. A second-order polynomial regression revealed that CR accounted for 63% of the variance in MAP ($F_{2,21} = 20.3$, $P < 0.0001$).

The MAP data described above were extracted during periods when pups were not emitting ultrasonic vocalizations. This distinction is particularly relevant at the lowest air temperature, 17°C, when ultrasound production is most prevalent. Because it was previously reported in 7- to 9-day-old rats that ultrasonic pulses were accompanied by mean increases in MAP of 3.8 mmHg (17), we further analyzed MAP for the pups in the present study during periods at 17°C when they were emitting ultrasonic vocalizations. Then, MAP was calculated as the difference in MAP during periods of ultrasound production and periods of quiet. Because ANOVA indicated no significant differences between the five experimental conditions ($F_{4,22} = 0.6$), the data were collapsed across groups. Consistent with the previous report, periods of ultrasound production were accompanied by mean increases in MAP of 3.5 ± 0.4 mmHg, significantly different from 0 ($t_{26} = 3.5$, $P < 0.0001$). Moreover, of the 27 pups for which data are available, 26 exhibited increased MAP during periods of ultrasound production.

A followup experiment was performed in which the effect of the three double block conditions on MAP was examined. Only two pups were tested in each of the double block conditions. Figure 3 presents box plots of MAP for the double block conditions at an air temperature of 17°C alongside the data from the five other conditions already discussed. It is clear that those double block conditions comprising prazosin and either losartan or Manning compound had a greater impact on MAP than did the double block condition comprising losartan and Manning compound. Although based on relatively few subjects, these data suggest that blockade of sympathetic neural output with prazosin is required to significantly impact MAP but that a maximal decrease in MAP requires blockade of sympathetic neural output as well as both of the two remaining hormonal systems.

DISCUSSION

It was previously demonstrated that week-old rats maintain MAP during cold exposure even as cardiac rate decreases as much as 50% below baseline values (17). Although that study did not address the mechanisms used by infant rats to maintain blood pressure, it was suggested that infant rats likely depend on increases in peripheral resistance to maintain arterial pressure. That suggestion was based in part on the demonstration in anesthetized infant rats under thermoneutral conditions that sympathetic neural outflow to the vasculature participates in the regulation of peripheral resistance and, consequently, arterial pressure (23). The present study extends this finding to unanesthetized infants during moderate and extreme cold exposure and directly addresses hormonal contributions to peripheral resistance. Therefore, the approach adopted here of employing single, double, and triple blockades of the SNS, RAS, and VP system allows us to conclude that all three systems contribute to the regulation of peripheral resistance during thermal challenge in rats as young as 1 wk of age.

As shown in Fig. 1, *A* and *B*, prazosin administration was associated with decreased BAT thermogenesis and
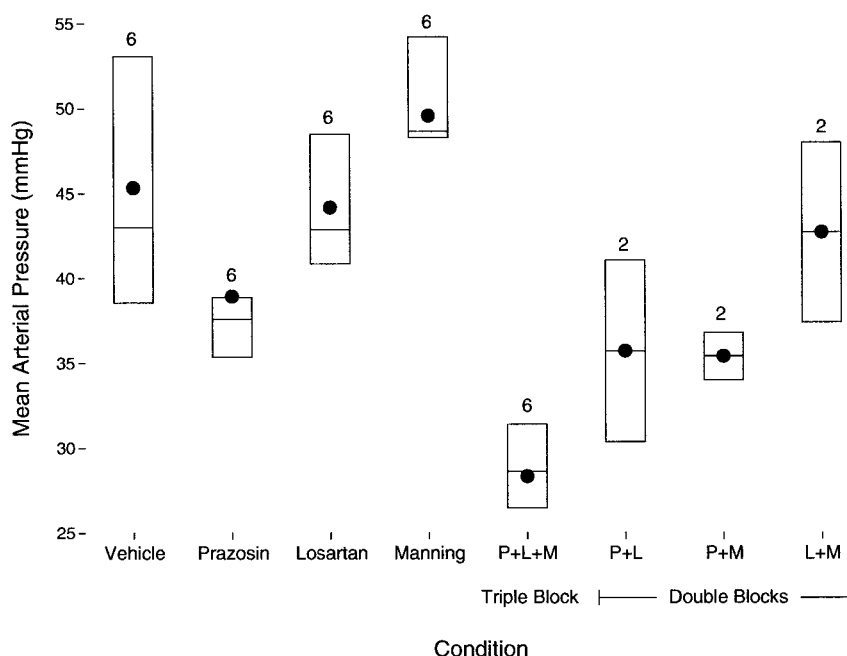

Fig. 3. Box plots depicting mean arterial pressure data from 8-day-old rats at an air temperature of 17°C. Data are from pups injected with vehicle, prazosin (P), losartan (L), Manning compound (M), combined administration of all 3 drugs (triple block; P + L + M), or paired combinations of the 3 drugs (double blocks; P + L, P + M, L + M). See legend of Fig. 1 for description of drugs and dosages. Each box presents mean value (●), median value (central horizontal line), and values at the 75th and 25th percentiles (top and bottom horizontal lines, respectively). The number of subjects in each group is indicated above each box.

oxygen consumption, resulting in increased bodily cooling. It is possible that prazosin suppressed heat production indirectly by reducing the pup's ability to control blood flow to BAT and to compartmentalize warmed blood within the thorax. It is also possible that prazosin directly suppressed BAT thermogenesis; α_1-adrenoceptors are present in BAT, although their role in BAT thermogenesis has not yet been convincingly demonstrated (10). In addition, although the α_1-adrenoceptor agonist, phenylephrine, activates BAT thermogenesis in adult rats (8), whether phenylephrine acts directly or indirectly to activate BAT is not clear. This study, however, was not designed to assess BAT thermogenesis or the mechanisms by which it is activated or inhibited. Regardless, this issue is of little concern to us here because although pups in the prazosin and triple block conditions cooled further than pups in the other conditions, only pups in the triple block condition exhibited significant decreases in MAP.

One aim of this study was to determine whether cardiac output decreases during extreme cooling as cardiac rate plummets. The direct measurement of cardiac output in infant rats during cold exposure, however, poses a number of technical difficulties. For example, the most widely used technique for measuring cardiac output (i.e., thermodilution) requires simultaneous implantation of arterial and venous catheters, which is a very difficult procedure in such small animals.

The relationship between cardiac rate and cardiac output can, however, be inferred indirectly. Specifically, by blocking the infant's ability to modulate vascular resistance during cold exposure, the relationship between decreased cardiac rate and cardiac output can be determined. First, we note that MAP is a function of cardiac output, peripheral resistance, arterial compliance, and blood volume. By preventing modulation of peripheral resistance in the triple block pups, MAP becomes a function of cardiac output, arterial blood volume, and arterial compliance. If we make the reasonable assumption that blood volume and arterial compliance remain constant during these tests, MAP becomes a function of cardiac output alone. Because cardiac output is a function of cardiac rate and stroke volume, MAP also becomes a function of cardiac rate and stroke volume. Given that MAP decreased as cardiac rate fell below 300 beats/min at the air temperature of 17°C (see Fig. 2), we can conclude that pups were unable to increase stroke volume in a compensatory fashion. This conclusion for altricial infant rats is consistent with the finding that contractile reserve is limited in precocial newborn lambs (22).

Although the triple block pups exhibited orderly decreases in MAP with decreasing cardiac rate, the relationship between the two variables was not very strong. First, cardiac rate accounted for only 63% of the variance in MAP, which is not nearly as strong as the relationship between interscapular temperature and cardiac rate. Second, although cardiac rate decreased 51% as air temperature decreased from 35°C to 17°C, MAP decreased only 28%. It may be, then, that some

other factor is buttressing MAP during extreme cold exposure. Increased stroke volume seems unlikely, especially if it is, like cardiac rate, sensitive to cooling of the heart muscle. Alternatively, it is possible that a fourth pressor mechanism, not affected by the agents used here, contributes to the regulation of arterial pressure.

One mechanism that could blunt the impact of decreasing cardiac rate on MAP is increased blood viscosity. Blood viscosity plays an important role in determining total peripheral resistance in normal and hypertensive subjects (12–14, 19, 28), and it is significantly increased by cooling in a wide variety of species (15, 21). Most significant for the present experiment, increases in blood viscosity have been measured in week-old rats under conditions identical to those used here (4). In that study, blood viscosity increased significantly at blood temperatures that correspond to those experienced by infant rats on exposure to the extreme air temperature of 17°C. Therefore, increased blood viscosity may play a passive role in maintaining MAP during extreme thermal challenge.

The decrease in cardiac output and increase in peripheral resistance during extreme cold exposure causes a shift in blood volume toward the venous side of the circulation, and the cold-induced increase in blood viscosity compromises venous return even as it may contribute to the maintenance of MAP. The resulting venous pooling, we have suggested (3), is counteracted by recruitment of the ACR that, through properly timed abdominal compressions, propels venous blood back to the heart (32, 33). Indeed, as already mentioned, ultrasonic vocalizations in infant rats are accompanied by substantial increases in intra-abdominal pressure and central venous pressure (6, 17). The present finding that MAP increases during periods of ultrasound production replicates a previous result (17), but it is not being suggested that the function of the ACR is to maintain MAP per se. Rather, the ultrasound-related increases in MAP reflect the increase in venous return that is the primary function of the ACR. Therefore, the maintenance of cardiac output would be protected by the ACR, and this would act in concert with the neural and hormonal mechanisms that elevate vascular resistance during extreme cooling to maintain perfusion of critical organs.

Perspectives

Altricial infant mammals face a number of severe challenges to thermal and cardiovascular homeostasis that are specific to their transient developmental predicament. They compensate for their small size and naked skin by producing heat using BAT and compartmentalizing the heat within the thorax for the maintenance of heart temperature (24). Thus, although still often referred to by many as poikilotherms, infant rats are actually highly capable thermoregulators, even outside the huddle (2). As shown here, infant rats also possess neural and hormonal mechanisms for modulating peripheral resistance and maintaining arterial

pressure in the cold. With extreme cooling, however, BAT capabilities are exceeded, resulting in bradycardia and decreased cardiac output. Nonetheless, pups are able to maintain arterial pressure, and in addition, they recruit the ACR to maintain venous return. This emerging, complex story of physiological competence has required a comprehensive and integrative approach, using unanesthetized animals and examining both behavioral and physiological mechanisms as they impact on thermoregulation and cardiovascular function. Still missing from this picture, however, is even a rudimentary understanding of cardiorespiratory function in infant rats during thermal challenge, especially regarding ventilation-perfusion relations.

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