Ventilatory Control in Infant Rats after Daily Episodes of Anoxia

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ABSTRACT. We asked whether the ventilatory control of the newborn rat was modified by preceding daily episodes of anoxia. At thermoneutrality (ambient temperature, 32°C) during anoxia, respiratory efforts lasted, on average, 28, 20, and 11 min at 1, 3, and 7 d, respectively. Therefore, we chose 10 min as the duration of a daily exposure to anoxia (100% N₂ breathing) from d 1 to d 6 (experimental group). During the 7 d of the study, body growth was not different between the two groups. On d 7, respiratory measurements were performed and compared with those of rats never exposed to anoxia (controls). In normoxia, oxygen consumption ($\dot{V}O_2$, measured with an open-flow system) and ventilation ($\dot{V}E$, measured by flow-plethysmography) were similar between control and experimental groups. Acute exposure to hypoxia (10 min of 10% O₂) resulted in a similar drop in $\dot{V}O_2$ and increase in $\dot{V}E$ in both groups. During the last exposure to anoxia on d 7, heart rate and breathing rate rapidly dropped; the attained degree of acidosis (blood pH and PCO₂) was also similar to that of control rats. The results of the present experimental approach suggest that, in the newborn rat, daily episodes of anoxia during the 1st postnatal wk have no appreciable impact on body growth, ventilatory control, and the response to additional hypoxia. (*Pediatr Res* 35: 490–493, 1994)

Abbreviations

Tamb, ambient temperature
C, control group
E, experimental group
 $\dot{V}O_2$, oxygen consumption
 $\dot{V}E$, ventilation
FIO₂, inspired O₂ concentration

The ability of the newborn mammal to sustain severe hypoxia, anoxia, or asphyxia has been known for many years; a brief hyperpneic response to oxygen lack is followed by apnea of variable duration, succeeded by gasping. If normoxia is restored before the last respiratory effort, the animal seemingly recovers by a process of autoresuscitation (1–4).

Whether the spontaneous restoration of breathing implies that the respiratory control mechanisms have fully recovered from the hypoxic event is likely to depend on the duration and severity of the episode. Breathing irregularities seem to be part of long-term neurologic sequelae in the most severe cases of perinatal hypoxia (5, 6). Prolonged hypoxia (FIO₂ = 10–12%) in the early

postnatal period of the rat resulted in short- and long-term consequences on respiratory mechanics, regulation of breathing pattern, and other aspects of lung structure and function (7–12). However, the corresponding effects of severe but brief events of lack of oxygen are not known. In particular, because repetitive apneic spells or severe hypoxic episodes are not uncommon in the clinical setting, we were interested in examining to what extent brief anoxic incidents influence ventilatory control and the respiratory response to a following hypoxic episode.

In this study, we characterized the pattern of respiratory and cardiac activities during anoxia in rats at 1, 3, and 7 d. On the basis of these results, a protocol of daily spells of anoxia has been applied to a second group of rats from d 1 to d 6. On d 7, their normoxic ventilation and gaseous metabolism and the responses to hypoxia and anoxia were compared with those of control rats with no history of anoxic episodes.

MATERIALS AND METHODS

Experiments were conducted on conscious Sprague-Dawley rats observing experimental protocols approved by the animal care committee of this institution. At d 1 after birth, the smallest and heaviest of the litter were culled, which left a litter of 12 pups.

Protocol 1. Measurements were performed to characterize the response of the rat to anoxia in the 1st postnatal wk. The general protocol consisted in measuring heart and respiratory rates during normoxia and during anoxia at Tamb of 32°C ($\pm 0.8^\circ\text{C}$ SEM), i.e. close to thermoneutrality (13). In an additional group of animals, the same measurements were performed at low Tamb ($24 \pm 0.4^\circ\text{C}$). At each age (d 1, 3, or 7 after birth), measurements were obtained from six to 15 pups of two to four litters. A separate group of 7-d-old rats ($n = 11$) was used for measurements of blood gases after 10 min of anoxia.

Protocol 2. A litter of pups was randomly assigned to C (two litters) or E group (two litters). The E pups were exposed to anoxia (100% N₂) for 10 min each day, from d 1 to d 6 inclusive. Anoxic exposures were obtained by placing the pups in a 350-mL chamber maintained at $32 \pm 1^\circ\text{C}$. A flow of N₂ (about 950 mL/min) was delivered through the chamber as the outflow gas concentration was monitored by an O₂ analyzer. The duration of anoxia (10 min) was chosen on the basis of the results of protocol 1 (see Results). During this period, the O₂ stores rapidly decrease and are almost completely depleted by the end of the exposure (Appendix). C pups were treated identically except that they were exposed daily to a flow of air in place of N₂.

On d 7, recordings of breathing pattern and gaseous metabolism ($\dot{V}O_2$) during normoxia and acute hypoxia (FIO₂ = 10%) were obtained for 10 pups per group. In an additional group of 7-d-old E rats ($n = 10$), blood gases were measured at 10 min of anoxia. The detailed breakdown of number of litters and animals used is provided in the Results section and figure legends.

Breathing and heart rates (protocol 1). Breathing efforts were monitored by airflow plethysmography, with a setup similar to that previously described (15, 16). In brief, the pup was prone in

Received May 5, 1993; accepted November 24, 1993.

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C.S. was recipient of a bursary from the Nihon University, Tokyo, Japan. The study was supported by MRC Canada, and partly by funds from the Canadian Foundation for the Study of Infant Deaths and the Quebec Lung Association.

a 20-mL box with the head emerging through a double layer of paraffin sealing film (Parafilm). Box Tamb was monitored by a small thermal probe (model 400, Yellow Springs Instrument Co., Yellow Springs, OH) and maintained by a large heating source around 32°C, which corresponds to thermoneutrality (13). For some measurements of protocol 1, Tamb was set at 24°C (cold). Airflow in the box was monitored by a small pneumotachograph connected to a differential pressure transducer. The front part of the plethysmograph was tightly connected to a second chamber, which was continuously flushed with the desired gas mixture (air or 100% N₂) (16). The O₂ concentration of the gas was continuously monitored by an O₂ analyzer (model OM-15, Sensor Medics, Dayton, OH).

ECG was measured by s.c. thread electrodes connected to a Grass amplifier (Grass Instrument Co., Quincy, MA). Airflow and ECG were recorded on paper (Gould pen recorder, Gould Inc., Cleveland, OH) at a speed of 5 mm/s. Breathing rate and heart rate during anoxia were computed from the airflow signal and the ECG, respectively, every 2 min for the first 10 min of anoxia and every 4 min thereafter.

$\dot{V}E$ and $\dot{V}O_2$ (protocol 2). The rat was placed in a double-chamber flow plethysmograph (16) similar to the setup used in protocol 1. The back chamber was used for measurements of breathing pattern by airflow plethysmography. The front chamber was used to measure $\dot{V}O_2$ by an open-flow system (14). This chamber had an inlet and outlet for gas flow; gas flow was controlled by a flowmeter at 100 mL/min volume of gas at standard temperature and pressure with no water vapor. The outflowing gas passed through a drying column (drierite) before being analyzed for fractional concentrations of O₂ and CO₂ by appropriate gas analyzers (Beckman OM-11 and Beckman LB-2, Beckman Instruments, Fullerton, CA). These concentrations were displayed on a computer monitor after real-time acquisition. Inflowing dry gas concentrations could be checked at any time. After measurements in normoxia, gas inflow was switched to hypoxia (10–30 min of 10% FiO₂). $\dot{V}O_2$ was computed as the product between the gas flow and the inflow-outflow O₂ concentration difference. Average difference between incurrent and excurrent gas concentrations was about 0.25–0.5%; the accuracy of the analyzer was such that the measurement error was less than 2%. Values of $\dot{V}O_2$ are reported as volume of gas at standard temperature and pressure with no water vapor, normalized by the animal weight in kilograms.

Breathing pattern in normoxia and hypoxia was monitored simultaneously with $\dot{V}O_2$ and recorded on paper (two-channel Gould pen recorder, Gould Inc.). Two hundred breaths were digitized with the help of a graphics tablet connected to a minicomputer for computation of tidal volume (V_T , mL) and total cycle duration (TTOT, s), from which breathing frequency (f , breaths/min = 60/TTOT) and minute ventilation ($\dot{V}E$, mL/min = $V_T \cdot f$) were calculated.

Colonic temperature and blood gases. Colonic temperature was measured with a fine chromel/alumel thermocouple (Omega 871, Omega Engineering, Inc., Stamford, CT) and was interpreted as representative of body core temperature. Blood gases were sampled during anoxic exposure. After a few minutes of anoxia, none of the pups responded to touch or pain stimuli; hence, it was considered ethically acceptable to withdraw blood by percutaneous puncture of the heart at 10 min of anoxia. A 0.1-mL sample was collected anaerobically and used for immediate blood gases determination (blood gas analyzer model 1302; Instrumentation Laboratory System, Lexington, MA); the values were corrected to the corresponding body core temperature of the animal (17).

Statistical analysis. Data are presented as means $\pm$ 1 SEM. A significant difference between the average value of two sets of data was evaluated by two-way analysis of variance or two-way repeated measurement analysis of variance. Two-tailed *t* test was performed on paired or unpaired data as appropriate. In all cases, a statistically significant difference was defined at $p < 0.05$.

RESULTS

Response to anoxia at d 1, 3, and 7 (protocol 1). Breathing and heart rates rapidly decreased at the onset of anoxia to very low values; then they increased slightly for a few minutes and then finally declined gradually toward zero (Fig. 1). The curves clearly differed according to postnatal age and Tamb. At thermoneutrality (*open symbols*), the last gasp and heart beat were after 28 and 45 min at d 1 and only after 11 and 29 min at d 7, respectively ($p < 0.05$). In the cold (*filled symbols*), they were delayed in all age groups, although at d 7 the difference did not meet our criteria of statistical significance. The times of the last breathing effort and heart beat significantly decreased with age, similar to what was observed at thermoneutrality. From these results, 10 min of anoxic exposure was considered the maximum duration that would allow survival of most pups of protocol 2.

At the end of 10 min of anoxia, blood gases of 7-d-old rats ($n = 11$) averaged 2 ± 1 mm Hg PO₂, 85 ± 5 mm Hg PCO₂, and pH 6.88 ± 0.02 ; colonic temperature was $34.3 \pm 0.2^\circ\text{C}$.

Effects of repeated anoxic episodes (protocol 2). Two of the 24 E pups died during anoxic exposure; both were 6 d old. Body growth of E pups was almost identical with that of C (Fig. 2). On d 7, no significant differences between E and C were seen in body weight (13.0 ± 0.3 g in C and 13.9 ± 0.3 g in E), $\dot{V}O_2$, $\dot{V}E$, breathing pattern, or colonic temperature (Table 1).

On exposure to acute hypoxia, $\dot{V}O_2$ dropped in both E and C by a similar amount. Hypoxic $\dot{V}E$ was slightly above the normoxic value because of an increase in respiratory rate; this change in breathing pattern was not significantly different between C and E (Table 1).

On d 7, an additional anoxic challenge in E ($n = 11$) determined an immediate drop in respiratory and heart rates, which stopped by 12 ± 1 min and 28 ± 2 min, respectively. At 10 min of anoxia, *i.e.* close to the time of the last gasp, blood PO₂ averaged ($n = 10$) 2 ± 1 mm Hg, PCO₂ 81 ± 5 mm Hg, and pH 6.93 ± 0.02 ; colonic temperature was $34.4 \pm 0.2^\circ\text{C}$.

DISCUSSION

The extraordinary ability of the newborn mammal to survive with no O₂ in the inspired air has long been known, and the dependence of anoxic resistance on postnatal age and temperature has also been well documented (18–21). The present study confirms these notions and indicates that in the rat anoxic resistance decreases rapidly during the 1st postnatal wk. Breathing efforts in anoxia followed a bimodal pattern, as noted by Adolph (1, 20), similar to what occurs during asphyxia, in which the late rise in frequency corresponds to gasping activity (3). Hence, the present results of the 1st protocol confirmed previous information and represented the basis for the 2nd protocol, in which the duration of the anoxic episodes was fixed at 10 min; this time corresponded to about one third of the gasping time at d 1 and to almost the whole gasping period at d 7, and it was tolerated by almost all pups. The two E pups that died (out of 24) did not survive the last anoxic exposure on d 6, which was in fact close to the maximum tolerable limit. Occurrence of apnea has been previously related to the severity of the hypoxia rather than the severity of the acidemia (22); the acidemia could further reduce brain metabolic rate (23), the low value of which is considered a key element for the ability of the developing brain to resist anoxia (21, 24).

The fact that body growth and metabolic rate of E pups did not differ from C are suggestive indications that no major body function was seriously compromised by the repeated anoxia. The normality in body growth is a finding that agrees with previous observations in rat pups exposed to severe episodes of asphyxia or hypoxia (25, 26). Some of the infants who survived severe asphyxia at birth presented apparently normal body and neurologic development by 3–7 y of age (27, 28).

The question that we proposed at the onset was to what extent daily occurrence of an anoxic spell may affect the regulation of

Fig. 1. Time course of breathing rate (left) and heart rate (right) during anoxia in rats of 1, 3, and 7 d of age at thermoneutrality ($32 \pm 0.8^\circ\text{C}$, open symbols) and in cold ($24 \pm 0.4^\circ\text{C}$, filled symbols). Symbols are group means; bars represent 1 SEM. Average times of the last gasp and heart beat are indicated. Number of animals in each group ranged between 6 and 15 from two to four litters.

breathing, an aspect not previously examined and yet important in the response to additional hypoxic or anoxic episodes. No differences in breathing pattern or metabolic rate were observed between C and E whether in normoxia or in hypoxia. In the 7-d-old E pups, duration of gasping and heart activities, and blood gases and body core temperature by the end of anoxia were also similar to those of untreated 7-d-old rats, indicating comparable metabolic adjustments during anoxia. Therefore, we conclude that daily anoxic episodes have no measurable effects on respiratory control.

The above conclusion, however, is limited by the characteristics of our experimental design. In particular, we did not consider other aspects of respiratory regulation, including the response to acute hypercapnia and its interaction with hypoxia, which may have revealed some differences in ventilatory control between C and E. Clinical anoxic episodes, either determined by prolonged periods of apnea or airway obstruction, are accompanied by

rapid CO_2 retention and acidosis; we have chosen to induce anoxia via N_2 breathing, therefore delaying the acidosis, which could have more readily compromised neuronal function. Hypothermia, which protects neonates from asphyxia (19) and greatly prolongs respiratory and cardiac activities in anoxia (Fig. 1), is likely to enhance further the tolerance to repeated anoxia. Finally, we cannot exclude that an even higher frequency of anoxic spells may have resulted in some alterations of respiratory control. In this respect, it was previously observed in the rat that moderate but continuous hypoxia during the 1st postnatal wk reduced body growth and resulted in numerous long-lasting alterations of the respiratory function, including aspects of ventilatory control (7, 8, 10).

In conclusion, the present results indicate that newborn rats can tolerate daily anoxic spells remarkably well, with no major effects on respiratory control.

Fig. 2. Body weights during the 1st postnatal wk in control (open symbols) and experimental rats (filled symbols). Symbols are means of 24 control and 24 experimental rats, each from two litters. Bars indicate 1 SEM; when not represented, SEM was within the size of the symbol. At no age did the values of the two groups significantly differ from each other.

Table 1. Values in normoxia and acute hypoxia in 7-d-old rats*

	FIO ₂ (%)	C	E
No. of animals		10	10
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	21	34 ± 1	37 ± 1
	10	18 ± 1†	20 ± 1†
$\dot{V}E$ (mL·kg ⁻¹ ·min ⁻¹)	21	1098 ± 55	1138 ± 27
	10	1392 ± 52†	1445 ± 71†
V_T (mL·kg ⁻¹)	21	7.9 ± 0.4	7.9 ± 0.3
	10	8.1 ± 0.4	7.6 ± 0.4
Frequency (breaths/min)	21	141 ± 4	148 ± 4
	10	177 ± 9†	194 ± 5†
$\dot{V}E/\dot{V}O_2$	21	33 ± 1	31 ± 1
	10	79 ± 4†	74 ± 5†
Colonic temperature (°C)	21	35.1 ± 0.3	35.5 ± 0.3
	10	34.4 ± 0.2	34.6 ± 0.3†

* Values are group mean ± 1 SEM. None of the experimental values differed significantly from control. V_T , tidal volume.

† Significant difference from 21%.

Acknowledgment. The authors thank Lina Naso for technical assistance.

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APPENDIX

BODY O₂ STORE IN A 6-G NEWBORN RAT

Blood. Total volume = 100 mL/kg. Hb = 0.09 g/mL blood, each binding 1.34 mL of O₂, with 100% desaturation. Blood O₂ (mL) = 0.006 kg · 100 mL/kg · 0.09 g Hb/mL · 1.34 mL O₂/g Hb = 0.07. If arterial and venous blood (one fourth and three fourths of total) maximal desaturation were only 50% and 90% of the maximal O₂ capacity, respectively, blood effective O₂ stores would be about 0.06 mL.

Muscle. Skeletal muscle mass = one third of body mass. Muscle myoglobin (Mb) = 45 g/kg muscle, each binding 1.34 mL O₂, with 100% desaturation. Muscle O₂ (mL) = 0.006 kg · 0.33 · 45 g Mb/kg · 1.34 mL O₂/g Mb = 0.12.

Lungs. Functional residual capacity = 50 mL/kg of air (0.21% O₂): Lung O₂ (mL) = 0.006 kg · 50 mL/kg · 0.21 = 0.063.

Total O₂ stores (blood + muscles + lungs) = 0.07 + 0.12 + 0.06 = 0.25 mL at body temperature and ambient pressure and saturated with water vapor, or 42 mL/kg.

During N₂ breathing, the O₂ in the lung is eliminated within a few breaths. If the newborn's aerobic metabolism remained around the normoxic value [about 40 mL·kg⁻¹·min⁻¹ (13)] the whole-body O₂ stores would be entirely depleted in less than 1 min. The rate of depletion is slower because of the rapid drop in $\dot{V}O_2$ at the onset of hypoxia (14) but probably completed after 10 min of anoxia, when arterial PO₂ averaged 2 mm Hg.