Postnatal age influences the ability of rats to autoresuscitate from hypoxic-induced apnea

JAMES E. FEWELL, FRANCINE G. SMITH, VIENNA K. Y. NG, VANESSA H. WONG, AND YINGHONG WANG
Department of Physiology and Biophysics, University of Calgary Health Sciences Centre, Calgary, Alberta T2N 4N1, Canada

Received 26 May 1999; accepted in final form 26 January 2000

Postnatal age influences the ability of rats to autoresuscitate from hypoxic-induced apnea. Am J Physiol Regulatory Integrative Comp Physiol 279: R39–R46, 2000.—Failure to autoresuscitate from apnea by gasping has been suggested to have a role in sudden infant death. Little is known, however, about the factors that influence the ability of gasping to sustain life during acute hypoxia in the newborn. The present experiments were carried out on 105 rat pups to investigate the influence of postnatal age on the time to last gasp during a single hypoxic exposure and on the ability to autoresuscitate from primary apnea during repeated hypoxic exposures. On days 1–2, 5–6, 10–11, 15–16, and 19–20 postpartum, each pup was placed into a temperature-controlled chamber regulated to 37 ± 1°C and was exposed either to a single period of hypoxia produced by breathing an anoxic gas mixture (97% N₂-3% CO₂), and the time to last gasp was determined, or repeated exposure to hypoxia was performed, and the ability to autoresuscitate from primary apnea was determined. Increases in postnatal age decreased the time to last gasp following a single hypoxic exposure and decreased the number of successful autoresuscitations following repeated hypoxic exposures. Thus our data provide evidence that postnatal age influences protective responses that may prevent death during hypoxia as may occur during episodes of prolonged sleep apnea.

autoresuscitation; hypoxia; 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” (43). 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 (43). Much of SIDS research has focused on determining the life-threatening event itself, whether it be cardiac arrhythmia, obstructive apnea, or hypoglycemia due to a systemic metabolic defect (26). Among the hypotheses for the cause of SIDS, those attributing cause to obstructive apnea as a life-threatening event seem to best explain the known facts about the majority of SIDS victims. These include evidence of chronic or recurrent hypoxia [e.g., elevated vitreous humor hypoxanthine levels (32)] and intrathoracic petechiae (3, 27). Intrathoracic petechiae, present in the majority of SIDS victims, are suggestive of rigorous respiratory efforts secondary to airway obstruction and/or hypoxia with large changes in intrathoracic pressure and subsequent microvascular rupture (1, 23).

In humans spontaneous recovery from obstructive sleep apnea can occur early as a result of arousal from sleep or later as a result of hypoxic gasping when it is known as autoresuscitation (22, 40). 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. (24). Peiper (30), Stevens (37), and Thach (40) have emphasized the importance of gasping in autoresuscitation during apnea in human infants and that repeated episodes of apnea may lead to autoresuscitation failure and death. Our current experiments focused on the latter of the two aforementioned protective responses and were carried out to investigate the influence of postnatal age on the ability of rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia as may occur during episodes of obstructive sleep apnea.

METHODS

One hundred and five 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, and 12:12-h light-dark cycle). Although 22°C is below the thermoneutral zone of newborn rats (39), 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

The respiratory response of both newborn (25) and adult (24) animals to acute hypoxia typically passes through four
To determine whether autoresuscitation had occurred, we compared heart rate and respiratory rate during the last 15 s of each 5-min period. If heart rate and respiratory rate were greater than 60% of control values, we deemed the autoresuscitation a success. If, however, heart rate and/or respiratory rate during the last 15 s of each 5-min period was less than 60% of control values, we deemed the autoresuscitation a failure.

**Experimental Apparatus**

The metabolic chamber used in our experiments consisted of a double-walled Plexiglas cylinder (length 30 cm, diameter 6 cm) into which flowed room air or 97% N₂-3% CO₂. Chamber ambient temperature was controlled 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.

**Experimental Measurements and Calculations**

During an experiment the electrocardiogram, respiratory movements, and chamber carbon dioxide or oxygen levels were recorded on a model 7 polygraph (Grass Instrument) at a paper speed of 10 mm/s. The electrocardiogram was recorded from multi-stranded stainless steel wire electrodes (AS 633, Cooner Wire) sewn across the chest wall; the wires were connected to a model 7 HIP5 high-impedance probe coupled to a model 7P03 wide band electroencephalogram, alternating current preamplifier (Grass Instrument). Respiratory movements were recorded from a model HgPC mercury in silicone rubber strain gauge (Davis) placed around the chest; the strain gauge was connected to an amplifier (Mountain Scientific Consulting), which was coupled to a model 7P03 Adapter Panel (Grass Instrument). Core temperature was measured using an 18-gauge copper/constantan thermocouple sheathed in Teflon (IT-18 Physitemp) interfaced with a BAT-12 thermometer (Physitemp). The thermocouple was inserted ~1 cm into the pup’s rectum and glued to its tail using tissue adhesive (Vetbond, 3M Animal Care Products). Chamber carbon dioxide and oxygen levels were measured using an Applied Electrochemistry carbon dioxide and oxygen analyzer (Ametek) coupled to a model 7P03 adapter panel.

**Statistical Analysis**

Statistical analyses were carried out using ANOVA followed by a Newman-Keul multiple comparison test to determine whether age, or time and age when appropriate, affected the measured variables. All results are reported as means ± SD, and P < 0.05 was considered to be of statistical significance.

**RESULTS**

**Experiment 1: Time to Last Gasp During a Single Anoxic Gas Challenge**

Postnatal age significantly influenced basal heart rate and respiratory rate with both variables increasing in value from days 1–2 and peaking on days 15–16 of age (Figs. 1 and 2). Furthermore, postnatal age significantly influenced the time to last gasp (Fig. 3) and the total number of gasps (Fig. 4) with both variables decreasing as postnatal age increased. Exposure to a single period of hypoxia resulted in a reproducible respiratory response (Fig. 5). Initially there was a
period of hyperpnea and arousal that preceded primary apnea (Fig. 5a); primary apnea was followed by a period of slower gasping of 1–2 gasps/min (Fig. 5c); finally there was a period of rapid gasping that eventually waned and gave way to terminal apnea and death (Fig. 5d). In all animals, gasping ceased before the appearance of arrhythmias or an isoelectric pattern on the electrocardiogram. Heart rate decreased during exposure to hypoxia at all postnatal ages.

Experiment 2: Core Temperature During a Single Anoxic Gas Challenge

Basal core temperatures during normoxia were (means ± SD): 1–2 days old, 37.8 ± 0.6°C; 5–6 days old, 38.0 ± 0.8°C; 10–11 days old, 38.0 ± 0.1°C; 15–16 days old, 39.0 ± 0.2°C; and 19–20 days old, 39.0 ± 0.1°C. The mean change in core temperature from control during a single anoxic gas challenge in each of the age groups is shown in the Fig. 6. The mean change in core temperature was significantly affected by time (P < 0.000) but not by postnatal age (P = 0.509).

Experiment 3: Autoresuscitation From Primary Apnea During Repeated Anoxic Gas Challenges

Postnatal age significantly influenced the ability of the rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia (Fig. 7). In 1- to 2-day-old pups, five autoresuscitation experiments were terminated following 75 successful autoresuscitations and two autoresuscitation experiments were terminated following 125 successful autoresuscitations. With increasing postnatal age, however, the number of successful autoresuscitations decreased. By days 9–10 of postnatal age, the number of successful autoresuscitations had reached its minimum and did not change significantly after this age. Before autoresuscitation failure, all successful autoresuscitations exhibited the same cardiorespiratory pattern (Fig. 8). Initially, there was a period of hyperpnea (Fig. 8a) and arousal (Fig. 8b), which preceded primary apnea and bradycardia (Fig. 8c); gasping (Fig. 8d) was followed by an increase in heart rate (Fig. 8e) and then restoration of a normal respiratory pattern (Fig. 8f).

The sequence of events leading to autoresuscitation failure, however, appeared to be different in newborn vs. older pups. In six of seven 5- to 6-day-old pups in which the electrocardiogram was successfully recorded, autoresuscitation failure followed atrioventricular dissociation after 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 six of seven 10- to 11-day-old pups, four of four 15- to 16-day-old pups, and in six of six 19- to 20-day-old pups, however, gasping ceased before signs of cardiac resuscitation appeared on the electrocardiogram.
DISCUSSION

Our experiments provide new information about factors that influence the ability of the newborn to survive hypoxia as may occur during prolonged sleep apnea. Novel findings in our study carried out on newborn and older rats were that postnatal age influenced 1) the time to last gasp during a single hypoxic exposure and 2) the ability of rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia. Thus our data provide evidence that postnatal age influences protective responses that prevent death during severe hypoxia as may occur during prolonged sleep apnea.

Postnatal age influenced the time to last gasp during a single hypoxic exposure with the time to last gasp decreasing as postnatal age increased. These results are in general agreement with the results of early studies (9, 14, 21, 36), most of which were carried out at ambient temperatures below thermoneutrality, which showed an inverse relationship between postnatal age and core temperature during hypoxia.
and gasping duration on exposure to a single period of hypoxia. Core temperature influences both the time to last gasp following exposure to a single period of hypoxia and the number of autoresuscitations during repeated exposure to hypoxia (33). Therefore, our current experiments were carried out at thermoneutrality to minimize deviations in basal core temperature from normal in this altricial species, which has a limited ability to regulate its core temperature when exposed to ambient temperatures outside its thermoneutral zone (29, 33). On exposure to a single period of hypoxia, our rat pups exhibited a triphasic gasping pattern following primary apnea as has been previously shown to occur by others in newborn rats (21, 44) and rabbits (6, 28) but not in mice (25). This triphasic gasping pattern consisted of an initial phase of rapid gasping (phase I) that was followed by a second phase of slower gasping (phase II); finally there was a third phase of rapid gasping (phase III), which eventually waned and gave way to terminal or secondary apnea and death. In all animals, terminal apnea preceded the appearance of arrhythmias or an isoelectric pattern on the electrocardiogram. Of course, the lack of arrhythmias or an isoelectric pattern on the electrocardiogram does not assure that an adequate blood pressure, which is essential for delivery of metabolic substrate (i.e., glucose) to the brain during oxygen lack, was maintained. Previous experiments by Swann et al. (38) on 4-day-old dogs and experiments by Cassin et al. (7) on 1-day-old dogs, rabbits, and cats, however, have shown that newborns of these species maintain their blood pressure at viable levels long after terminal apnea.

As far as we are aware, the neurophysiological basis for the inverse relationship between postnatal age and gasping duration in rats on exposure to a single period of hypoxia is unknown. It may, however, result from the influence of various neurotransmitters and/or neuromodulators [e.g., excitatory amino acids (19, 41), nitric oxide (20)], which may affect the firing patterns of neurons in the lateral tegmental field of the medulla, the proposed neural substrate underlying gasping in the rat (11, 42). For example, the initiation and duration of gasping following exposure to a single period of hypoxia may be determined by accumulation of excitatory amino acids such as aspartate and glutamate. In support of this contention, Gozal and Torres (19) have shown that although administration of MK-801, a non-competitive N-methyl-D-aspartate glutamate receptor antagonist, does not alter the hyperpneic component of the respiratory response to hypoxia (i.e., hyperpnea, primary apnea, gasping, secondary or terminal apnea), it does alter the latency and duration of gasping in rat pups following exposure to a single period of hypoxia. Furthermore, Gozal et al. (20) have recently shown that administration of N-nitro-L-arginine methyl ester, an L-arginine analog that prevents the formation of nitric oxide, increases the duration of gasping in an age-dependent fashion during exposure to a single period of hypoxia; the effect was greater in older than in newborn rat pups. These data, coupled with evidence of increased neuronal nitric oxide synthase present in the lateral tegmental field of the medulla with increasing postnatal age (20), suggest that nitric oxide partici-
Peiper (30), Stevens (37), and Thach (40) have emphasized the importance of gasping in autoresuscitation during apnea in human infants and that repeated episodes of apnea may lead to autoresuscitation failure and death. As far as we are aware, our experiments provide the first data on postnatal age and autoresuscitation from primary apnea during repeated exposures to hypoxia in rat pups. We found that there were dramatic changes in the ability of rat pups to autoresuscitate during the first 10 days of postnatal life, but thereafter the number of successful autoresuscitations during repeated exposure to hypoxia was fairly constant. In fact, on days 1 and 2 of postnatal life, autoresuscitation failure did not occur despite the fact that some pups were exposed to 125 episodes of hypoxia! Our results are qualitatively similar to those of Gershan et al. (13) who found that young BALB/c mice were more tolerant to repeated exposure to hypoxia than were older BALB/c mice; the number of successful autoresuscitations from primary apnea decreased with increasing postnatal age.

Why autoresuscitation failure occurs is unclear, but clinical reports provide evidence that autoresuscitation can fail following repeated apneic episodes (30, 31, 35, 37). Jacobi and Thach (25) have recently defined the cardiorespiratory events that occur during successful autoresuscitation from hypoxic apnea in mice. These cardiorespiratory events consisted of three sequential stages: stage I, gasping with marked bradycardia; stage II, cardiac resuscitation with a rapid increase in heart rate to greater than 60% of baseline; and stage III, respiratory resuscitation with an increase in respiratory rate to greater than 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. (13), that repeated exposure to hypoxia led to autoresuscitation failure in day 5 and older pups, but the sequence of events leading to autoresuscitation failure was different in the younger vs. the older pups. In six of seven, 5- to 6-day-old pups in which the electrocardiogram was successfully recorded, autoresuscitation failure followed atrioventricular dissociation, 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 six of seven 10- to 11-day-old pups, four of four 15- to 16-day-old pups, and six of six 19- to 20-day-old pups, however, gasping ceased before signs of cardiac resuscitation appeared on the electrocardiogram. Thus during the final hypoxic exposure, in the majority of 5- to 6-day-old pups, stages I and II of autoresuscitation occurred before autoresuscitation failure, whereas in the older pups, only stage I of autoresuscitation occurred before autoresuscitation failure. Interestingly, recent reports by Poets et al. (31) and Sridhar et al. (35) have provided evidence that some SIDS infants display stage I of autoresuscitation but that gasping fails to produce cardiac resuscitation (i.e., stage II of autoresuscitation) with resulting death. Although the mechanism of autoresuscitation failure following repeated exposure to hypoxia is unclear, it may have ultimately resulted from the depletion of metabolic substrates or from the accumulation of metabolic by-products of hypoxia. Gershman et al. (13) have suggested that the following events accompany the three stages of autoresuscitation: first, introduction of air into the lungs by gasping; second, transport of oxygen from the lung to the heart; third, response of the heart by increasing heart rate and cardiac output; and fourth, response of the nervous system to reoxygenation and increased perfusion. Considering this, we would speculate that the failure of gasping to produce autoresuscitation during the final episode of hypoxia may have resulted from different mechanisms in the younger and older animals.

In the young animals (i.e., 5- to 6-day-old pups), stages I and II of autoresuscitation occurred before the onset of atrioventricular dissociation, the loss of ventricular depolarization, and ultimately death; gasping continued throughout. This would suggest that cardiac output was maintained during hypoxia and that gasping resulted in the transport of oxygen from the lungs to the heart, resulting in reoxygenation of the atrial pacemaker cells. The subsequent arrhythmia may have resulted from the accumulation of a metabolic by-product of hypoxia that affects atrioventricular conduction, such as adenosine (4, 5).

In the older animals, only stage I of autoresuscitation occurred before autoresuscitation failure despite continued gasping. This would suggest that cardiac output was not maintained during hypoxia and that gasping did not result in the transport of oxygen from the lungs to the heart. The inability to maintain cardiac output during hypoxia may have resulted from the depletion of the cardiac metabolic substrate glycogen, the cardiac stores of which are greater in the newborn than in older animals, and which occurs during hypoxia (8, 34). These mechanisms of autoresuscitation failure and their age dependence are speculative and warrant further investigation.

This work was done during the tenures of J. E. Fewell as a Senior Medical Scholar of the Alberta Heritage Foundation for Medical Research and F. G. Smith as a Scholar of the Heart and Stroke Foundation of Canada. V. K. Y. Ng and V. H. Wong were supported by Summer Research Studentships from the Alberta Heritage Foundation for Medical Research.

This study was supported by the Medical Research Council of Canada.

These data were presented in poster format at Experimental Biology 99, Washington, DC, and has been published in abstract form (FASEB J 13: 494, 1999).

REFERENCES