Further evidence that BAT thermogenesis modulates cardiac rate in infant rats

GRETA SOKOLOFF, ROBERT F. KIRBY, AND MARK S. BLUMBERG
Department of Psychology, University of Iowa, Iowa City, Iowa 52242

Sokoloff, Greta, Robert F. Kirby, and Mark S. Blumberg. Further evidence that BAT thermogenesis modulates cardiac rate in infant rats. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R1712–R1717, 1998.—Previous research in infant rats suggested that brown adipose tissue (BAT), by providing warm blood to the heart during moderate cold exposure, protects cardiac rate. This protective role for BAT thermogenesis was examined further in the present study. In *experiment 1*, 1-wk-old rats in a warm environment were pretreated with saline or chlorisondamine (a ganglionic blocker), and then BAT thermogenesis was stimulated by injection with the β_3-agonist CL-316243. In *experiment 2*, pups were pretreated with chlorisondamine and injected with CL-316243, and after BAT thermogenesis was stimulated the interscapular region of the pups was cooled externally with a thermode. In both experiments, cardiac rate, oxygen consumption, and physiological temperatures were monitored. Activation of BAT thermogenesis substantially increased cardiac rate in saline- and chlorisondamine-treated pups, and focal cooling of the interscapular region was sufficient to lower cardiac rate. The results of these studies support the hypothesis that BAT thermogenesis contributes directly to the modulation of cardiac rate.

cardiovascular system; nonshivering thermogenesis; thermoregulation

IN MAMMALIAN INFANTS, brown adipose tissue (BAT) thermogenesis is the primary means of endogenous heat production (9, 12, 17, 21). During moderate cold exposure in infant rats, BAT thermogenesis is regulated stably (6) and has been shown to protect sleep-related behaviors and to suppress production of ultrasonic vocalizations (8, 19). The relationship between BAT thermogenesis and cardiac rate regulation has also been investigated. Specifically, we found that, during moderate cold exposure, BAT thermogenesis was activated and cardiac rate was maintained (7, 13). In contrast, when BAT thermogenesis was overwhelmed during extreme cold exposure or was blocked with a ganglionic blocker, cardiac rate decreased as a function of air temperature.

Our previous work (7, 13) provided strong evidence of a relationship between BAT thermogenesis and cardiac rate, although the results were primarily descriptive in nature. In the present experiments, BAT was activated pharmacologically in infant rats at a thermoneutral air temperature to further examine the effects of BAT thermogenesis on cardiac rate. In *experiment 1*, 7- to 8-day-old rats were pretreated with saline or the ganglionic blocker chlorisondamine; chlorisondamine was administered to prevent the activation of other neural mechanisms that could potentially influence cardiac rate. Pups were then injected with CL-316243 (a β_3-adrenoceptor agonist) to stimulate BAT thermogenesis, and cardiac rate was measured. In *experiment 2*, 8-day-old chlorisondamine-treated rats were again injected with CL-316243. After BAT thermogenesis was activated, the region of skin overlying BAT was cooled with a thermode and cardiac rate was monitored. The results of both experiments support the hypothesis that BAT thermogenesis protects cardiac rate during cold challenge.

METHODS

Subjects. Seventeen 7- to 8-day-old male rat pups from twelve litters were used: twelve 7- to 8-day-old pups in *experiment 1* and five 8-day-old pups in *experiment 2*. At the time of testing, pups in *experiment 1* weighed 16.7–22.2 g and pups in *experiment 2* weighed 17.1–21.5 g. Pups used in both experiments were born to Harlan Sprague-Dawley female rats maintained in the animal colony at the University of Iowa. Mothers and their litters were housed in standard laboratory cages (48 × 20 × 26 cm) in which food and water were available ad libitum. Litters were culled to eight pups within 3 days after birth (day of birth = *day 0*). All animals were maintained on a 12:12-h light-dark schedule with lights on at 0600.

Test environment. For *experiments 1* and *2*, pups were placed inside a double-walled glass metabolic chamber (for dimensions and detailed description see Ref. 7). Briefly, by pumping temperature-controlled water through the walls of the chamber, air temperature was controlled. Access holes on the side of the chamber as well as connectors on the lid allowed for the passage of air throughout the chamber and connections for all physiological recordings. Once inside the chamber, the pups were placed on a polyethylene mesh platform.

Temperature measurements. Air temperature (T_a) and physiological temperatures were measured using chromel-constantan thermocouples (Omega, Stamford, CT). All thermocouples were calibrated to within 0.1°C of a mercury thermometer and fed into a computerized data acquisition system (National Instruments, Austin, TX). T_a values were obtained using the acquired computer average of two thermocouples placed beneath the mesh platform: one near the center of the chamber and one close to the outer diameter. Physiological temperatures were attained using thermocouples attached to the dorsal skin surface of the animal by use of the adhesive collodion. One thermocouple was attached in the interscapular region directly above the BAT pad, providing a measure of interscapular temperature (T_{is}). A second thermocouple was attached ~1 cm rostral to the base of the tail in the lumbosacral region; this thermocouple provided a measure of skin temperature (T_{back}) distant from the site of heat production.

Oxygen consumption measurements. Compressed air passed through a regulator and was split into two lines. One line passed through a digital flowmeter (Omega); the air in the line was then humidified and circulated through the metabolic chamber at 300 ml/min. The air was drawn from the chamber and desiccated, then it was drawn through one of two channels of an electrochemical oxygen analyzer (Ametek, Pittsburgh, PA). The second line of air flowed directly from

the regulator to the second channel of the oxygen analyzer. The oxygen content of each airstream was measured simultaneously, and the percent difference in concentration was computed to within 0.001%. The percent difference was then fed into the computerized data acquisition system and transformed to oxygen consumption ($\dot{V}O_2$) in milliliters of oxygen per kilogram per minute.

Thermode. In *experiment 2* the temperature of the interscapular region was manipulated using a custom-built thermode. The conductive surface and the base of the thermode were fashioned from the head of a brass flat-head screw (0.6 cm diameter). A piece of plastic tubing (1 cm long, 0.6 cm ID) was fitted over the head of the screw and sealed in place with cyanoacrylate (Surehold, Chicago, IL). The height of the thermode was stabilized with a plastic sheath that fit tightly inside the tubing above the conductive area. Two small pieces of silicone tubing (0.2 cm OD) were fit into the top of the body of the thermode, and the top was then sealed with cyanoacrylate. Two longer pieces of silicone tubing (7 cm long, 0.2 cm ID) were placed over the smaller tubes emerging from the top of the thermode. Silicone sealant was used to prevent any leaks from the thermode to the intake and outlet tubes. From the inside of the metabolic chamber the thermode was attached to a connector protruding from one of the chamber's side access holes. On the outside of the chamber a second water circulator was connected to the intake and outlet tubes of the thermode. The thermode temperature was controlled by circulating water through the body of the thermode.

Data acquisition. For both experiments, thermal and $\dot{V}O_2$ measures were acquired four times per minute by use of a customized LabView (National Instruments, Austin, TX) data acquisition program for Macintosh. Electrocardiogram (ECG) data were acquired simultaneously on a second data acquisition system by use of one of two methods. For one method, raw ECG data were acquired at a rate of 1,000/s, and the times between successive R waves were determined after the test (7). For the other method, interbeat intervals were calculated at the time of data acquisition at a rate of 30/min. These two methods yielded identical results. Finally, thermal, $\dot{V}O_2$, and ECG measures were recorded simultaneously.

Drugs. The β_3-agonist CL-316243 (Wyeth-Ayerst Research, Pearl River, NY) and chlorisondamine hydrochloride (Ciba-Geigy, Summit, NJ) were dissolved in isotonic saline before use. All drug injections were administered at a volume of 1 μl/g body wt sc.

Procedure. On the day of testing a pup was removed from its home cage. Each pup had been fed recently, as evidenced by the presence of a milk band visible through the abdominal skin. The pup was weighed and lightly anesthetized with ether (exposure ≤1 min). After anesthetization the pup was placed in an incubator maintained at ~35–36°C. Three ECG leads were implanted transcutaneously, and the thermocouples for physiological temperature measures were also attached; leads and thermocouples were secured to the skin with collodion. In *experiment 2*, after the thermocouples were attached the thermode for cooling the interscapular region was secured directly over the site of the interscapular thermocouple with collodion. The pup was then placed inside the metabolic chamber at a T_a of ~35°C and allowed to acclimate for 45 min. The ECG leads were connected to a differential amplifier (A-M Systems, Everett, WA) that filtered and amplified the signal before it was acquired by the computer.

In *experiment 1*, pups received an injection of saline vehicle or chlorisondamine hydrochloride (5 mg/kg sc) 30 min after being placed in the metabolic chamber. The chamber was resealed, allowing the oxygen analysis system to stabilize. Before the end of the acclimation period (42–43 min), data collection began. The β_3-agonist CL-316243 (0.1 mg/kg sc) was injected 45 min after the pup was placed in the chamber. After the administration of CL-316243, thermal, $\dot{V}O_2$, and ECG data were recorded for 60 min with no further manipulations.

In *experiment 2*, pups were injected with chlorisondamine (5 mg/kg) 30 min after being placed in the chamber. As in *experiment 1*, baseline recording began near the end of the 45-min acclimation period. After ~1 min of data collection, pups received an injection of CL-316243 (0.1 mg/kg). Cooling of the interscapular region with the thermode began 45 min after the β_3-agonist was administered. The temperature of the water circulated through the thermode was 28°C, which produced a T_{is} of 32.2–34.9°C. After 45 min with the thermode on, the water circulator was turned off and the pup was allowed to reheat for 45 min, after which there was a final period of data acquisition; this final period was included to ascertain whether the β_3-agonist was still activating BAT thermogenesis.

For both experiments, T_a was maintained at ~35°C. All pups were removed from the metabolic chamber after testing, the chamber was resealed, and the oxygen analysis system was allowed to rezero, thus verifying minimal drift in the system.

Data analysis. Thermal, metabolic, and cardiac data were imported into StatView 4.5 for the Macintosh. Interbeat interval was converted to cardiac rate in beats per minute, and mean cardiac rates for each pup were calculated from 30 data points at each data acquisition period of the experiment. Similarly, the thermal and metabolic data for that same period were used, giving four data points averaged over a 60-s period. For *experiment 1* a repeated-measures ANOVA was used to test for differences in the variables across time, and α was set at 0.05. Data were analyzed from 1-min periods immediately before and 5, 10, 15, 30, 45, and 60 min after administration of the β_3-agonist. For $\dot{V}O_2$, data were analyzed immediately before and 15, 30, 45, and 60 min after administration of the β_3-agonist; $\dot{V}O_2$ data were unavailable at the 5- and 10-min time points, because the oxygen analysis system requires 15 min to restabilize after opening. Post hoc paired *t*-tests were used to test for differences between the baseline value (pre-β_3-agonist) and each subsequent time point. For *experiment 2*, paired *t*-tests were used to test for differences between successive experimental periods. For both experiments, a Bonferroni correction was used to adjust α for multiple comparisons ($\alpha = 0.008$ and 0.0167 for *experiments 1* and *2*, respectively).

RESULTS

Experiment 1. Stimulation of BAT thermogenesis by the β_3-agonist increased T_{is} significantly in saline- and chlorisondamine-pretreated pups ($86.8 \leq F_{6,30} \leq 142.12$, $P < 0.0001$; Fig. 1). Administration of the drug produced rapid increases in T_{is}. For pups pretreated with saline, T_{is} increased significantly over baseline within 5 min of drug administration ($t_5 = 11.7$, $P < 0.0001$). For pups pretreated with chlorisondamine, T_{is} increased significantly over baseline within 10 min of drug administration ($t_5 = 16.1$, $P < 0.0001$). For both groups the increases in T_{is} were maintained for the remainder of the test period ($10.7 \leq t_5 \leq 19.0$, $P < 0.0001$). The average increase in T_{is} after administration of the β_3-agonist was ~2.5°C higher than baseline for both groups.

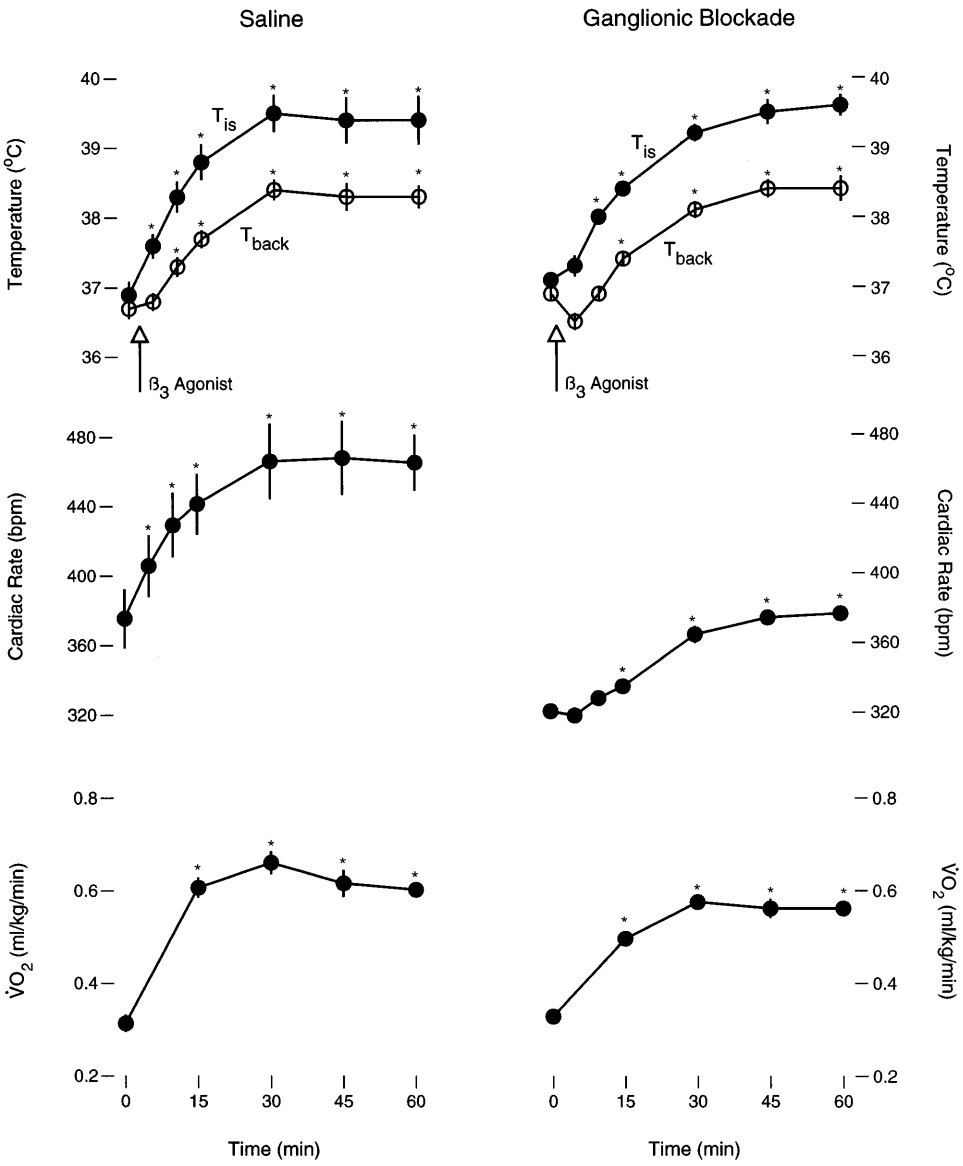

Fig. 1. Interscapular temperature (T_{is}), skin temperature (T_{back}), cardiac rate, and O_2 consumption ($\dot{V}O_2$) for 1-wk-old rats pretreated with saline or chlorisondamine and then injected with β_3-agonist. Thermal data are presented together: ●, T_{is}; ○, T_{back}. Activation of brown adipose tissue thermogenesis by β_3-agonist led to an increase in cardiac rate in both groups. bpm, Beats/min. Values are means ± SE. *Significantly different from baseline.

Administration of the β_3-agonist also increased T_{back} significantly for pups in the saline and chlorisondamine groups ($58.3 \leq F_{6,30} \leq 106.3$, $P < 0.0001$). Increases in T_{back}, however, were of smaller magnitude and were delayed in comparison to increases in T_{is}. T_{back} did not differ significantly from baseline until 10 min after administration of the β_3-agonist for pups pretreated with saline ($t_5 = 15.0$, $P < 0.0001$) and not until 15 min after injection for pups pretreated with chlorisondamine ($t_5 = 5.2$, $P < 0.005$). As with the increases in T_{is}, T_{back} remained elevated over baseline for the rest of the test period for both groups ($8.7 \leq t_5 \leq 12.4$, $P < 0.0005$).

Cardiac rate increased significantly in both groups after stimulation of BAT thermogenesis with the β_3-agonist ($39.8 \leq F_{6,30} \leq 99.6$, $P < 0.0001$). The change in cardiac rate was significant within 5 min of drug administration for pups pretreated with saline ($t_5 = 8.4$, $P < 0.0005$) and 15 min for pups pretreated with chlorisondamine ($t_5 = 5.7$, $P < 0.005$). For both groups the increases in cardiac rate continued and cardiac rate remained higher than baseline values for the remainder of the test period ($5.4 \leq t_5 \leq 17.2$, $P < 0.001$). The average increase in cardiac rate from baseline values was similar in both groups (24 and 17% for saline and chlorisondamine, respectively).

$\dot{V}O_2$ increased significantly in both groups after stimulation of BAT thermogenesis with the β_3-agonist ($79.1 \leq F_{4,20} \leq 126.3$, $P < 0.0001$). Within 15 min, $\dot{V}O_2$ was significantly greater than baseline values and remained elevated for the entire test period ($10.5 \leq t_5 < 29.4$, $P < 0.0001$). It is not known whether pups increased $\dot{V}O_2$ significantly at the 5- and 10-min time points. This unavailability of data resulted from the opening of the chamber to inject the β_3-agonist and the time then required for the oxygen analysis system to restabilize.

Experiment 2. As in *experiment 1*, T_{is} increased 2°C over baseline values after administration of the β_3-agonist ($t_4 = 6.5$, $P < 0.005$; Fig. 2). When cool water

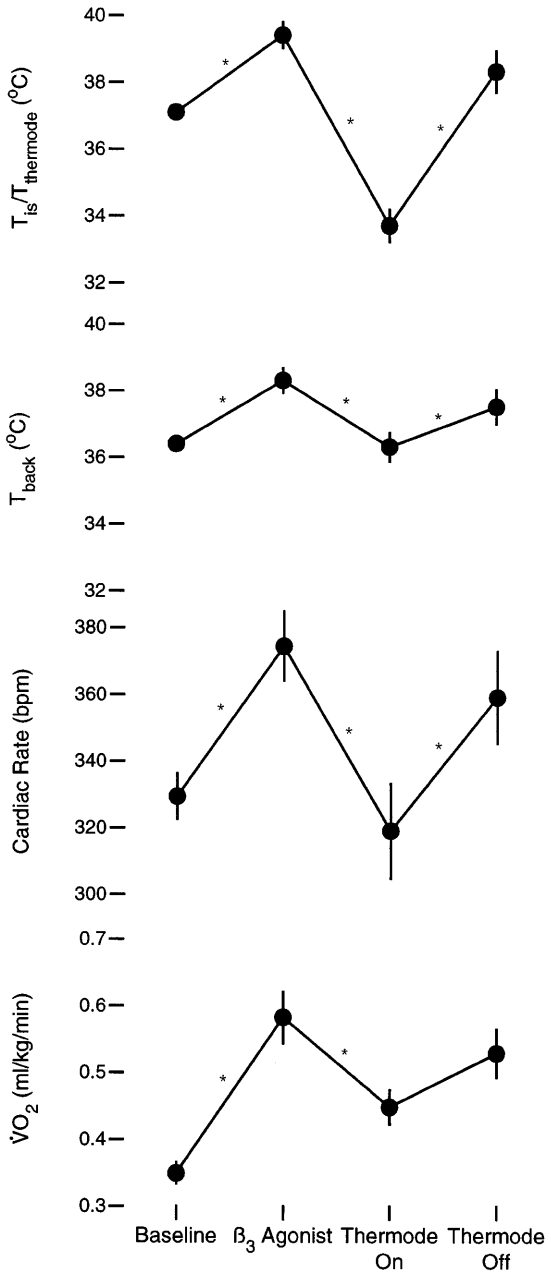

Fig. 2. T_{is}/thermode temperature ($T_{thermode}$), T_{back}, cardiac rate, and $\dot{V}O_2$ for 1-wk-old rats after pretreatment with chlorisondamine, after administration of β_3-agonist, during interscapular cooling with thermode, and after thermode was turned off and pups were allowed to reheat. Activation of brown adipose tissue thermogenesis by β_3-agonist increased cardiac rate, and this effect was counteracted by focal cooling of interscapular region. Values are means ± SE. * Significantly different from previous time period.

was circulated through the thermode, even with BAT thermogenesis stimulated, the interscapular region was cooled significantly ($t_4 = 13.1$, $P < 0.0005$). When circulation of water through the thermode was terminated, T_{is} again increased significantly ($t_4 = 19.2$, $P < 0.0001$).

T_{back} also increased significantly after administration of the β_3-agonist ($t_4 = 7.1$, $P < 0.005$). In addition, cooling with the thermode produced a significant decrease in T_{back} ($t_4 = 5.0$, $P < 0.01$) and, once interscapular cooling ended, T_{back} again increased significantly ($t_4 = 7.2$, $P < 0.005$).

Figure 2 also presents the cardiac rate data for pups in *experiment 2*. Cardiac rate increased from baseline values after stimulation of BAT thermogenesis with the β_3-agonist ($t_4 = 5.5$, $P < 0.01$). When the interscapular region was cooled with the thermode, cardiac rate decreased significantly ($t_4 = 5.2$, $P < 0.01$). Finally, when the thermode was turned off, cardiac rate again increased significantly ($t_4 = 7.6$, $P < 0.005$).

$\dot{V}O_2$ followed a pattern similar to T_{is}, T_{back}, and cardiac rate. After administration of the β_3-agonist, $\dot{V}O_2$ increased significantly ($t_4 = 5.4$, $P < 0.01$). Cooling with the thermode was sufficient to decrease $\dot{V}O_2$ ($t_4 = 7.3$, $P < 0.005$). When the thermode was turned off, $\dot{V}O_2$ did not increase significantly ($t_4 = 3.6$, $P > 0.02$); $\dot{V}O_2$ was, however, significantly greater than its baseline level ($t_4 = 4.4$, $P = 0.01$), suggesting that the β_3-agonist was still activating BAT thermogenesis through the end of the experiment.

DISCUSSION

Many years ago, it was demonstrated that cardiac rate is responsive to changes in temperature in vivo and in vitro (1, 11, 14). When the thermogenic function of BAT was elucidated, anatomic studies led to the suggestion that BAT thermogenesis provides heat focally to vital organs in the thoracic cavity (16, 18). Previous work from our laboratory built on these earlier studies to demonstrate that, in infant rats, BAT thermogenesis protects against bradycardia during moderate, but not extreme, cold exposure (7, 13). In addition, when pups were injected with a ganglionic blocker and thus BAT thermogenesis was inhibited, cardiac rate fell in lock step with decreasing T_{is} (7). These results provided the first evidence in support of the hypothesis that interscapular BAT thermogenesis protects cardiac function by supplying warmed venous blood to the heart.

The results of the present study provide further support for the hypothesized link between BAT thermogenesis and the maintenance of cardiac rate during cold exposure. In *experiment 1*, pharmacological activation of BAT thermogenesis by use of a selective β_3-agonist led to an increase in cardiac rate in pups tested in a thermoneutral environment. Moreover, this tachycardia was observed even in pups pretreated with a ganglionic blocker, indicating that changes in cardiac rate during BAT thermogenesis do not require neural control of the heart. Because the dose of chlorisondamine used here was identical to that used in previous experiments to completely block BAT thermogenesis (7, 20), we are confident that the sympathetic blockade was complete in the present experiments. *Experiment 2* showed that cooling of the interscapular region reverses the tachycardia produced by pharmacological activation of BAT thermogenesis. It should be stressed that these results should not be interpreted to mean that thermal manipulation of other areas of the body could not have produced similar results. Rather, the

present experiment was designed only to determine whether cooling the region that overlies the interscapular BAT pad is sufficient to reverse the tachycardia induced by the β_3-agonist. Again, because this effect of focal cooling was observed in ganglionically blocked animals, it is apparent that neural mechanisms are not required. The results of both experiments provide strong evidence that manipulation of T_{is}, by the activation of BAT thermogenesis or by cooling of the overlying skin, modulates cardiac rate.

Cardiac rate can be increased by the nonselective stimulation of β-adrenoceptors. However, CL-316243 has a high selectivity for β_3-adrenoceptors and low affinity for β_1- and β_2-receptors, precluding direct effects on cardiac rate or secondary effects mediated through the activation of baroreceptors (4). Tavernier et al. (22) and Berlan et al. (3) found that the β_3-adrenoceptor agonists BRL-37344 and CGP-12177 increase cardiac rate in adult dogs. However, both agents induced hypotension, and their ability to increase cardiac rate was eliminated after sinoaortic denervation, indicating that the tachycardia was produced by activation of baroreceptor mechanisms. Because, in the present study, increases in cardiac rate after CL-316243 administration were similar in ganglionically blocked and nonblocked animals, the alterations in cardiac rate could not have been due to a baroreceptor-mediated increase in sympathetic outflow. In addition, preweanling rats do not develop effective baroreceptor mechanisms regulating cardiac rate until ~12–15 days of age (2, 10). Therefore, in the present study it is not likely that the increases in cardiac rate after activation of BAT were mediated by the nonselective activation of β-adrenoceptors.

It is apparent that cardiac rate is determined by a combination of factors that includes cardiac temperature and autonomic activation (7). With respect to the determinants of cardiac temperature, interscapular BAT is ideally suited for the efficient delivery of warmed blood to the heart (17). Nonetheless, it must be stressed that cardiac temperature can be influenced by the flow of venous blood from all regions of the body and that the temperature of interscapular BAT will be more or less important for the determination of cardiac temperature, depending on the ability of infant rats to regulate blood flow to and from the extremities.

Perspectives

Cold challenge poses a serious threat to isolated infants. Because infant rats, like the young of most altricial mammals, are unable to shiver effectively, BAT thermogenesis is the primary means of producing heat in response to decreasing ambient temperature. The results of this study, coupled with those of previous studies in rats (7, 13) and Golden hamsters (5), suggest a primary role for BAT in the defense of cardiac function during cold exposure. By heating the blood before it is returned to the heart, BAT thermogenesis allows cardiac rate to be maintained and heated blood to be supplied to the appropriate tissues of the body through the reapportioning of blood flow.

The authors thank Wyeth-Ayerst Research for the donation of CL-316243.

This research was supported by National Institute of Mental Health Grant MH-50701 to M. S. Blumberg.

Address reprint requests to R. F. Kirby.

Received 20 August 1997; accepted in final form 2 February 1998.

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