Thermoregulation in rats during early postnatal maturation: importance of nitric oxide

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Malik, Sulleman S. and James E. Fewell. Thermoregulation in rats during early postnatal maturation: importance of nitric oxide. Am J Physiol Regul Integr Comp Physiol 285: R1366–R1372, 2003; 10.1152/ajpregu.00280.2003.—Experiments were carried out to determine the role of nitric oxide in mediating autonomic and behavioral thermoregulatory control in rat pups on postnatal days 1–2, 5–6, and 10–11. For an experiment, each pup received a subcutaneous injection of vehicle, Nω-nitro-arginine methyl ester (L-NAME; 100 mg/kg), or Nω-nitro-arginine methyl ester (L-NAME; 100 mg/kg) before being placed in a metabolic chamber or in a thermocline with a linear temperature gradient of 23 to 43°C. In the metabolic chamber, oxygen consumption and core temperature were measured as ambient temperature was decreased from 40 to 15°C over a 60-min period. Decreasing ambient temperature elicited an increase in oxygen consumption in all age groups that received vehicle or D-NAME. The lower critical temperature and peak oxygen consumption upon exposure to cold after vehicle were 41 ± 10 ml kg⁻¹ min⁻¹ at 30°C, 43 ± 12 ml kg⁻¹ min⁻¹ at 28°C, and 55 ± 11 ml kg⁻¹ min⁻¹ at 25°C in the 1- to 2-, 5- to 6-, and 10- to 11-day-old pups, respectively. Administration of L-NAME abolished the oxygen consumption response to cold in the 1- to 2- and 5- to 6-day-old pups and significantly attenuated the oxygen consumption response to cold in the 10- to 11-day-old pups. Selected ambient temperature in the thermocline was not significantly affected by prior administration of D-NAME or L-NAME compared with vehicle. Thus our data provide evidence that the nitric oxide system plays a role in mediating autonomic but not behavioral thermoregulatory control in rat pups during early postnatal maturation.

autonomic thermoregulation; behavioral thermoregulation; brown adipose tissue; core temperature; newborn rats; nitric oxide; nonshivering thermogenesis; shivering thermogenesis

RATS ARE BORN RELATIVELY IMMATURE in comparison with other eutheria mammals (20). Typically, in this “altricial” species, there are many newborns in the litter, and they are poorly developed, naked, and blind. Similar to other newborn mammals, their primary requirements are food, protection, and warmth. Rats are homeotherms and employ both their somatomotor nervous system (e.g., shivering thermogenesis, behavioral thermoregulation) and the sympathetic portion of their autonomic nervous system (e.g., nonshivering thermogenesis in brown adipose tissue, changes in vasomotor tone) as thermoregulatory effectors to maintain a stable core temperature. When given the opportunity, adult rats will preferentially utilize behavioral thermoregulation as a more energy-efficient thermoregulatory effector over shivering and nonshivering thermogenesis to increase their core temperature when exposed to a novel stimulus (9).

Before birth, the rat fetus resides in the warm environment of the uterus. In all species studied to date [i.e., humans (2, 27), baboons (17), sheep (1), dogs (2), and rabbits (12)], core temperature of the fetus is ~0.5°C higher than that of the mother. Immediately after birth, however, the newborn must expend energy not only for growth but also to maintain its core temperature at or near the central nervous system “set point” temperature. Considering the energy requirements for growth during early postnatal life and the fact that most newborns have limited food supplies, it would be of an advantage for the newborn rat to use behavioral thermoregulation rather than nonshivering thermogenesis as a thermoregulatory effector.

The specific aim of the present experiments was to define the autonomic and behavioral thermoregulatory profiles of newborn and older rats to test the hypothesis that rats have the mechanisms in place shortly after birth that allow them to optimize their energy expenditure for thermoregulation by selecting a thermal environment that requires the lowest metabolic oxygen requirements. In addition, given the recent evidence that nitric oxide functions as a peripheral and central mediator in temperature regulation (6, 7, 24), we also carried out experiments to determine the role of nitric oxide in mediating autonomic and behavioral thermoregulatory control in rat pups during early postnatal maturation.

METHODS

Experiments were carried out on Sprague-Dawley rat pups (Charles River Breeding Laboratories) from primigravida dams. Each pup was born by spontaneous vaginal delivery and was housed with its dam and siblings in an environmental chamber (22 ± 1°C, 20–30% relative humidity and 12:
12-h light-dark cycle) until study. The dams had continuous access to food (Lab Diet 5001) and tap water.

Experimental Series

Two experimental series were carried out on rat pups at 1–2, 5–6, and 10–11 days of age. The first experimental series (experimental series I: control) was carried out on pups that had not received a prior injection. The second experimental series (experimental series II: nitric oxide) was carried out on vehicle-injected, d-NAME-injected (100 mg/kg), or L-NAME-injected (100 mg/kg) pups. All experimental procedures were carried out in accordance with the Guide for the Care and Use of Experimental Animals provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

Experimental series I: Control. For an experiment, a pup was removed from the litter and placed in the thermocline for 60 min. The pup was then returned to its cage and siblings for 60 min before being studied in the metabolic chamber.

Experimental series II: Nitric oxide. For an experiment, a pup was removed from the litter, injected subcutaneously with either vehicle (saline), d-NAME, or L-NAME, and returned to the litter. After 30 min, the pup was removed again from the litter and studied in either the thermocline or metabolic chamber.

Fig. 1. Oxygen consumption of 1–2-, 5–6-, and 10–11-day-old (d) rat pups in experimental series I (control) as ambient temperature in the metabolic chamber was decreased from 40 to 15°C. Values are means ± SD; n = 16 pups at each age. *P < 0.05 vs. minimal oxygen consumption (●) by repeated-measures ANOVA and Newman-Keuls test (ANOVA; age, P = 0.000; ambient temperature, P = 0.000; age × ambient temperature, P = 0.000).

Fig. 2. Core temperatures of 1–2-, 5–6-, and 10–11-day-old rat pups in experimental series I (control) as ambient temperature in the metabolic chamber was decreased from 40 to 15°C. Values are means ± SD; n = 11 pups at 1–2 days of age and n = 16 pups at 5–6 and 10–11 days of age. *P < 0.05 vs. core temperature at minimal oxygen consumption (●) by repeated-measures ANOVA and Newman-Keuls test (ANOVA; age, P = 0.000; ambient temperature, P = 0.000; age × ambient temperature, P = 0.000).

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Experimental Protocols

Thermocline. The rat pups were placed at an initial ambient temperature of 35°C, and selected ambient temperature was recorded at 5-min intervals throughout the 60-min period. Reported values represent measurements made at the end of the 60-min period.

Metabolic chamber. Measurements of core temperature and total body oxygen consumption were made at 1°C decrements as chamber ambient temperature was decreased from 40 to 15°C over a 60-min period.

Condition of Observations

Thermocline. The thermocline used in our experiments consisted of a sealed Plexiglas cylinder (180 cm long; internal diameter of 11.5 cm) with a plastic grid along the bottom into which flowed room air at 1 l/min. A linear temperature gradient from 43 to 23°C was produced by circulating hot and cold water (Endocal RTE-8DD) in two copper coils wrapped around the cylinder.

Metabolic chamber. The metabolic chamber used in our experiments consisted of a double-walled Plexiglas cylinder (10.3 cm long; 2.5 cm internal diameter; internal volume of 50 ml) with a plastic grid along the bottom into which flowed room air at 298.5 ml/min. Chamber ambient temperature was controlled by a computer-operated circulating water bath (model 1174; VWR PolyScience) that circulated water through the space between the outer chamber walls.

Experimental Measurements

Thermocline. Selected ambient temperature was determined by observing the position of the pup in the calibrated thermocline.

Metabolic chamber. Core temperature was measured using a 21-gauge copper/constantan thermocouple sheathed in Teflon (IT-18 Physitemp) interfaced with a BAT-12 thermometer (Physitemp). The thermocouple was inserted 1 cm in the pup’s rectum and glued to its tail using tissue adhesive (3M Animal Care Products; Vetbond). Total body oxygen consumption was calculated from the difference in oxygen concentrations between the inflow and outflow gases (Applied Electrochemistry S-3A/O2 Analyzer; Ametek) and the flow rate. All values of oxygen consumption were expressed at STPD.

Fig. 3. Influence of vehicle, \(N^{G}\)-nitro-D-arginine methyl ester (D-NAME), and \(N^{G}\)-nitro-L-arginine methyl ester (L-NAME) on oxygen consumption of 1- to 2-day-old (A), 5- to 6-day-old (B), and 10- to 11-day-old (C) rat pups in experimental series II (nitric oxide) as ambient temperature in the metabolic chamber was decreased from 40 to 15°C. Values are means ± SD; \(n = 10\) at each age. *\(P < 0.05\) vs. minimal oxygen consumption (●) by repeated-measures ANOVA and Newman-Keuls test (ANOVA; age, \(P = 0.000\); drug, \(P = 0.000\); ambient temperature, \(P = 0.000\); age × drug, \(P = 0.099\); age × ambient temperature, \(P = 0.000\); drug × ambient temperature, \(P = 0.000\); age × drug × ambient temperature, \(P = 0.000\)).
**Experimental Compounds**

D-NAME (Sigma) and L-NAME (Sigma) were dissolved in isotonic saline (0.9% sodium chloride; ASTRA) to a final concentration of 5 mg/ml and administered at a dose of 100 mg/kg according to the protocol of Gozal et al. (11). Previous experiments by Traystman et al. (26) have shown that systemic administration of approximately one-half of this dose of L-NAME inhibits brain nitric oxide synthase activity at 30 min by ~90% in adult cats, dogs, and piglets. Vehicle was isotonic saline.

**Statistical Analysis**

Statistical analysis was carried out using an ANOVA for repeated measures, followed by a Newman-Keul's multiple-comparison test. All results are presented as means ± one SD; *P* < 0.05 was considered to be of statistical significance.

**RESULTS**

**Experimental Series I: Control**

Decreasing ambient temperature in the metabolic chamber from 40 to 15°C elicited an increase in oxygen consumption in all age groups (Fig. 1). The lower critical temperature and peak oxygen consumption upon exposure to cold were 31°C and 45 ± 9 ml·kg⁻¹·min⁻¹, 30°C and 53 ± 15 ml·kg⁻¹·min⁻¹, and 26°C and 55 ± 18 ml·kg⁻¹·min⁻¹ in the 1- to 2-, 5- to 6-, and 10- to 11-day-old pups, respectively. Despite the increase in oxygen consumption upon exposure to cold, none of the age groups of animals maintained their core temperature as ambient temperature was decreased (Fig. 2).

Selected ambient temperatures in the thermocline at the end of the 60-min experimental period were 36 ± 1°C (range 33–38°C), 35 ± 2°C (range 32–37°C), and 35 ± 2°C (range 30–38°C) in the 1- to 2-, 5- and 6-, and 10- to 11-day-old pups, respectively. These ambient temperatures elicited oxygen consumption rates in the metabolic chamber that were not significantly different from minimal values in each age group.

**Experimental Series II: Nitric Oxide**

Decreasing ambient temperature in the metabolic chamber from 40 to 15°C elicited an increase in oxygen consumption in all age groups (Fig. 1). The lower critical temperature and peak oxygen consumption upon exposure to cold were 31°C and 45 ± 9 ml·kg⁻¹·min⁻¹, 30°C and 53 ± 15 ml·kg⁻¹·min⁻¹, and 26°C and 55 ± 18 ml·kg⁻¹·min⁻¹ in the 1- to 2-, 5- to 6-, and 10- to 11-day-old pups, respectively. Despite the increase in oxygen consumption upon exposure to cold, none of the age groups of animals maintained their core temperature as ambient temperature was decreased (Fig. 2).

Selected ambient temperatures in the thermocline at the end of the 60-min experimental period were 36 ± 1°C (range 33–38°C), 35 ± 2°C (range 32–37°C), and 35 ± 2°C (range 30–38°C) in the 1- to 2-, 5- and 6-, and 10- to 11-day-old pups, respectively. These ambient temperatures elicited oxygen consumption rates in the metabolic chamber that were not significantly different from minimal values in each age group.

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**Fig. 4.** Influence of vehicle, D-NAME, and L-NAME on core temperatures of 1- to 2-day-old (A), 5- to 6-day-old (B), and 10- to 11-day-old (C) rat pups in experimental series II (nitric oxide) as ambient temperature in the metabolic chamber was decreased from 40 to 15°C. Values are means ± SD; *n* = 10 pups at each age. *P* < 0.05 vs. minimal oxygen consumption (*F*) by repeated-measures ANOVA and Newman-Keuls test (ANOVA; age, *P* = 0.000; drug, *P* = 0.979; age × drug, *P* = 0.000; drug × ambient temperature, *P* = 0.000; age × drug × ambient temperature, *P* = 0.0166; ambient temperature, *P* = 0.000; age × drug, *P* = 0.979; age × ambient temperature, *P* = 0.000; drug × ambient temperature, *P* = 0.000; age × drug × ambient temperature, *P* = 0.913).
consumption in all age groups after administration of saline (i.e., vehicle) or \( \text{d-NAME} \). Administration of \( \text{l-NAME} \), however, abolished the oxygen consumption response to cold in the 1- to 2- and 5- and 6-day-old pups and significantly attenuated the oxygen consumption response to cold in the 10- to 11-day-old pups (Fig. 3). Again, despite the increase in oxygen consumption upon exposure to cold, none of the age or drug groups of animals maintained their core temperature as ambient temperature was decreased (Fig. 4).

Selected ambient temperatures in the thermocline at the end of the 60-min experimental period after saline, \( \text{d-NAME} \), and \( \text{l-NAME} \) were 36 ± 2°C (range 35–40°C), 36 ± 2°C (range 34–41°C), and 36 ± 2°C (range 34–39°C) in the 1- to 2-day-old pups, 36 ± 3°C (range 30–42°C), 35 ± 3°C (range 29–38°C), and 36 ± 3°C (range 29–41°C) in the 5- to 6-day-old pups, and 36 ± 2°C (range 34–41°C), 34 ± 3°C (range 30–37°C), and 33 ± 3°C (range 30–41°C), respectively, in the 10- to 11-day-old pups. These ambient temperatures elicited oxygen consumption rates in the metabolic chamber that were not significantly different from minimal values in each age and drug group. Movement patterns of the 1- to 2-, 5- to 6-, and 10- to 11-day old pups after injection of saline are shown in Fig. 5. Neither movement pattern during the 60-min experimental period nor selected ambient temperature at the end of the 60-min experimental period was affected by the prior injection of \( \text{d-NAME} \) or \( \text{l-NAME} \) compared with that observed after saline.

**DISCUSSION**

Our experiments provide new information about mechanisms of thermoregulatory control during early postnatal maturation in rats. Novel findings in our study were that 1) rats at all postnatal ages exhibited a metabolic response to a decrease in ambient temperature, the magnitude of which increased with increasing postnatal age, 2) despite the metabolic response, none of the age groups of animals maintained their core temperature upon exposure to cold, 3) rats selected an ambient temperature in the thermocline that placed oxygen consumption at levels comparable to those observed at ambient temperatures in which minimal oxygen consumption occurred in the metabolic chamber, and lastly 4) nitric oxide is essential in mediating autonomic but not behavioral thermoregulatory control early in postnatal life.

Our data provide evidence that rats have the neurophysiological mechanisms in place shortly after birth that allow them to optimize their energy expenditure for thermoregulation by selecting a thermal environment that places oxygen consumption at levels comparable to those observed at ambient temperatures in which minimal oxygen consumption occurred in the metabolic chamber. We observed, as did Kleitman and Satinoff (15), that even the youngest animals were active and moved position by crawling and/or rolling along the alley of the thermocline to select their preferred ambient temperature. Can rat pups achieve this optimal thermal environment in the nest? Mortola and Dotta (18) found that the core temperature of 2-day-old rat pups upon removal from the undisturbed nest averages 35.9 ± 0.5°C, which suggests that they can. This has important ramifications for survival and for growth because it suggests that, by utilizing behavioral thermoregulation, the rat pup not only avoids the hazards of hypothermia but also avoids wastage of energy on heat production and thus more will be available for growth. This factor is of considerable importance, for most newborn mammals have limited food reserves. Estimates of energy expenditures for postnatal growth in some rapidly growing species may be as much as 30–35% of the basal metabolic rate (16).

Decreasing the ambient temperature from 40°C to 15°C over a 60-min period elicited a metabolic response, assessed indirectly by changes in total body oxygen consumption, in all age groups of animals, the
magnitude of which increased with increasing postnatal age. The increased oxygen utilization most likely resulted from activation of nonshivering thermogenesis in brown adipose tissue, which is the primary source of heat production in most newborns upon exposure to cold (13, 20). In the rat, catecholamines, as determined by the Falck fluorescence histochemical method, are discernable in brown adipose parenchymal nerves by 2–3 days of age (8). Our data are in agreement with and extend those Mortola and Dotta (18), who measured total body oxygen consumption in 2-day-old rat pups, and Taylor (25) who measured total body oxygen consumption in 4 h- to 21-day-old rat pups as the ambient temperature was decreased from ~40 to 15°C, respectively. Our experimental design is unique in that we measured core temperature concurrently with total body oxygen consumption as the ambient temperature was changed in the metabolic chamber. We found that, despite the fact that the maximal total body oxygen response increased and the lower critical temperature decreased with increasing postnatal age, none of the age groups of pups was able to maintain its core temperature as ambient temperature was decreased. This indicates that the mechanisms that control heat transfer between the pup and environment, if existent at all, are basically rendered ineffective by the large surface area-to-volume ratio of this immature species during the first 10 days of postnatal life. Furthermore, it indicates that, within the limits of our experimental measurements, a thermoneural zone cannot be defined in rat pups during the first 10 days of postnatal life if one uses the classical definition [i.e., the range of ambient temperature within which the metabolic rate is at a minimum and within which temperature regulation (i.e., a stable core temperature) is achieved by nonevaporative physical processes alone (4, 14)].

Administration of the l-arginine analog L-NAME abolished the oxygen consumption response to cold in the 1- to 2- and 5- to 6-day-old pups and significantly attenuated the oxygen consumption response to cold in the 10- to 11-day-old pups. It is possible that the 10- to 11-day-old pups exhibited a small increase in total body oxygen consumption upon exposure to cold after L-NAME as a result of subtle changes in muscle tone even though overt shivering was not observed. In the newborn, shivering thermogenesis usually appears as an effector for heat generation as the ability to generate heat from nonshivering thermogenesis in brown adipose tissue declines (5). In the rat, brown adipose tissue weight and the total amount of uncoupling protein expressed as GDP binding per unit newborn surface area increases after birth, reaching a maximum by 4–6 days of postnatal age, and then declines (20). This possibility warrants further investigation.

In summary, our experiments provide new information on autonomic and behavioral thermoregulatory profiles of newborn rats during early postnatal maturation and provide evidence that rats have mechanisms in place shortly after birth that allow them to optimize their energy expenditure for thermoregulation by selecting a thermal environment that corresponds to the lowest metabolic oxygen requirements. Furthermore, our experiments provide evidence that nitric oxide is essential in mediating autonomic but not behavioral thermoregulatory control in rats during early postnatal life.

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DISCLOSURES

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