PRIOR EXPOSURE TO HYPOXIC-INDUCED APNEA IMPAIRS PROTECTIVE RESPONSES OF NEWBORN RATS IN AN EXPOSURE DEPENDENT FASHION: INFLUENCE OF NORMOXIC RECOVERY TIME

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ABSTRACT

Experiments were carried out to determine if prior exposure to hypoxic-induced apnea impairs protective responses of newborn rats. Ninety-five, 5 to 6 day-old rat pups were instrumented for respiratory measurements and placed prone in a metabolic chamber regulated to 37.0°C. The time to first and last gasp as well as the number of gasps were determined upon exposure to unrelenting hypoxia after each pup had experienced 0, 1, 2, 3, 4, 9 or 14 hypoxic-induced apnea / autoresuscitation cycles (HIA/AR) at 5-minute intervals. Prior exposure to HIA/AR did not significantly alter the time to first gasp but it decreased the time to last gasp after two HIA/AR and the number of gasps after three HIA/AR upon exposure to unrelenting hypoxia. When the normoxic recovery time following 9 HIA/AR was varied from 5 to 120 minutes, the time to last gasp as well as the total number of gasps increased upon exposure to unrelenting hypoxia but only at 120 minutes (i.e., the number of gasps was similar but the time to last gasp was still decreased compared to that observed in naïve animals exposed to unrelenting hypoxia). Thus, prior exposure to hypoxic-induced apnea as may occur during obstructive sleep apnea or positional asphyxia decreases the number and duration of potential autoresuscitation producing gasps upon exposure to unrelenting hypoxia for a period of up to and exceeding 120 minutes, respectively. The mechanism by which prior exposure to hypoxic-induced apnea influences the duration and number of hypoxic-induced gasps is unknown.
INDEX TERMS

Apnea

Autoresuscitation

Hypoxic Gasping

Rat

Sudden Infant Death Syndrome
INTRODUCTION

The respiratory response of newborn mammals [e.g., mice (21), rabbits (3,6,10,22), rats (8,14), and sheep (42)] to unrelenting hypoxia typically passes through four stages: hyperpnea, primary apnea, gasping, and secondary apnea. The onset of gasping following primary apnea occurs when the PaO$_2$ decreases to ~8-10 torr; this is true during hypercapneic hypoxia produced by airway obstruction or during hypocapneic hypoxia produced by inhalation of a hypoxic gas mixture (16,22). Peiper (25), Stevens (38), and Thach (40) have emphasized the importance of hypoxic gasping in "self-resuscitation" (1) or "autoresuscitation" (16) in human infants and that repeated exposure to hypoxia may lead to autoresuscitation failure and death. Recent reports by Poets et al (26) and Sridhar et al (35) -- where home memory monitor recordings of sudden infant deaths have been analyzed -- have documented failure of hypoxic gasping to effect “autoresuscitation” and prevent death in a number of apneic infants. Considering the importance of gasping as the last operative mechanism used by mammals to ensure survival during exposure to severe hypoxia, it is important to be knowledgeable of factors that influence the onset, duration and number of potential autoresuscitation producing gasps as a first step in understanding the integrated physiology of successful autoresuscitation as well as the pathophysiology of failed autoresuscitation from hypoxic-induced apnea.

We have recently reported that naïve 5 to 6 day-old rat pups -- studied at thermoneutrality -- display a triphasic pattern of gasping upon exposure to unrelenting hypoxia (8). In these animals, hypoxic-induced primary apnea was followed by a period of rapid gasping that lasted 1 to 2 minutes; this period of rapid gasping was followed by a period of slower gasping of 1 to 2 per gasps per minute that lasted 6 to 8 minutes;
finally, there was a period of rapid gasping which eventually waned and gave way to secondary apnea and death. In our experience, gasping occurs within 60 to 90 seconds of the onset of hypoxia, and naïve 5 to 6 day-old rat pups may gasp up to 15 minutes and exhibit as many as 86 potential autoresuscitation producing gasps. We and others have shown that one or more of the previously mentioned gasping characteristics are modulated in this age-range of rat pups by factors such as core temperature (28), glucose (44), catecholamines (45), nitric oxide (13), and glutamate (12). Given that all of these factors may be altered in one way or the other by exposure to hypoxia, our current experiments have been carried out to determine if prior exposure to hypoxic-induced apnea, such as may occur during prolonged obstructive apnea or positional asphyxia, influences gasping upon exposure to unrelenting hypoxia. Specifically, our experiments were designed to test the hypothesis that prior exposure to 1, 2, 3, 4, 9 and 14 HIA/AR modulates the onset, duration and number of potential autoresuscitation producing gasps upon exposure to unrelenting hypoxia in an exposure dependent fashion. Furthermore, we have done experiments to determine if the aforementioned gasping characteristics upon exposure to unrelenting hypoxia return to normal within a 120-minute normoxic recovery period following exposure to 9 HIA/AR.

METHODS

Ninety five, 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 to 30% relative humidity in a 12:12 hour light/dark cycle) until an experiment. Although 22°C is below the thermoneutral zone of newborn rats (23), each pup had the
opportunity to huddle with its siblings and dam in the nest, and thus to thermoregulate behaviorally.

All experimental procedures described herein 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**

*Experimental Series I – Prior Exposure to Hypoxic-Induced Apnea / Autoresuscitation Cycles and Gasping Characteristics During Exposure to Unrelenting Hypoxia:* For an experiment, each 5 to 6 day-old pup was removed from its mother and siblings, weighed and instrumented for measurement of cardiovascular and respiratory variables. Afterwards, the pup was positioned prone in a metabolic chamber regulated to 37.0±0.1°C into which flowed room air at a rate of 1 liter per minute. The time to first and last gasp as well as the total number of gasps to unrelenting hypoxia (i.e., 97% N₂ and 3% CO₂) was determined 5 minutes after each pup had experienced 0 (n=13), 1 (n=7), 2 (n=7), 3 (n=6), 4 (n=9), 9 (n=9) or 14 (n=9) HIA/AR at 5-minute intervals. For each HIA/AR, the gas which flowed into the chamber was changed from room air to 97% N₂ and 3% CO₂ until primary apnea occurred; the gas was then changed back to room air and autoresuscitation was effected by gasping. 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 liter per minute. We have previously shown that naïve 5 to 6 day-old pups studied at a thermoneutral temperature of 37.0°C tolerate an average of 15 episodes of HIA/AR before autoresuscitation failure (8).
During an experiment, stages of the respiratory response to hypoxia were directly observed on the polygraph tracing.

**Experimental Series II – Normoxic Recovery Time and Gasping Characteristics During Exposure to Unrelenting Hypoxia Following 9 Hypoxic-Induced Apnea / Autoresuscitation Cycles:** For an experiment, each 5 to 6 day-old pup was removed from its mother and siblings, weighed and instrumented for measurement of cardiovascular and respiratory variables. Afterwards, the pup was positioned prone in a metabolic chamber regulated to 37.0±0.1°C into which flowed room air at a rate of one liter per minute. The time to first and last gasp as well as the total number of gasps to unrelenting hypoxia was then determined after each pup had experienced a normoxic recovery period of 5 minutes (n=7), 15 minutes (n=7), 30 minutes (n=7), 60 minutes (n=7) or 120 minutes (n=7) following 9 HIA/AR at 5-minute intervals. To determine whether or not gasping had indeed returned to “normal”, comparisons were also made with data obtained in pups (n=13) from Experimental Series I that had not experienced hypoxic-induced apnea before being exposed to unrelenting hypoxia.

**Experimental Apparatus**

The metabolic chamber used in our experiments consisted of a double-walled plexiglass cylinder (30 cm long - internal diameter 6 cm) into which flowed room air or 97% N₂ and 3% CO₂. Chamber ambient temperature was regulated to 37.0±0.1°C by circulating water from a temperature controlled bath (Neslab - Endocal Refrigerated Circulating Bath RTE-8DD) through the space between the walls. We have previously shown that 37.0±0.1°C is the preferred ambient temperature of naïve 5 to 6 day-old rat pups 20-30 minutes after they are placed in a thermocline with a linear temperature gradient of 25°C to 40°C (8).
Experimental Measurements and Calculations

During an experiment, the electrocardiogram, respiratory movements and chamber CO₂ levels were recorded on a Model 7 polygraph (Grass Instrument Company) at a paper speed of 10 mm sec⁻¹. A bipolar lead II electrocardiogram was recorded from multistranded stainless steel wire electrodes (AS 633, Cooner Wire Company) sewn on the right shoulder (- electrode) and the left thigh (+ electrode) as described by Osborne (24); the electrodes were connected to a Model 7HIP5 High Impedance Probe coupled to a Model 7P5 Wide Band EEG A.C. Preamplifier (Grass Instrument Company). Respiratory movements were recorded from a mercury-in-silicone rubber strain gauge (Model HgPC, D.M. Davis, Inc.) placed around the chest; the strain gauge was connected to a bridge amplifier (Biomedical Technical Support Center, University of Calgary) that was coupled to a Model 7P03 Adapter Panel (Grass Instrument Company).

Statistical Analysis

Statistical analysis was carried out by ANOVA and Newman Keul’s multiple comparison tests. All results are reported as means ± one standard deviation, and p<0.06 was considered to be of statistical significance.

RESULTS

Experimental Series I – Prior Exposure to Hypoxic-Induced Apnea / Autoresuscitation Cycles and Gasping Characteristics During Exposure to Unrelenting Hypoxia: Prior exposure to HIA/AR at 5-minute intervals did not significantly alter the time to first gasp (ANOVA p=0.344) (Figure 1) but it decreased the time to last gasp (ANOVA p<0.001) (Figure 2) after two HIA/AR and the total number of gasps (ANOVA p<0.001) (Figure 3)
after three HIA/AR upon exposure to unrelenting hypoxia. Exposure of naïve pups to unrelenting hypoxia resulted in a reproducible respiratory response as previously reported (8): Initially there was a period of hyperpnea and arousal which preceded primary apnea; primary apnea was followed by a period of rapid gasping (phase I of gasping) that was followed by a period of slower gasping (phase II of gasping) of one to two per gasps per minute; finally there was a period of rapid gasping (phase III of gasping) which eventually waned and gave way to secondary apnea and death. The three phases of gasping became less identifiable as the number of prior HIA/AR were increased prior to exposure to unrelenting hypoxia (Figure 4).

Experimental Series II – Normoxic Recovery Time and Gasping Characteristics During Exposure to Unrelenting Hypoxia Following 9 Hypoxic-Induced Apnea / Autoresuscitation Cycles: Normoxic recovery time following 9 HIA/AR significantly influenced the time to last gasp and the total number of gasps (Figures 5 & 6) upon exposure to unrelenting hypoxia but only at 120 minutes. After a normoxic recovery period of 120 minutes, the total number of gasps was similar but the time to last gasp was still decreased compared to that observed in naïve animals exposed to unrelenting hypoxia.

DISCUSSION

Our experiments provide new information about factors that influence the newborn’s respiratory response to hypoxia. Novel findings of our study were that although prior exposure to hypoxic-induced apnea did not alter the time to first gasp, it significantly decreased the time to last gasp as well as the total number of gasps upon exposure to unrelenting hypoxia in an exposure dependent fashion. When the normoxic recovery
time following 9 HIA/AR was varied from 5 to 120 minutes, the time to last gasp as well as the total number of gasps upon exposure to unrelenting hypoxia recovered partially but only at 120 minutes (i.e., the total number of gasps was similar but the time to last gasp was still decreased compared to that observed in naïve animals exposed to hypoxia). Thus, prior exposure to hypoxic-induced apnea as may occur during prolonged obstructive sleep apnea or positional asphyxia decreases the number and duration of potential autoresuscitation producing gasps upon exposure to unrelenting hypoxia for a period of up to and exceeding 120 minutes, respectively.

Newborn mammals display a characteristic respiratory response consisting of hyperpnea, primary apnea, gasping, and secondary apnea upon exposure to unrelenting hypoxia [e.g., mice (21), rabbits (3,6,10,22), rats (8,14), and sheep (42)]. The onset of gasping following primary apnea occurs when the PaO$_2$ decreases to ~8-10 torr whether produced by airway obstruction resulting in hypercapneic hypoxia or inhalation of a hypoxic gas mixture resulting in hypocapneic hypoxia (16,22). As seen in the present study, naïve 5 to 6 day-old rats exhibit a triphasic pattern of gasping following primary apnea which eventually wanes and gives way to secondary apnea and death (8,13). As far as we are aware, the neurophysiological basis for the three phases of gasping which 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 (9,36,43) -- 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. In the present study, the three phases of gasping became less
discernible as the number of prior HIA/AR were increased prior to exposure to unrelenting hypoxia.

Although the mechanism of the altered gasping pattern following prior exposure to hypoxic-induced apnea is unknown, it may have resulted from substrate depletion, altered neuroendocrine function or synthesis and release of neuromodulators that influence hypoxic gasping. As previously mentioned, a number of factors have been shown to govern the time to last gasp in rats during early postnatal development upon exposure to unrelenting hypoxia including core temperature (28), glucose (37,44), catecholamines (45), excitatory amino acids (12) and nitric oxide (13). We have previously shown that exposure to hypoxia induces a “regulated” decrease in core temperature (4) and that core temperature influences the time to last gasp as well as the total number of gasps in 5-6 day-old rat pups upon exposure to unrelenting hypoxia. Variations in core temperature, however, are unlikely to have altered the gasping pattern following prior HIA/AR in our current experiments as core temperature was clamped at or near 37°C by regulating environmental temperature (23), and it is an increase rather than a decrease in core temperature that elicits a decrease in the time to last gasp by (28).

As oxygen levels decrease to very low levels, a transition from aerobic to anaerobic metabolism occurs throughout the body and energy for processes such as gasping is provided by glycolysis via the Embden-Meyerhof pathway which utilizes carbohydrate (e.g., glucose and/or glycogen) as a substrate. With regard to provision of substrate, Stafford and Weatherall (37) have shown that neither liver glycogen nor blood glucose levels determine survival time (i.e., the time to last gasp) when newborn rats are exposed to nitrogen. Brain glucose levels, however, can decrease dramatically during
anoxia despite normal or elevated plasma (and liver) glucose levels indicating an imbalance between brain glucose supply and demand (19). For example, experiments carried out by Holowach-Thurston et al (19) on intact newborn mice at 37°C have revealed that even though plasma glucose levels double during a 6-minute exposure to anoxia, brain glucose decreases by ~72%. Brain glucose is likely a relatively important substrate for glycolysis in the newborn as compared to the adult as basal brain glycogen is low and remains relatively stable during the first few minutes of anoxia perhaps due to the absence of enzymes (e.g., phosphoglucomutase) required for the utilization of brain glycogen (20,29,30). In rats pups, supplemental glucose has been shown to increase the time to last gasp during anoxic exposure when administered after the first few days of postnatal life (17,18,37,44). Thus, it is possible that the altered gasping pattern observed in our present experiments following repeated HIA/AR may have resulted from inadequate energy production via glycolysis secondary to low brain glucose levels. This postulate warrants investigation as does an investigation of the rate at which brain glucose is replenished in the newborn following bouts of hypoxic-induced apnea.

Hypoxia is a potent stimulus for the adrenomedullary secretion of catecholamines which mediate important respiratory, cardiovascular and metabolic adaptations to oxygen lack during the perinatal period (5,27). In the rat, which is born relatively immature, functional innervation of the adrenal medulla by the splanchnic nerves is not apparent until the second week of postnatal life (31,32,34). Adrenal chromaffin cells, however, possess a developmentally regulated oxygen-sensing mechanism -- similar to that of carotid body type I cells (41) -- which mediate a “non-neurogenic” release of catecholamines in response to hypoxia until splanchnic control of adrenomedullary
catecholamine secretion is functional (27). The role of catecholamines in hypoxic-induced gasping was shown in experiments carried out by Yuan, Runold and Lagercrantz (45) who reported that the time to last gasp upon exposure to unrelenting hypoxia was decreased in 1 and 8 day-old rat pups following adrenalectomy compared to that observed in sham-operated controls. Considering this and the evidence that hypoxia causes adrenal catecholamine depletion in rat pups (27), the altered gasping pattern observed in our present experiments following HIA/AR may have resulted from adrenal catecholamine depletion and the lack of a “normal” adrenal catecholamine response. This postulate warrants investigation as does an investigation of the rate at which adrenal catecholamines are replenished in the newborn following bouts of hypoxic-induced apnea. Although Slotkin and Kirshner (33) have shown that it takes up to 96 hours for adrenal vesicular catecholamines to return to control levels following insulin administration in adult rats, as far as we are aware, the rate at which adrenal vesicular catecholamine replenishment occurs in the newborn following hypoxic-induced depletion is unknown.

Gozal et al (13) and Gozal and Torres (12) have shown that nitric oxide and glutamate -- signaling molecules that influence neuronal excitability -- play important roles in initiating and modulating the pattern of gasping in rat pups during exposure to anoxia. In their experiments, pretreatment of 5 day-old rat pups with N-nitro-L-arginine -- a nitric oxide synthase blocker -- significantly increases the time to first gasp and gasping duration without altering the total number of gasps (2). Pretreatment of 5 day-old rat pups with MK801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate, a noncompetitive NMDA glutamate receptor channel antagonist) also prolonged the duration of primary apnea and increased the time to last gasp.
Thus, their data support the postulate that these neuromodulators favor the early appearance of gasps but limits anoxic tolerance during exposure to anoxia. Given that exposure to anoxia results in massive glutamate release (39) and activation of the brain nitric oxide system (e.g., (7)), it is possible that these neuromodulators played a role in modulating gasping in our experiments following repetitive HIA/AR in our experiments. This warrants further investigation.

The results of our experiments extend the observations of Gozal et al (11) who recently reported that prolonged exposure to intermittent hypoxemia in the fetus or newborn alters the gasping pattern of 5 day-old pups upon exposure to unrelenting hypoxia. In their experiments, intermittent fetal hypoxemia was produced by alternating the dam’s fraction of inspired oxygen between 0.21 and 0.10 at 90 second intervals from day 5 of gestation to term, and intermittent newborn hypoxemia was produced by alternating the pup’s fraction of inspired oxygen between 0.21 and 0.10 at 90 second intervals within 12 hours of parturition until day 5 of postnatal life. Neither perturbation altered the time to first gasp but both decreased the time to last gasp as well as the total number of gasps at day 5 of postnatal life when exposed to unrelenting hypoxia. In the current experiments, decreases in the time to last gasp and the total number of gasps were produced by prior exposure to as few as 2 and 3 HIA/AR, respectively, which induces a more severe level of hypoxia. Although the mechanisms of action may be different, it is interesting that such different low oxygen exposure regimens in essence have the same effect on hypoxic gasping in the 5 day-old rat pup.

In infants, spontaneous recovery from obstructive sleep apnea or positional asphyxia during sleep 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 "autoresuscitation" (15,40). Peiper (25),
Stevens (38), and Thach (40) have emphasized the importance of gasping in “self-resuscitation” or “autoresuscitation” during apnea in human infants, and that repeated episodes of apnea might lead to autoresuscitation failure and death. Why autoresuscitation fails is unclear, but our current experiments show that the duration and number of potential autoresuscitation producing gasps upon exposure to unrelenting hypoxia are decreased by prior exposure to hypoxia and that this “impairment” lasts upward of 120 minutes. If oxygen does not become available immediately upon the initiation of gasping – as conceivably may occur in infants, hypoxic secondary to obstructive sleep apnea or positional asphyxia – a decrease in the duration and/or number of gasps could diminish the chance of a successful autoresuscitation.
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FIGURE LEGENDS

Figure 1. Lack of influence of prior exposure to 0, 1, 2, 3, 4, 9, or 14 hypoxic-induced apnea / autoresuscitation cycles on the time to first gasp upon exposure to unrelenting hypoxia in 5 to 6 day-old rat pups. Each bar represents mean ± one standard deviation. p=0.344 by ANOVA
Figure 2. Influence of prior exposure to 0, 1, 2, 3, 4, 9, or 14 hypoxic-induced apnea /autoresuscitation cycles on the time to last gasp upon exposure to unrelenting hypoxia in 5 to 6 day-old rat pups. Each bar represents mean ± one standard deviation. p<0.001 by ANOVA; *p<0.06 versus 0 prior hypoxic-induced apneas by Newman-Keuls.
Figure 3. Influence of prior exposure to 0, 1, 2, 3, 4, 9, or 14 hypoxic-induced apnea /autoresuscitation cycles on the total number of gasps upon exposure to unrelenting hypoxia in 5 to 6 day-old rat pups. Each bar represents mean ± one standard deviation. 
p<0.001 by ANOVA; *p<0.06 versus 0 prior hypoxic-induced apneas by Newman-Keuls.
Figure 4. Influence of prior exposure to 0, 4, 9, or 14 hypoxic-induced apnea / autoresuscitation cycles on the gasping pattern in 5 to 6 day-old rat pups upon exposure to unrelenting hypoxia.
Figure 5. Influence of normoxic recovery time on the time to last gasp upon exposure to unrelenting hypoxia in 5 to 6 day-old rat pups after 9 hypoxic-induced apnea / autoresuscitation cycles. Each bar represents mean ± one standard deviation. p<0.001 by ANOVA; *p<0.06 versus 0/NA from Experimental Series I by Newman-Keuls; †p<0.06 versus 9/5 by Newman-Keuls.
Figure 6. Influence of normoxic recovery time on the total number of gasps upon exposure to unrelenting hypoxia in 5 to 6 day-old rat pups after 9 hypoxic-induced apnea / autoresuscitation cycles. Each bar represents mean ± one standard deviation. p<0.001 by ANOVA; *p<0.06 versus 0/NA from Experimental Series I by Newman-Keuls; †p<0.06 versus 9/5 by Newman-Keuls.