Inhibitory effects of hyperthermia on mechanisms involved in autoresuscitation from hypoxic apnea in mice: a model for thermal stress causing SIDS

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Kahraman, Levent, and Bradley T. Thach. Inhibitory effects of hyperthermia on mechanisms involved in autoresuscitation from hypoxic apnea in mice: a model for thermal stress causing SIDS. J Appl Physiol 97: 669–674, 2004; 10.1152/japplphysiol.00895.2003.—The physiological mechanisms that might be involved in an association between heat stress and sudden infant death syndrome (SIDS) are obscure. We tested the hypothesis that a combination of acute hypoxia and elevated body temperature (T_B) might prevent autoresuscitation from hypoxic apnea (AR). We exposed 21-day-old mice (total = 216) to hyperthermia (40.5–43.5°C), hypoxia, or a combination of the two. Neither hyperthermia alone (40.5–42.5°C) nor hypoxia alone was found to be lethal, but the combination produced failure to AR during the first hypoxic exposure with increasing frequency as T_B increased. The ability to withstand multiple hypoxic exposures was also reduced as T_B increased. In contrast, heat stress causing moderate T_B increase (40.5°C) had no effect on survival. Increased T_B (43.5°C) reduced gasping duration and number of gasps. It increased heart rate during anoxia but did not alter gasping rate. Furthermore, the oxygen-independent increase in heart rate observed before gasping failure was usually delayed until after the last gasp in hyperthermic animals. Mild dehydration occurred during T_B elevation, but this did not appear to be a primary factor in AR failure. We conclude that a thermal stress, which by itself is nonlethal, frequently prevents AR from hypoxic apnea. This may be due, at least in part, to decreased gasp number and duration as well as to hyperthermia-related asynchrony of reflexes regulating heart and gasping frequencies during attempted AR.

Sudden Infant Death Syndrome (SIDS) occurs in infants during a relatively brief period of development with peak incidence at 2–3 mo of age. It has been suggested that thermal stress with or without actual increase in body temperature (T_B) may be causal in many SIDS cases (6, 12). The primary evidence for this comes from epidemiological studies. These studies have found increased environmental temperature and/or history of heavy sweating in association with some SIDS cases (6, 26, 27). Despite this, the potential physiological mechanisms linking heat stress to SIDS are unclear.

Healthy infants have frequently been observed to autoresuscitate (AR) from prolonged apnea without apparent ill effects, indicating that this can be an important mechanism for surviving severe episodes of hypoxia (22, 32). Failure to AR from hypoxic apnea by gasping is well documented in SIDS cases, and it has been proposed that such failure could be a critical SIDS causal mechanism (21, 25). Examination of terminal recordings of infants dying of SIDS reveals that hypoxic apnea is followed by gasping, and yet AR fails during the first attempt. In contrast, infants with other diagnoses often have repeated successful AR attempts immediately before death (25).

It has previously been shown in 5-day-old rats that hyperthermia changes AR patterns and that, compared with control animals, death occurs more rapidly but only after repeated hypoxic exposures with repeated resuscitations (24). In contrast, Swiss Webster mice (21–23 days old) have been shown to be prone to AR failure during a relatively narrow window of development and after the first exposure to hypoxia, providing a model more closely resembling the AR failure occurring in SIDS (4, 10, 14, 15). In the present study, we tested four hypotheses in 21- to 23-day-old C57BL/6 mice, which are normally competent in AR. These hypotheses were the following: 1) moderate thermal stress sufficient to elevate T_B does not impair AR, 2) T_B values that are in and of themselves well tolerated can seriously impair AR, 3) AR failure during hyperthermia often occurs after the very first hypoxic exposure, 4) dehydration resulting from hyperthermia is a significant factor in AR failure, and 5) hyperthermia impairs heart rate- and gasping rate-regulating mechanisms that potentially contribute to successful AR.

METHODS

Animals. Unanesthetized, 21- to 23-day-old, C57BL/6 weanling mice (Jackson Laboratories) were used in the experiments (n = 296). They were kept with their mothers until the time of the experiment. Animals were weighed before and after the experiment. These studies were approved by the Institutional Animal Studies Committee.

Protocol 1. In these experiments, we studied the effects of different T_B values on the number of times mice were able to AR after repeated hypoxic exposures. Each litter included randomly chosen animals that were allocated to each of three experimental groups: 1) hypoxia and hyperthermia, 2) hypoxia only, and 3) hyperthermia only control animals. Animals were not restrained. Colonic temperatures (T_B) were continuously monitored via a copper-constantan thermocouple inserted ~1.5 cm into the rectum (model BAT-12, Sensortek, Clifton, NJ).

In the first experimental group (n = 102), hyperthermia was induced by an infrared heat source. Animals were heated at a rate of ~0.8 ± 0.1°C/min. The mean temperature of chamber wall was 43.9 ± 0.75°C. A continuous flow of room airflow (22°C) passed through the chamber at 2 l/min except during anoxia. Four target temperatures were studied: 40.5, 41.5, 42.5, and 43.5°C. Immediately after the target temperature was reached, hypoxic apnea was induced...
by sudden introduction of a 97% N₂-3% CO₂ gas mixture into the chamber at the same flow rate (9, 10, 14). Then a flow of 21% O₂ (balance N₂) was started at the onset of the apnea to allow for successful AR (10). In groups 1 and 2 once gasping ceased and eupneic respiration started, apnea was induced again before the animal was fully awake. This process was repeated until the animal failed to AR (10). Thus the survival rate (% successful AR) after the first episode and total number of successful ARs per animal were determined. The peak TB studied (43.5 °C) was below hyperthermic seizure threshold, although it is close to the threshold documented in past studies of hyperthermia-induced seizures in rats and mice (13, 19, 20).

The second group (hypoxia only, n = 82) was exposed to anoxic gas mixture without preheating. As with the first group, the mice rapidly lost consciousness and became apneic, at which point a flow of room air was resumed. The third group consisted of animals heated to the various target temperatures and then promptly allowed to cool off (n = 58).

Protocol 2. In these experiments, mice (n = 54) were warmed to a TB of 43.5 °C and were exposed to continuous anoxia so that time to last gasp, which is a measure of an animal’s tolerance to anoxia, could be measured (14, 24). During this second set of experiments, respiration was recorded using a semiquantitative plethysmograph closely similar to one previously used by our laboratory (16). A 20-ml cylindrical custom-made polypropylene chamber that covered most of the trunk of the animal was used. Latex sheets over both ends provided a seal so that pressure oscillations inside the chamber caused by respirations could be recorded. The pressure changes within the plethysmograph were detected by a differential pressure transducer and recorded on a chart recorder (Beckman Instruments, Schiller Park, IL). The electrocardiogram was recorded after subcutaneous placement of thoracic, fine-wire electrodes (38 gauge, Isomid, Belden, Chicago, IL) that were connected to a differential alternating-current preamplifier (model P15, Grass Instrument, Chicago, IL). During these experiments, we gathered data on respiratory responses such as time to last gasp, number and frequency of gasps, as well as heart rate changes during the gasping period.

The heart rate data were analyzed to find the severity and the duration of bradycardia. As used in prior studies, the point at which heart rate increased to 60% of the baseline (averaged over 2 s) was used as an end point for some of the analyses (9, 10).

Statistics. Categorical variables were analyzed by χ² analysis. Unpaired t-test or ANOVA was used for continuous variables. A value of P < 0.05 was considered significant. Values are shown as means ± SE.

RESULTS

General characteristics of animals. Weight and initial TB for animals studied in repeated hypoxia experiments were similar in test (hypoxia + hyperthermia) and control groups (Table 1).

Effect of thermal stress on dehydration. Increased weight loss occurred in the hyperthermia groups (Table 1, Fig. 1). With increasing hyperthermia, there was a relative increase in weight loss (r = 0.87, P < 0.01; Fig. 1).

Table 1. Baseline characteristics of animals studied during the first series of experiments (repeated hypoxic exposures)

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Test Group (Hypoxia and Hyperthermia)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypoxia only</td>
<td>Hyperthermia only</td>
</tr>
<tr>
<td>Weight, g</td>
<td>8.97±0.14</td>
<td>8.83±0.13</td>
</tr>
<tr>
<td>Weight loss, % of body weight</td>
<td>0.8±0.23</td>
<td>1.41±0.13</td>
</tr>
<tr>
<td>Initial temperature, °C</td>
<td>36.26±0.06</td>
<td>36.4±0.08</td>
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Values are means ± SE. There were no significant differences between the groups except for the weight loss that occurred during the experiments.

Effects of colonic temperature on survival. AR was highly successful in control animals at normal colonic temperatures. Increased colonic temperature started affecting the survival rate adversely at a TB of 41.5 °C in the hyperthermia and hypoxia group (Fig. 2). The first trial survival of mice exposed to heat and hypoxia varied with TB in a dose-dependent fashion (range 100–25%; P < 0.001). The number of hypoxic trials before AR failure occurred was also decreased in hyperthermic animals, and this also was dose dependent (P < 0.001; Fig. 3).

Significantly, hypoxic stress alone resulting in moderate elevation of TB (40.5 °C) and increased weight loss had no effect on survival either during the first or multiple exposures (Figs. 2 and 3). There was no effect of sex on survival.

Effects of colonic temperature on gasping. During the protocol 2 experiments using continuous anoxia, several signifi-
significant differences were observed between the control and test animals (Fig. 4). The total number of gasps as well as time to last gasp were significantly decreased ($P < 0.01$) (Fig. 5). Notably, however, we did not find a significant difference in gasp frequency (Fig. 5; $P = 0.32$).

**Heart rate changes in hyperthermic-hypoxic mice.** Baseline respiratory and heart rates were increased in hyperthermic compared with normothermic mice (253 ± 7.33 vs. 186 ± 6.04 breaths/min and 796 ± 15.95 vs. 692 ± 15.25 beats/min in hyperthermic and normothermic mice, respectively; $P < 0.01$). Hypoxic bradycardia at onset of hypoxic apnea was found to be less pronounced in hyperthermic mice (168 ± 12.95 vs. 260 ± 8.87 beats/min; $P < 0.01$; Fig. 6). Increases in heart rate after hypoxic apnea to at least 60% of prehypoxic levels were frequently observed in both normothermic and hyperthermic mice. However, this would more often occur before the last gasp in normothermic compared with hyperthermic mice, in which it usually occurred after the last gasp ($P < 0.01$; Fig. 7).

**DISCUSSION**

Physiological mechanisms involved in AR have been studied for well over 300 years, and yet a role for AR in survival apart from the immediate perinatal period has, for the most part, been ignored. A departure from this thinking was the proposal of French et al. (7) that failure to AR from apnea might be causal in SIDS. More recently, it was proposed that thermal stress, even when not associated with increased $T_B$, could be causal in SIDS; however, mechanisms whereby this could be fatal in the absence of overt heatstroke have remained largely obscure (6, 12). We chose to evaluate $T_B$ values below the seizure threshold in mice as appropriate in testing this model because infants frequently experience febrile seizures due to elevated $T_B$, and these are considered benign (13). Accordingly, our ranges of $T_B$ used in this study are consistent with those experienced by many healthy infants.

Our findings are in several respects consistent with those of Sederevich and Fewell (24) in neonatal rats with respect to hyperthermia causing decreased gasping time, decreased number of gasps, and increased heart rate. We found increased susceptibility to repeated anoxic exposures as they did. However, in marked contrast to their findings, we have shown that young mice frequently fail to AR on the first exposure to anoxia when heat stressed. In several other respects, we were able to provide new information. We found the dehydration that occurs during the thermal exposure has little effect if any on survival. In addition, we found evidence of incoordination of cardiovascular and respiratory control during attempted AR when mice were overheated. Finally, we were able to show that moderate heat stress alone did not adversely effect AR, a finding contrary to what the thermal stress theory would predict in this model.

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**Fig. 3.** Number of successful AR with repeated hypoxic exposures in mice with normothermic and hyperthermic temperatures. Error bars represent SE. $P < 0.001$.

**Fig. 4.** Effects of elevated body temperature ($T_B$) on the preterminal oxygen-independent increase in heart rate. **A:** tracings of respirations (Resp) and electrocardiogram (ECG) in a mouse with normal $T_B$. The 2 traces at bottom are continuations of the 2 traces at top. Dots mark heart beats. G, gasps. **B:** hyperthermic mouse. Note increase in cardiac rate before last gasp in normothermic but not in hyperthermic mouse and prolonged gasping in the normothermic mouse.
Probably the biggest factor contributing to the decreased number of ARs during repeated hypoxia is a progressive decrease in cardiac glycogen stores. These are essential for successful AR (4). In past work, it was found that cardiac glycogen is almost nil at the time of the last of several AR attempts (4). In mice, cardiac glycogen stores reach a nadir at 19–22 days (4). The increased susceptibility of 21-day-old Swiss mice to AR failure on first anoxic exposure, compared with other mouse strains, has been attributed to their exceptionally low cardiac glycogen at this stage of development (4). The mice used in the present study presumably reach a nadir in cardiac glycogen during this developmental period; however, as shown in our study, unlike Swiss mice, they are competent in AR at normal TB (15). Increased TB increases cardiac rate and would be expected to increase anaerobic metabolism in the C57BL/6 mice used in this study. Whether this would be sufficient to lower cardiac glycogen to levels normally occurring in same-aged Swiss mice is unclear. In any event, 21-day-old mice appear to be a better model than 5-day-old rats for heat stress-associated SIDS because, unlike deaths in infants due to other causes, SIDS infants die without evidence of prior successful ARs (21, 24).

One of the arguments that heat stress may be a major factor in SIDS is that a substantial number of SIDS infants are found wet with perspiration at death (12). It is well known that infants are particularly vulnerable to dehydration and hypovolemia when exposed to excessive environmental temperatures, yet the role of dehydration in heat-stressed SIDS infants has not been considered. The mice in the present study experienced mild dehydration during brief exposure to elevated ambient temperature. This can largely be attributed to their increased salivation and fur-wetting behavior (8, 28). Because AR is dependent on maintenance of a critical level of blood pressure, dehydration and associated hypovolemia and hypotension would be expected to impair AR competence (29). In the present study, dehydration was probably not a major factor in AR failure because mortality increased between 40.5 and 41.5°C TB, whereas weight loss did not change over this range. Furthermore, mild dehydration (5–10%) is generally well tolerated in infants. However, with longer heat exposures producing larger fluid losses, dehydration would be expected to play an increasingly important role in AR failure.

If cardiac and respiratory reflexes involved in AR were accelerated to the same degree during TB elevation, consistent

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**Fig. 5.** Number of gasps (A) and time to last gasp and gasp frequency (B) in control and hyperthermic (colonic temperature = 43.5°C) animals during continuous anoxia. Error bars represent SE. Number of gasps and time to last gasp were significantly different (*P < 0.01). Gasp frequency was not significantly different between the 2 groups (P = 0.32).

**Fig. 6.** Heart rates (HR) in normothermic and hyperthermic animals at various stages during hypoxia last gasp (LG). Error bars represent SE. *P < 0.01.
with the thermal Q_{10} effect, one might expect that AR would still occur as T_{B} increases, only at a faster rate (18). In fact, during maturation, although adult mice have a much shorter anoxic survival than infant mice, they are capable of AR during hypoxia equally well. This has been attributed to both increased heart rate and gasping frequency in the adult (9). Coordination of heart rate and gasping frequency appears to be crucial for successful AR during maturation (9). However, in the present case, with T_{B} elevation, heart rate and gasping frequency during AR were not coordinated as they are during maturation because heart rate increased but gasping frequency did not. Hence, cardiovascular and respiratory reflexes were not equally affected by T_{B}, and this may have prevented AR. Yet another example of incoordination of reflexes was evident in the preterminal heart rates. It is well documented that increasing heart rate once gasping has started is required for successful AR. Oxygen delivery to the arterial pacemaker, which produces an increase in heart rate, is provided by gasping (1). However, we like others (1, 2) observed an oxygen-independent increase in heart rate occurring late in the AR attempt. An increase in heart rate to 60% of control is usually associated with successful AR in mice when oxygen is made available at onset of the gasping phase (10, 14). Therefore, an oxygen-independent heart rate increase of this magnitude might be beneficial in AR. It is certain, however, that this would be of little or no benefit if it occurred after the last gasp.

Another and perhaps an even more important reason for AR failure in overheated, hypoxic mice may involve the different effects of heat and hypoxia on peripheral blood flow. It is well established that normal thermoregulation when T_{B} increases is associated with increased blood flow to the skin and extremities (17, 30). In contrast, hypoxia decreases such blood flow (3, 5). This decrease is regarded as a protective reflex because the redistribution of blood flow serves to decrease cardiac work load while preserving blood flow to vital internal organs (3, 5). Just how these opposing vascular reflexes are regulated when hypoxia and hyperthermia coexist is unclear. A very rapid change from excessive peripheral vasodilation to vasoconstriction would be required to maintain blood pressure, and therefore any diminution or retardation of the hypoxic redistribution of blood flow could significantly impair AR.

Finally, recent studies indicate that glottic closure occurs immediately after a gasp (31). This in theory could be beneficial in successful AR. In any event, it is noteworthy that hyperthermia has been shown to markedly affect glottic closure-regulating mechanisms in immature species (11).

The thermal stress theory for SIDS, particularly that occurring in the absence of elevated T_{B}, has yet to be proven. Our findings do not add support for the theory that moderate heat stress alone can cause AR failure. It does seem probable, however, that some deaths diagnosed as SIDS result, in part at least, from elevated T_{B} resulting from increased environmental temperatures, febrile illnesses, overbundling, and prone sleeping position or a combination of these factors (23, 26, 27). In home-monitored SIDS deaths, gasping is present but bradycardia persists and AR is unsuccessful (21, 25). If an hypoxic event such as that associated with sleep apnea were to coincide with increased T_{B}, then our findings that elevated T_{B} can prevent AR in a manner similar to that occurring in SIDS would offer a plausible explanation for an association between heat stress and SIDS.

We conclude that an environmental thermal stress with moderate T_{B} elevation has no effect on survival during AR but that further T_{B} increase, which by itself is nonlethal, can often prevent AR from hypoxic apnea. The mechanism whereby thermal stress impairs AR may be related, at least in part, to decreased gasp number and duration as suggested previously (24). Our findings suggest that altered cardiorespiratory adaptations to hypoxia, including asynchrony of reflexes regulating heart rate and gasping frequency, may also be important in the adverse effects of hyperthermia on AR, whereas the effects of elevated T_{B} on fluid loss appears to be less important.

GRANTS

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REFERENCES


