Influence of core temperature on autoresuscitation during repeated exposure to hypoxia in normal rat pups

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Serdarevich, Christine, and James E. Fewell. Influence of core temperature on autoresuscitation during repeated exposure to hypoxia in normal rat pups. J. Appl. Physiol. 87(4): 1346-1353, 1999.—Failure to autoresuscitate by hypoxic gasping during prolonged sleep apnea has been suggested to play a role in sudden infant death. Furthermore, thermal stress brought about by a contribution of infection, overwrapping, or excessive environmental heating has been shown to be associated with an increased risk of sudden infant death, particularly in prone sleeping infants. The present experiments were carried out on newborn rat pups to investigate the influence of “forced” changes in core temperature on their time to last gasp during a single hypoxic exposure and on their ability to autoresuscitate during repeated exposure to hypoxia. On day 5 or 6 postpartum the pups were placed in a temperature-controlled chamber regulated to 33, 35, 37, 39, or 41°C and exposed to a single period of hypoxia (97% N₂-3% CO₂) and their time to last gasp was determined, or they were exposed repeatedly to hypoxia and their ability to autoresuscitate from primary apnea was determined. Increases in core temperature brought about by changes in ambient temperature from 33 to 41°C decreased the time to last gasp after a single hypoxic exposure and decreased the number of successful autoresuscitations after repeated hypoxic exposures. Thus our data support the hypothesis that forced changes in core temperature brought about by changes in ambient temperature influence protective responses in newborns that may prevent death during hypoxia, as may occur during single or repeated episodes of prolonged sleep apnea.

apnea; gasping; sudden infant death syndrome

THE SUDDEN INFANT DEATH syndrome (SIDS) is defined as “the sudden death of an infant under one year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history” (39) and is a leading cause of death beyond the neonatal period during the 1st yr of life in North America. SIDS is thought to occur during sleep or during the transition from sleep to wakefulness and occurs primarily between 2 wk and 8 mo of age, with 90% of SIDS cases occurring in the first 6 mo of life (39).

Thermal stress brought about by a contribution of infection, overwrapping, or excessive environmental heating has been shown to be associated with an increased risk of SIDS in a number of anecdotal reports (3–5, 9, 31, 32, 34, 37) and case-control studies (11, 28, 29); the issue is complex, inasmuch as thermal stress appears to be a risk factor exclusively in prone sleeping infants (11, 28). Infants who sleep prone and in the face-straight-down position are at risk of rebreathing their expired air, resulting in significant asphyxia (7, 23) and obstructive apnea from occlusion of the nares and/or from backward pressure on the mandible, which may lead to reduced oropharyngeal airway dimensions (36). Very little is known, however, about the possible consequences of hyperthermia (i.e., a “forced” increase in core temperature above the central nervous system thermoregulatory “set point” caused by overwrapping or excessive environmental heating) or fever (i.e., a “regulated” increase in core temperature that follows an increase in central nervous system thermoregulatory set point caused by pyrogens released during an infection) on baseline cardiorespiratory control or on protective responses that prevent hypoxia and death during rebreathing or apnea.

An inability to recover from prolonged sleep apnea has long been postulated as a possible factor in SIDS (18, 20, 21). In humans, recovery from sleep apnea is thought to occur early as a result of arousal from sleep or later as a result of hypoxic gasping, when it is termed “autoresuscitation” (18, 35). With this and the aforementioned information on thermal stress taken into consideration, one possible consequence of a change in core temperature may be an alteration in protective responses that prevent hypoxia (i.e., arousal) and death (i.e., autoresuscitation) during prolonged sleep apnea. Our present experiments have focused on the latter of these protective responses and have been carried out to test the hypothesis that forced changes in core temperature brought about by changes in ambient temperature influence the ability of newborn rats to “autoresuscitate” from primary apnea during repeated exposure to hypoxia, as may occur during episodes of prolonged sleep apnea.

METHODS

Fifty-three, 5- to 6-day-old Sprague-Dawley rat pups were studied. Each pup, born by spontaneous vaginal delivery, was housed with its mother and siblings (22 ± 1°C, 20–30% relative humidity, 12:12-h light-dark cycle) until an experiment. Although 22°C is below the thermoneutral zone of newborn rats, each pup had the opportunity to select its ambient temperature between experiments by huddling with its siblings and/or mother (i.e., behavioral thermoregulation). Experimental Protocols

Experiment I: time to last gasp. For an experiment, each 5- to 6-day-old pup was removed from its mother and siblings, weighed, and placed in a metabolic chamber regulated to 33,
35, 37, 39, or 41°C into which flowed room air at a rate of 1 l/min. At the end of a 30-min stabilization period, the gas that flowed into the chamber was changed from room air to 97% N₂-3% CO₂ and the time to last gasp was determined. At the beginning of the hypoxic exposure the chamber was flushed with the hypoxic gas mixture until the gas concentrations in the chamber had stabilized; the flow rate was then lowered to 1 l/min. Five pups from each of seven litters were studied at one of the aforementioned ambient temperatures. The sequence of ambient temperatures was randomly selected for the five experiments carried out on each litter.

Experiment II: autoresuscitation. For an experiment, each 5- to 6-day-old pup was removed from its mother and siblings, weighed, and placed in a metabolic chamber regulated to 33, 35, 37, 39, or 41°C into which flowed room air at a rate of 1 l/min. At the end of a 30-min stabilization period, the gas that flowed into the metabolic chamber was changed from room air to 97% N₂-3% CO₂ until primary apnea occurred; the gas was then changed back to room air, and the ability of the pup to autoresuscitate by gasping was determined. This procedure was repeated at 5-min intervals until death occurred. Again, when the gas mixture was changed, the flow rate was increased until the gas concentrations in the chamber had stabilized; the flow rate was then lowered to 1 l/min. Three pups from each of six litters were studied at one of the aforementioned ambient temperatures. The sequence of ambient temperatures was randomly selected for the three experiments carried out on each litter.

The respiratory response of newborn (22) and adult (19) animals to acute hypoxia typically passes through four stages: hyperpnea, primary apnea, gasping, and terminal apnea. During an autoresuscitation experiment, primary apnea was detected by observing respiratory movements on the polygraph tracing. Autoresuscitation was deemed to occur when heart rate and respiratory rate returned to >60% of control levels within 5 min.

Experimental Apparatus

Metabolic chamber. The metabolic chamber used in our experiments consisted of a double-walled Plexiglas cylinder (30 cm long, 6 cm ID) into which flowed room air or 97% N₂-3% CO₂. Chamber ambient temperature was controlled by circulating water from a temperature-controlled bath (Neslab, Endocal Refrigerated Circulating Bath RTE-8DD) through the space between the walls.

Experimental Measurements and Calculations

During an experiment the electrocardiogram, respiratory movements, and chamber CO₂ levels were recorded on a polygraph (model 7, Grass Instrument) at a paper speed of 10 mm/s. The electrocardiogram was recorded from multistranded stainless steel wire electrodes (AS 633, Cooner Wire) sewn across the chest wall that were connected to a high-impedance probe (model 7HIP5, Grass Instrument) coupled to a wide-band electroencephalogram alternating-current preamplifier (model 7P5, Grass Instrument). Respiratory movements were recorded from a mercury-in-silicone rubber strain gauge (model HgPC, D. M. Davis) placed around the chest, which was connected to a bridge amplifier (Mountain Scientific Consulting, Calgary, AB, Canada), which was coupled to an adapter panel (model 7P03, Grass Instrument). Chamber CO₂ levels were measured using an Applied Electrochemistry CO₂ analyzer (Ametek), which was coupled to an adapter panel (model 7P03). Core temperature was measured using an 18-gauge copper-constantan thermocouple sheathed in Teflon (model 1T-18, Physitemp) interfaced with a thermometer (model BAT-12, Physitemp). The thermocouple was inserted ~1 cm into the pup’s rectum and glued to its tail by use of tissue adhesive (Vetbond, 3M Animal Care Products). Core temperature was recorded in each animal immediately before the first hypoxic exposure.

Statistical Analysis

Statistical analyses was carried out using a Pearson product-moment correlation coefficient to determine the relationship between core temperature and ambient temperature and the relationship between heart rate, respiratory rate, time to last gasp, total number of gasps, number of successful autoresuscitations, and core temperature. Values are means ± SD, and P < 0.05 was considered to be of statistical significance.

RESULTS

Ambient Temperature and Core Temperature

Core temperature mirrored ambient temperature, as evidenced by the strong correlation between the two variables (Fig. 1).

Core Temperature and Basal Heart Rate and Respiratory Rate

Core temperature significantly influenced basal heart rate (Fig. 2A), with basal heart rate increasing as core temperature increased. Basal respiratory rate, on the other hand, did not vary in a significant fashion with core temperature (Fig. 2B).

Experiment I: Time to Last Gasp

Core temperature significantly influenced the time to last gasp (Fig. 3A) and the total number of gasps (Fig. 3B). Exposure to a single period of hypoxia resulted in a reproducible respiratory response (Fig. 4). Initially, there was a period of hyperpnea and arousal that preceded primary apnea; primary apnea was followed by a period of rapid gasping that was followed by a period of slower gasping of one to two gasps per minute; finally, there was a period of rapid gasping that eventually waned and gave way to terminal apnea and death. As core temperature changed, it was primarily the period of slow gasping that appeared to change; the length of the period of slow gasping varied inversely with core temperature (Fig. 5). In all animals, gasping
ceased before the appearance of arrhythmias or an isoelectric pattern on the electrocardiogram. Heart rate decreased during exposure to hypoxia, the magnitude of which was influenced by core temperature (Fig. 6). With increasing core temperature, heart rate remained higher during hypoxia.

Experiment II: Autoresuscitation

Core temperature significantly influenced the ability of the rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia (Fig. 7). With increasing core temperature, the number of successful autoresuscitations decreased. Before autoresuscitation failure, all successful autoresuscitations exhibited the same cardiorespiratory pattern illustrated in Fig. 8. Initially, there was a period of hyperpnea and arousal that preceded primary apnea and bradycardia; gasping was followed by an increase in heart rate and then restoration of a normal respiratory pattern. The mechanism of autoresuscitation failure, however, in a number of the pups that were studied at an ambient temperature of 41°C appeared to be different from that in the pups studied at ambient temperatures of 33 and 37°C. In all pups that were studied at ambient temperatures of 33 and 37°C, autoresuscitation failure followed atrioventricular dissociation subsequent to early cardiac resuscitation, as evidenced by an initial return of heart rate toward control; the atrioventricular dissociation and ultimate loss of ventricular depolarization preceded the cessation of gasping (Fig. 9). In three of the six pups that were studied at an ambient temperature of 41°C, however, gasping ceased before signs of cardiac resuscitation appeared on the electrocardiogram.

DISCUSSION

Our experiments provide new information about factors that influence the newborn’s ability to survive hypoxia, as may occur during prolonged sleep apnea. Novel findings in our study were 1) that core temperature altered the gasping pattern and the time to last gasp during a single hypoxic exposure and 2) that core temperature altered the ability of rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia. Thus our data provide evidence that core temperature influences protective responses that prevent death during severe hypoxia, as may occur during prolonged sleep apnea.

Core temperature influenced the time to last gasp during a single hypoxic exposure, with the time to last gasp decreasing as the core temperature increased. These results are in keeping with the results of earlier studies where ambient temperatures and thus core temperatures of newborn rats were varied over different and/or greater ranges (30): ambient temperatures of 20–40°C (10), ambient temperatures of 24–34°C (1), and core temperatures of ~10 to ~38°C. On exposure to a single period of hypoxia, our rat pups exhibited a
triphasic gasping pattern after primary apnea, as has been previously shown to occur by others in newborn rats (17, 40) and rabbits (6, 25), but not in mice (22), when the animals were studied at ambient temperatures below their thermoneutral zone. This triphasic gasping pattern consisted of an initial phase of rapid gasping of six to eight gasps per minute that was followed by a second phase of slower gasping of one to two gasps per minute; finally, there was a third phase of rapid gasping that eventually waned and gave way to terminal or secondary apnea and death. As core temperature changed, it was primarily the second phase of slow gasping that was influenced, the length varying inversely with core temperature. As far as we are aware, the neurophysiological basis for the three phases of gasping that follow primary apnea is unknown. It may, however, result from firing of different populations of neurons in the lateral tegmental field of the medulla, the proposed neural substrate underlying gasping in the rat (12, 38), which have different thresholds and/or latencies to the hypoxic stimulus, or perhaps it results from the influence of various neuromodulators on the firing pattern of a single population of neurons during hypoxia.

A number of factors other than core temperature have been shown to influence the time to last gasp in rats during exposure to hypoxia at a given postnatal age. These include blood glucose levels (30, 40), adrenal catecholamines (41), excitatory amino acids (15), and nitric oxide (16). To our knowledge, however, none of these factors other than nitric oxide has been shown to solely influence the second phase of slow gasping in a fashion similar to what we have observed in our present experiments. Specifically, Gozal et al. (16) showed that pretreatment of 5-day-old rat pups with N-nitro-L-arginine, a nitric oxide synthase blocker, significantly increases the second phase of slow gasping but not the first or third phase of rapid gasping observed on exposure to nitrogen. Thus it is possible that nitric oxide may have played a role in modulating the second phase of slow gasping that was observed during hypoxia after changes in core temperature in our experiments. The fact that increases in core temperature specifically shortened the second phase of gasping, rather than truncating the third phase, supports the contention that factors other than a sole exhaustion of energy substrate mediate the response. Interestingly, it is a decrease in the length of phase 2 of slow gasping that is primarily responsible for the decrease in time to last gasp that occurs in rats in response to hypoxia during postnatal maturation (17; unpublished observation).

Peiper (27), Stevens (33), and Thach (35) emphasized that gasping is important in "self-resuscitation" or autoresuscitation during apnea in human infants and that repeated episodes of apnea may lead to autoresuscitation failure and death. The process of recovery from hypoxia by gasping was first termed self-resuscitation in 1969 by Adolph (2) and then autoresuscitation in 1975 by Guntheroth et al. (19). Gershan et al. (13) recently defined the cardiorespiratory events that occur during successful autoresuscitation from hypoxic ap-
nea in mice: 1) gasping with marked bradycardia, 2) cardiac resuscitation with a rapid increase in heart rate to >60% of baseline, and 3) respiratory resuscitation with an increase in respiratory rate to >60% of baseline. We observed a similar sequence of events during successful autoresuscitation in our rat pups. Likewise, we found, as did Gershan et al. (14), that repeated exposure to hypoxia led to autoresuscitation failure, which was associated with cardiac arrhythmia (i.e., atrioventricular dissociation) that preceded cessation of gasping in normal animals. Another novel finding in our present experiments was that increasing core temperature not only impaired the ability of rat pups to autoresuscitate after repeated exposure to hypoxia, but it altered the sequence of events leading to autoresuscitation failure. In all pups that were studied at ambient temperatures of 33 and 37°C, autoresuscitation failure followed atrioventricular dissociation subsequent to early cardiac resuscitation, as evidenced by an initial return of heart rate toward control; the atrioventricular dissociation and ultimate loss of ventricular depolarization preceded the cessation of gasping. In three of the six pups that were studied at an ambient temperature of 41°C, however, gasping ceased before signs of cardiac resuscitation appeared on the electrocardiogram. The mechanism responsible for the changes in the physiology of this protective response with increases in core temperature is unknown and warrants further investigation.

Our experiments were carried out on an altricial species, the rat. Typically, in this species, there are many newborns in the litter, and they are poorly developed, naked, blind, and helpless. Although the rat pup is considered to be a homeotherm, the range of ambient temperatures over which it can maintain its core temperature is narrow (26). Thus, as seen in our present experiments, it was relatively easy to force changes in core temperature by changing ambient conditions.
temperature. The resulting changes in core temperature most likely elicited parallel changes in metabolic rate via a Q_{10} effect (24) as well as changes in the concentrations of various neurotransmitters in the central nervous system (8). Whether changes in ambient temperature would have the same effect on these variables and on the protective responses to hypoxia in a more precocial newborn such as the guinea pig, which is more adept at maintaining its core temperature over a wide range of ambient temperatures, remains to be determined.

**Perspectives**

The results of our experiments provide insight into how a forced elevation in core temperature may place infants at an increased risk of SIDS. Thermal stress brought about by a contribution of infection, overwrapping, or excessive environmental heating has been shown to be associated with an increased risk of SIDS...
in a number of anecdotal reports (3–5, 9, 31, 32, 34, 37) and case-control studies (11, 28, 29). As previously discussed, an inability to recover from prolonged sleep apnea has long been postulated as a factor in SIDS (18, 20, 21), and recovery from sleep apnea is thought to occur early as a result of arousal from sleep or later as a result of hypoxic gasping, when it is known as autore-
suscitation (18, 35). Given the recent results of our present experiments, we would speculate that a forced increase in core temperature places infants who have apnea from whatever cause at increased risk for severe hypoxia and death because of an impairment of protective responses that terminate apnea and restore normal tidal ventilation.

Fig. 8. Continuous polygraph tracing showing a successful autorecussitation of a 5-day-old rat pup from primary apnea when studied at an ambient temperature of 37°C. During exposure to hypoxia, an initial period of hyperpnea and arousal (A) preceded primary apnea and bradycardia (B); gasping (C) was followed by an increase in heart rate (D) and then restoration of a normal respiratory pattern (E).

Fig. 9. Continuous polygraph tracing showing autorecussitation failure in a 5-day-old rat pup that was studied at an ambient temperature of 37°C. During exposure to hypoxia, an initial period of hyperpnea (A) and arousal (B) preceded primary apnea and bradycardia (C); gasping (D) was followed by an increase in heart rate of normal sinus rhythm (E) that gave way to atrioventricular dissociation (F); and ultimate loss of ventricular depolarization preceded cessation of gasping.
REFERENCES