Age-dependent core temperature responses of conscious rabbits to acute hypoxemia

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Fewell, James E., Sarah H. M. Wong, and Kim C. Crisanti. Age-dependent core temperature responses of conscious rabbits to acute hypoxemia. J Appl Physiol 89: 259–264, 2000.—Experiments were carried out on chronically instrumented newborn and older rabbits to characterize their core temperature (Tc) responses to acute hypoxemia and to differentiate “forced” vs. “regulated” thermoregulatory responses. Three age ranges of kits were studied: 4–6, 9–11, and 28–30 days of age. During an experiment, Ta, selected ambient temperature (Ta), and oxygen consumption were measured from kits studied in a thermocline during a control period of normoxemia, an experimental period of normoxemia or hypoxemia (fraction of inspired oxygen 0.10), and a recovery period of normoxemia. We reasoned that no change or a decrease in Ta while Tc decreased during hypoxemia would indicate a regulated thermoregulatory response, whereas an increase in Ta while Tc decreased during hypoxemia would indicate a forced thermoregulatory response. Ta decreased during acute hypoxemia in the older kits but not in the 4–6-day-old kits; the decrease in Ta was accentuated on postnatal days 28–30 compared with postnatal days 9–11. Ta decreased or stayed the same during exposure to acute hypoxemia. Our data provide evidence that postnatal maturation influences the Tc response of rabbits to acute hypoxemia and that the decrease in Tc during hypoxemia in the older kits results from a regulated thermoregulatory response.

autonomic thermoregulation; behavioral thermoregulation; forced thermoregulatory response; postnatal maturation; regulated thermoregulatory response

IN NEWBORNS AND ADULTS of a number of species, core temperature decreases during acute hypoxemia (9, 23, 25). Clark and Fewell (6) have shown that the decrease in core temperature during acute hypoxemia in newborn and older guinea pigs, which are born relatively mature (27), results from a “regulated” rather than a “forced” thermoregulatory response (14). This then represents an example of “rheostasis” (26) or regulation around a shifted central nervous system thermoregulatory “set point” rather than a failure of “homeostasis” (4). Neither activation of afferents from the carotid baroreceptors and/or chemoreceptors (11) nor liberation of endogenous opioids (7) appears to mediate this core temperature response in guinea pigs. Recent experiments from our laboratory, however, support the hypothesis that adenosine plays an age-dependent role in mediating the regulated decrease in core temperature that occurs in newborn and older guinea pigs during acute hypoxemia (8).

Previous experiments carried out on anesthetized and/or acutely instrumented newborn rabbits, which are born relatively immature (27), have shown that acute hypoxemia causes a decrease in core temperature and oxygen consumption when the animals are studied at ambient temperatures below thermoneutrality (1, 3, 32) but not when they are studied at thermoneutrality (3). Thus it appears that the thermoregulatory response to acute hypoxemia may be different in newborn rabbits compared with newborn guinea pigs. We do not know, however, whether this difference is related to “maturity at birth” of the two species or to the fact that the rabbits were studied under anesthesia and/or after acute instrumentation. The present experiments, which were carried out on chronically instrumented, conscious newborn and older rabbits, have been designed to test the hypothesis that maturity at birth influences the thermoregulatory response to acute hypoxemia during early postnatal development.

METHODS

One hundred and three New Zealand White rabbits were studied. Each kit, born by spontaneous vaginal delivery, was housed with its mother and siblings in the vivarium of the University of Calgary’s Animal Resource Centre (22 ± 1°C, 20–30% relative humidity, and 12:12-h light-dark cycle). Although 22°C is below the thermoneutral zone of newborn rabbits (20, 21), each kit had the opportunity to huddle with its siblings in the nest and thus to thermoregulate behaviorally.

Surgical Preparation

Fifty-eight rabbits underwent one operation before study. Within 2–3 days of an experiment, each kit was anesthetized by inhalation of halothane (2.0% for induction and for maintenance) in oxygen. In 49 kits (experiment A), a paramedian laparotomy was done, and a battery-operated biotelemetry device (PhysioTel TA10ETA-F20, Data Sciences International, or VM-FH, Mini-Mitter) was inserted into the perito-
neal cavity for later measurement of core temperature. Innine kits (experiment B), a catheter was inserted into a carotid artery for later determination of arterial blood gases and pH.

All surgical and experimental procedures were carried out in accordance with the Guide to 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 Protocols**

*Experiment A.* For an experiment, each kit was weighed and placed in a thermocline. After a stabilization period of normoxemia, measurements were made during a control period. A period of five consecutive measurements at 2-min intervals in which core temperature did not vary more than ±0.2°C was considered to be a suitable control period. After the control period, measurements were made at 12-min intervals during a 120-min experimental period of normoxemia or hypoxemia produced by decreasing the fraction of inspired oxygen from 0.21 to 0.10 and then during a 120-min recovery period of normoxemia.

Three age ranges of kits were studied: 4–6 days of age (n = 17; 73 ± 21 g), 9–11 days of age (n = 16; 124 ± 28 g), and 28–30 days of age (n = 16; 558 ± 105 g). The animals in each age range were randomly assigned to experience either normoxemia or hypoxemia during the experimental period.

*Experiment B.* For an experiment, each kit was placed in a metabolic chamber. After a stabilization period, arterial blood was sampled at the end of a 15-min control period of normoxemia and at the end of a 15-min experimental period of hypoxemia for determination of blood gases and pH. Measurements were made in three animals at each of the aforementioned age ranges.

*Experiment C.* For an experiment, each kit was anesthetized, and blood was obtained via cardiac puncture for determination of hematocrit. Measurements were made from seventeen 4- to 6-day-old animals, twelve 9- to 11-day-old animals, and sixteen 28- to 30-day-old animals.

**Experimental Apparatus**

*Thermocline.* The thermocline used in our experiments consisted of a sealed Perspex cylinder (2 m long, 0.12 m ID) with a plastic grid along the bottom into which flowed room air. A linear temperature gradient from 6 to 40°C was produced by circulating hot and cold water (Endocal refrigerated circulating bath RTE-8DD, Neslab) into two copper coils wrapped around the cylinder. Gas of the desired oxygen concentration flowed through the thermocline from the cold end at a constant rate (i.e., room air at 1.412 l/min; 10% oxygen in nitrogen at 1.490 l/min). Each time the gas mixture was changed, the metabolic chamber was flushed by increasing the gas flow rate.

**Experimental Measurements and Calculations**

*Experiment A.* For measurement of core temperature, platform antennas (PhysioTel CTR 86, Data Sciences International), which received the output frequency (Hz) from the previously implanted biotelemetry device, were placed under the thermocline. The received output was then fed into a peripheral processor (Dataquest III, Data Sciences International) connected to an IBM computer. Selected ambient temperature was determined by observing the position of the rabbit in the thermocline. Oxygen consumption was calculated as the difference between the inflow and outflow (dry) oxygen concentration (Applied Electrochemistry S-3A/O2 analyzer, Ametek) and the flow rate. All values of oxygen consumption were normalized by the weight of the animal in kilograms and expressed at STPD.

*Experiment B.* Arterial blood gases and pH were measured on a blood-gas analyzer (NOVA Stat 3) and corrected for core temperature.

*Experiment C.* Blood was collected in microhematocrit capillary tubes (Scientific Products), centrifuged, and read in triplicate on a Readacrit centrifuge (Clay Adams).

**Statistical Analysis**

Statistical analysis was carried out by using a three-factor ANOVA for repeated measures followed by a Student-Newman-Keuls multiple-comparison test to determine whether age, gas, or time affected core temperature, selected ambient temperature, or oxygen consumption. All results are reported as means ± SD; P < 0.05 was considered to be of statistical significance.

**RESULTS**

*Experiment A*

Basal core temperature (as determined during the normoxic control period) increased, selected ambient temperature decreased, and oxygen consumption normalized by the weight of the animal did not change significantly during the first month of postnatal life (Fig. 1).

Postnatal age significantly influenced the thermo-regulatory response to acute hypoxemia (Fig. 2). Core temperature decreased during acute hypoxemia in the older kits but not in the 4- to 6-day-old kits; the decrease in core temperature was accentuated on postnatal days 28–30 compared with postnatal days 9–11. Furthermore, core temperature increased above control values during recovery from hypoxemia in the 4- to 6-day-old kits but not in the older kits. Selected ambient temperature decreased transiently during exposure to acute hypoxemia but only in the oldest kits (Fig. 3). Oxygen consumption increased during exposure to acute hypoxemia but only in the youngest kits (Fig. 4). There were no changes in any of the variables during the normoxic experimental period.

*Experiment B*

Decreasing the fraction of inspired oxygen from 0.21 to 0.10 produced hypocapnic hypoxemia in all of the kits, with the arterial PO2 decreasing from 55 ± 3 to
22 ± 1, 64 ± 5 to 23 ± 3, and 70 ± 8 to 25 ± 5 Torr and the arterial \( \text{PCO}_2 \) decreasing from 37 ± 7 to 24 ± 2, 39 ± 3 to 30 ± 6, and 34 ± 7 to 26 ± 4 Torr in the 4- to 6-day-old, 9- to 11-day-old, and 28- to 30-day-old kits, respectively.

**Experiment C**

Hematocrit values were 45 ± 4, 43 ± 5, and 35 ± 4% in the 4- to 6-day-old, 9- to 11-day old, and 28- to 30-day-old kits, respectively.

**DISCUSSION**

Our experiments provide new information about thermoregulatory control in rabbits during postnatal maturation, as well as their core temperature response to acute hypoxemia. Novel findings were as follows. 1) Core temperature increased, selected ambient temperature decreased, and oxygen consumption normalized by the weight of the animal did not change during the first month of postnatal life. 2) Core temperature decreased during acute hypoxemia in the older kits but not in the 4- to 6-day-old kits; the decrease in core temperature, which was accentuated on postnatal days 28–30 compared with postnatal days 9–11, was accompanied by a decrease or no change in selected ambient temperature. 3) Core temperature increased during the recovery period of normoxemia after hypoxemia in the 4- to 6-day-old kits but not in the older kits. Thus postnatal maturation influences the core temperature responses of rabbits to acute hypoxemia. Furthermore, our data provide evidence that the decrease in core temperature during acute hypoxemia in the older kits resulted from a regulated thermoregulatory response.
which provide the first measurements of core temperature by biotelemetry during early postnatal life in rabbits, suggest that the central nervous system thermoregulatory set point increases during early postnatal development. The decrease in selected ambient temperature that we observed during postnatal maturation most likely reflects a change in the thermoneutral environment of the rabbit because thermal efficiency increases during the first month of life. In general, our results on selected ambient temperature and oxygen consumption are in keeping with those of Hull and Hull (21) and Hull et al. (20). Experiments relating behavioral and autonomic thermoregulation have shown that most species, including mice (15), guinea pigs (16), and golden hamsters (19), select an ambient temperature that is equal to or slightly above the lower critical limit of their thermoneutral zones. Although this has not been precisely determined for the rabbit, previous experiments by Hull et al. have shown that rabbits from 1 to 14 days of postnatal life select an ambient temperature in a thermal gradient that corresponds to the ambient temperature at which minimal oxygen consumption occurs as ambient temperature is varied in a metabolic chamber.

Core temperature decreased during acute hypoxemia in the older kits but not in the 4- to 6-day-old kits. The interpretation of mechanisms of the core temperature response to acute hypoxemia was based on the
fact that newborn and older rabbits (i.e., homeothermic endotherms) can employ their somatomotor nervous system [e.g., behavioral thermogenesis by voluntary movement and shivering thermogenesis in skeletal muscle (20, 21, 29)] as well as the sympathetic portion of their autonomic nervous system [e.g., nonshivering thermogenesis in brown adipose tissue and dry heat loss or gain secondary to circulatory convection (5, 27, 30)] for thermoregulation. As in other species, a shift from nonshivering to shivering thermogenesis for heat production occurs in rabbits during early postnatal maturation (27, 30). Given that the degree of hypoxemia used in our experiments did not affect gross motor activity, we reasoned that, if the thermoregulatory response was forced such that autonomic thermoregulatory effectors were impaired by a limited oxygen supply and core temperature decreased below the central nervous system thermoregulatory set point [i.e., regulated hypothermia (14), then the animal would move to a warmer region of the thermocline in an attempt to restore core temperature to set point temperature. Alternatively, if the thermoregulatory response was regulated such that the decrease in core temperature followed a decrease in the central nervous system thermoregulatory set point [i.e., unregulated hypothermia (14)], then the animal would either move to a cooler region in the thermocline in an attempt to accelerate heat loss or would not move. This strategy has previously been used by others in investigations of the neuropharmacology of temperature regulation in adult animals (14, 17, 18, 24, 28).

In the 4- to 6-day-old kits, neither core temperature nor selected ambient temperature changed during the experimental period, suggesting that exposure to acute hypoxemia did not alter the central nervous system thermoregulatory set point. In these animals, it is likely that dry heat loss secondary to circulatory convection, resulting from hypoxic-induced vasodilation in the ear (5), balanced the heat produced by an increase in metabolic rate, as evidenced by an increase in total body oxygen consumption. The increase in metabolic rate during hypoxemia most likely resulted from the thermogenic action of catecholamines, which are released during acute hypoxemia (31) and which have an effect on brown adipose tissue that is maximum in newborn kits and at a minimum by 20 days of postnatal life (12, 30). The increase in core temperature above basal level early in the recovery period after hypoxemia most likely resulted from a transient carryover effect of the catecholamine-induced increases in metabolic rate and heat production in the absence of continued hypoxic vasodilation and resulting heat loss from the ear.

In the older kits, however, as discussed above, core temperature decreased at a time when selected ambient temperature either stayed the same or decreased. This suggests that exposure to acute hypoxemia in these age groups produced a decrease in the central nervous system thermoregulatory set point. In these animals, it is likely that dry heat loss secondary to circulatory convection was the primary source of heat loss during acute hypoxemia (5). Although the mechanism of this age-dependent core temperature response to acute hypoxemia is unknown, our data, as well as data in the literature, allow us to speculate. Previous experiments from our laboratory suggest that the decrease in the central nervous system thermoregulatory set point during acute hypoxemia results from a decrease in the transport of oxygen to the brain and the resulting release of adenosine rather than from stimulation of the peripheral arterial chemoreceptors (8, 11). In fact, experiments by Gautier et al. (13) on adult cats and the experiments from our laboratory (11) on newborn and older guinea pigs provide evidence that stimulation of the peripheral chemoreceptors may actually attenuate the decrease in core temperature that occurs during exposure to acute hypoxemia.

Oxygen transport to the brain is determined by cerebral blood flow and arterial oxygen content. Although we are unaware of data on maturational changes in the cerebral blood flow response to acute hypoxemia in rabbits, Feuer et al. (10) have shown that acute hypoxemia, produced by decreasing the fraction of inspired oxygen from 0.21 to 0.12, produces a maximal cerebral blood flow increase of 93 ± 17% in 2- to 3-wk-old rabbits. In our experiments, it is likely that hypocapnic hypoxemia produced by decreasing the fraction of inspired oxygen from 0.21 to 0.10 elicited an increase in cerebral blood flow in all three age ranges of rabbits.

It is possible that maturational changes in the hemoglobin content (present study) and in the hemoglobin oxygen affinity (2) altered the arterial oxygen content during acute hypoxemia in the three age ranges of rabbits, even though their arterial Po2 values were similar (i.e., 22 ± 1, 23 ± 3, and 25 ± 5 Torr in the 4- to 6-day-old, 9- to 11-day-old, and 28- to 30-day-old kits, respectively). We found that hematocrit values decreased from 45 ± 4% in the 4- to 6-day-old rabbits to 35 ± 4% in the 28- to 30-day-old rabbits. Furthermore, Bard and Shapiro (2) have shown that rabbits, which lack fetal-type hemoglobin (22), display marked changes in hemoglobin oxygen affinity during the first month of postnatal life that correlate with red blood cell 2,3-diphosphoglycerate levels. This results in values of arterial Po2, at which hemoglobin is 50% saturated with oxygen (p50) of ~16, 19, and 25 Torr in 4- to 6-day-old, 9- to 11-day-old, and 28- to 30-day-old kits, respectively. Taken together, these changes would produce higher arterial oxygen contents in the youngest kits compared with the older kits at a given level of hypoxemia and would perhaps explain the age-dependent thermoregulatory responses. This postulate warrants investigation.

Our laboratory previously found that decreasing the fraction of inspired oxygen from 0.21 to 0.10 produced hypocapnic hypoxemia (11) and a regulated decrease in core temperature in 2-, 5-, 10-, 15- and 26-day-old guinea pigs (6); the core temperature response was accentuated in the older compared with the younger animals. Thus similar blood-gas changes elicited a regulated decrease in core temperature in newborn guinea pigs but not in newborn rabbits. Like rabbits, guinea pigs do not have fetal hemoglobin (22). By 2 days of
postnatal age, however, they have red blood cell 2,3-diphosphoglycerate levels that are similar to that observed in the adult (2). Furthermore, their p50 at 5 days of age is ~21 Torr compared with that of ~24 Torr in the adult guinea pig. This would perhaps result in hypoxemia producing lower arterial oxygen contents in newborn guinea pigs compared with newborn rabbits, effecting different thermoregulatory responses. From a thermoregulatory standpoint, both newborn rabbits and newborn guinea pigs are considered precocial (27).

Regardless of the mechanism underlying the age-dependent thermoregulatory response to hypoxemia, our data provide evidence that postnatal maturation influences the core temperature response of rabbits to acute hypoxemia and that the decrease in core temperature during hypoxemia in the older kits results from a regulated thermoregulatory response. This response, which is prevalent throughout the animal kingdom (33), should not be viewed as a failure of homeostasis (4) but rather as an example of rheostasis (26) or regulation around a shifted set point to optimize oxygen supply and oxygen demand in an attempt to ensure survival. It is likely, however, that more severe hypoxemia would result in failure of rheostasis and force further changes in core temperature.

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