Spectral Analysis of Diaphragmatic EMG during the Neonatal Biphasic Hypoxic Ventilatory Response

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ABSTRACT. The newborn infant monkey consistently demonstrates a biphasic ventilatory response to hypoxemia. We have previously shown that the ventilatory depression during the late portion of this biphasic response is secondary to a decline in inspiratory volume that cannot be explained solely by central neural depression. We hypothesized that hypoxemia caused the diaphragm to fatigue, thereby accounting for the late ventilatory depression during the biphasic neonatal hypoxic response. Diaphragmatic fatigue has been reported to be associated with a decrease in the centroid frequency (Fc) of the electromyogram derived through frequency spectral analysis. Therefore, we analyzed the power spectral density of the diaphragmatic electromyogram recorded from percutaneously implanted crural diaphragmatic electrodes in five 2-day-old infant monkeys while they breathed room air and after 5 min exposure to two levels of hypoxemia during the late ventilatory depression. A fast Fourier transform of EKG free diaphragmatic electromyogram was used to compute the power spectral density and the Fc. The Fc during room air breathing was statistically equivalent to the Fc observed after five minutes exposure to 12% FiO₂ ($p = 0.79$), and 8% FiO₂ ($p = 0.74$) when ventilation was falling. In conclusion, our data demonstrate that changes in the centroid frequency are not present during the biphasic ventilatory decline that occurs with the hypoxic ventilatory response in newborn monkeys. Thus, diaphragmatic fatigue, as defined by a decline in Fc, does not occur during the neonatal biphasic hypoxic response. (*Pediatr Res* 21: 238-241, 1987)

Abbreviation

Fc, centroid frequency

The hypoxic ventilatory response of the human neonate and the newborn of other animal species is characterized by a transient increase in minute ventilation followed by a return to baseline values despite the continued presence of arterial hypoxemia (1-5). Recent studies performed in subhuman primates and kittens have shown that the ventilatory decline cannot be attributed solely to central neural depression since diaphragmatic

and phrenic neural activity remain elevated even as ventilation falls (1, 4).

Data collected on the newborn monkey suggest that the biphasic ventilatory pattern may result from changes in respiratory mechanics (6). A fall in dynamic lung compliance and an increase in functional residual capacity occur during the late portion of the ventilatory decline (6). These changes could account for a reduction in inspired volume for a given level of neural drive in that the lung is less distensible and the diaphragm a less efficient pressure generator at higher lung volume. Consistent with this hypothesis is the fact that the late ventilatory decline during hypoxia is due to a fall in tidal volume (4, 6). Diaphragmatic contractility may also be impaired by hypoxemia beyond that attributable to a shift in length-tension status. Specifically, acute hypoxemia has been reported to depress diaphragmatic force-output in the neonatal piglet (7). These data raise the possibility that hypoxemia causes the diaphragm to fatigue, thereby accounting for the late ventilatory depression during the biphasic neonatal hypoxic response.

Diaphragmatic fatigue has been reported to be associated with a decrease in the Fc of the electromyogram derived through frequency spectral analysis (8). Therefore, we analyzed the power spectrum of the crural diaphragmatic electromyogram to calculate the centroid frequency of infant monkeys during the biphasic hypoxic ventilatory response. We hypothesized that the late ventilatory decline during the biphasic neonatal hypoxic response is associated with a decrease in the numeric value of the Fc, indicating diaphragmatic fatigue.

METHODS

Eight *Macaca nemestrina* infants were delivered by cesarian section at 160 days of gestation (0.95 term) at which time an umbilical artery cannula was placed. All infants were healthy in appearance and had normal blood gases and respiratory rates during the 36-48 h prior to study.

The infant monkeys were instrumented and monitored as previously described (4). Briefly, a flexible, nonkinking tracheostomy tube of latex rubber was placed on the morning of the study under a light dose of ketamine anesthesia (5 mg/kg). The subjects were then put in a prone position on a sponge rubber mattress molded to their shape which allowed the infant the freedom to raise its head and limited movement of its appendages. A humidified gas flow (1.5 liter/min) was provided through a T-piece connected to the tracheal tube opening and during inspiration, tidal volume was obtained as the integral of the flow decrease sensed downstream by a hot-wire anemometer (9).

Two insulated stainless steel wires (0.01 inch diameter) were percutaneously placed in the crural diaphragm of our subjects

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under local anesthesia (chloroprocaine HCl) (10). A third electrode was placed subdermally at the base of the buttocks to provide an electrical ground. Clear, strong signals indicated the electrodes were contained within the crura, 4 mm or less from another as documented by postmortem examination.

Electromyographic signals were recorded differentially to ground and amplified 5000 to 10,000 times on a Grass preamplifier (P511J) prior to recording on an FM tape recorder (Honeywell 5600C). The EMG signal was processed utilizing a Service Associates 414 Analog Processor where it was filtered to allow passage of frequencies between 30 and 3000 Hz. Raw and processed signals were displayed on a Tektronix 5111A storage oscilloscope. Diaphragmatic EMG signals were free of electrocardiographic interference in five of the eight animals and they served as our study subjects. The fast Fourier transform, power spectral density, and Fc of the EMG signal were obtained by utilizing a Hewlett Packard dynamic signal analyzer (model 3561A).

The signal analyzer sampling was triggered by the onset of diaphragmatic EMG activity. The frequency span of the entire power spectrum was from 0 to 1000 Hz, resulting in a time record duration of 400 ms and a display resolution of 2.5 Hz. The onset of EMG sampling was adjusted in order to include the early phase of the diaphragmatic EMG. It should be noted, thus, that the 400-ms period during which diaphragmatic EMG was sampled represented a majority fraction of the overall period of EMG activity for each inspiration. Twenty inspiratory EMG signals were averaged to obtain the power spectrum for each experimental condition.

The EMG signals were sampled at a rate of 2560 Hz to satisfy the Nyquist requirement and minimize aliasing. A Hanning window was utilized to minimize leakage. High pass filtering at 30 Hz was used to minimize motion artifact (11). The Fc was computed from the power spectrum between 60 and 500 Hz ($\geq 95\%$ of inspiratory diaphragmatic EMG power in 2-day-old infant monkeys is contained between these two frequencies). Fc was calculated and defined as that frequency at which the power above and below it are equal (11).

A minimum of 2 h passed after surgical preparations before initiation of the study in order to allow ample time for elimination of anesthetic effects (12). The protocol called for measurement of ventilatory variables (4) and diaphragmatic EMG when infants breathed room air and after a 5-min exposure to a hypoxic gas mixture. Two levels of hypoxia were tested in order to arrive at a PaO_2 between 20–25 torr or 17–19 torr after the initial hyperventilatory phase. This typically represented a step change from room air to 12 or 8% FiO_2 , respectively. Hypoxemia induced by 12% FiO_2 is sufficient to induce electrocortical depression (13). Since the brain stem might be more resistant than the cortex to a hypoxic insult, the second more severe level of hypoxemia ($\text{PaO}_2 = 17\text{--}19$ torr) was also tested. A Corning 168 analyzer was used to measure arterial blood bases and pH. Studies were performed during sleep when no rapid eye movements were visible. At least 20 min recovery time was provided between trials with longer periods allowed if arterial blood gas values did not return to normal after this time allotment.

Statistical comparisons were made using the Student's two-tailed *t* test for paired data (Minitab Release 80.1, Penn State Univ., 1980). A *p* value < 0.05 was considered to indicate a statistically significant difference.

RESULTS

As previously reported (4) all the infant monkeys demonstrated a typical biphasic response to both stimulus levels of hypoxia. The late ventilatory decline was due to a fall in tidal volume which occurred in the face of elevated levels of diaphragmatic EMG activity.

Figure 1 is a representative tracing of crural diaphragmatic EMG signals under baseline conditions and after 5 min of

Fig. 1. A representative tracing of crural diaphragmatic EMG signals in one animal under baseline conditions and after 5 min of hypoxemia demonstrating the absence of EKG contamination.

hypoxemia and demonstrates the absence of EKG contamination.

Figure 2 documents the power spectrum and centroid frequency of one representative monkey under baseline conditions (A), after 5 min of hypoxemia induced by breathing 12% FiO_2 (B), and after 5 min of hypoxemia induced by breathing 8% FiO_2 (C). The Fc did not decrease during the hypoxic exposures when compared to its baseline value.

Table 1 documents the centroid frequencies of the five infant monkeys under the baseline condition ($\text{PaO}_2 = 66 \pm 12$ torr) and after 5 min of hypoxemia induced by breathing 12% FiO_2 ($\text{PaO}_2 = 23 \pm 3$ torr). No significant shift in the Fc was noted after the 5-min hypoxic exposure ($p = 0.79$).

Table 2 documents the Fc of the five infant monkeys under the baseline condition ($\text{PaO}_2 = 67 \pm 10$ torr) and after 5 min of hypoxemia induced by breathing 8% FiO_2 ($\text{PaO}_2 = 18 \pm 1$ torr). Again, no significant shift in the Fc was noted between these two conditions ($p = 0.74$).

The coefficient of variation of the Fc for the study animals ranged from 7 to 12% during the baseline period, from 8 to 15% during 12% FiO_2 exposure, and from 6 to 19% during 8% FiO_2 exposure.

DISCUSSION

This investigation demonstrates that significant changes in the Fc of the crural diaphragmatic electromyogram do not occur during the late ventilatory decline in the biphasic hypoxic ventilatory response in newborn monkeys. Thus, diaphragmatic fatigue, as defined by a decline in the Fc, does not occur during the neonatal biphasic hypoxic response.

It should be noted that our definition of fatigue is limited to the case involving a power spectral shift to lower frequencies. This definition rests on observations that fatigue in limb skeletal muscle is associated with characteristic changes in the power spectrum of the frequency components of the electromyogram. These changes include an increase in the total power, an increase in low frequency power, and a decay in high frequency power (14–16). Similar changes in diaphragmatic electromyogram signals have been noted during loaded breathing and/or fatiguing exercise in adult human volunteers (8, 17–19). The mechanisms underlying the spectral shift in the EMG during muscle fatigue are uncertain. Central and peripheral factors may contribute to this phenomenon (20). Central factors may include increasing synchronization and a mean slowing of the motor unit firing rate (21, 22). Peripheral factors may include a slowing of conduction in the terminal nerve fibers (23) and a slowing in muscle action potential conduction velocity (24).

Fig. 2. The power spectrum and centroid frequency of one representative infant monkey under baseline condition (A), after 5 min of hypoxemia induced by breathing 12% FiO_2 (B), and after 5 min of hypoxemia induced by breathing 8% FiO_2 (C). The centroid frequency is indicated by the dotted vertical line.

Recent work, however, has raised questions regarding the exact relationship between shifts in the electromyogram power frequency spectrum and muscle fatigue. Sandercock *et al.* (25) examining single motor unit action potentials during fatigue observed that changes in motor unit action potential amplitude and duration correlated with changes in tension development at high frequencies of stimulation (80 Hz) but not low frequencies of stimulation (10 Hz) (25). Since the electromyogram signal is dependent on the summation and cancellation of individual motor unit action potentials, they hypothesized that the electro-

Table 1. Fc determinations baseline versus 5 min of 12% FiO_2

Fc (Hz) baseline	Fc (Hz) 12% FiO_2
172.5	167.5
177.5	190.0
185.0	190.0
165.0	165.0
195.0	187.5
179 $\pm$ 12*	180 $\pm$ 13*

* Mean $\pm$ 1 SD.

Table 2. Fc determinations baseline versus 5 min of 8% FiO_2

Fc (Hz) baseline	Fc (Hz) 8% FiO_2
175.0	180.0
175.0	185.0
190.0	187.5
175.0	162.5
195.0	190.0
182 $\pm$ 10*	181 $\pm$ 11*

* Mean $\pm$ 1 SD.

myogram will provide a reasonable estimate of high frequency fatigue (*i.e.* loss of tension at high frequencies of neural stimulation that results from failure of excitation of skeletal muscle fibers) but an unreliable measure of low frequency fatigue (*i.e.* loss of tension at low frequencies of neural stimulation that results from a failure of excitation-contraction coupling). Moxham *et al.* (26) generated *in vivo* data in support of this hypothesis during their examination of changes in the diaphragmatic electromyogram power spectrum with fatigue in adult humans. They observed that only high frequency fatigue (*i.e.* a decline in transdiaphragmatic pressure development at high frequencies of phrenic nerve stimulation) was associated with shifts to lower frequencies in the electromyogram frequency spectrum. Low frequency fatigue (*i.e.* a decline in transdiaphragmatic pressure at low frequencies of phrenic nerve stimulation) was not associated with any shifts in the electromyogram frequency spectrum. Whether a similar phenomenon occurs in the neonate and/or during severe hypoxemia is unknown. Further investigation is needed to define the relationship between electromyographic and mechanical activities within a neonatal context during a documented fatigue state.

Since diaphragmatic fatigue appears to be absent during the neonatal biphasic hypoxic ventilatory response, we suggest that the late ventilatory decline during hypoxia is secondary to a combination of various factors. These include alterations in 1) pulmonary mechanics, 2) diaphragmatic mechanics, and 3) central neural drive. Previous work in our laboratory demonstrated that dynamic lung compliance falls and functional residual capacity increases as ventilation declines during the biphasic response (6). These mechanical changes would account for a reduction in inspired volume for a given level of neural drive in that 1) the lung is less distensible and 2) the diaphragm is in a less favorable force-length configuration making it a less efficient pressure generator. These findings alone could theoretically explain the late ventilatory depression during the biphasic neonatal hypoxic response.

In addition, the small decline in neural activity that does occur after the peak ventilatory response may play an etiological role in the late ventilatory depression particularly since investigators have demonstrated that an unfavorable diaphragmatic force-length configuration elicits a compensatory increase in central neural drive in adults (27). No such augmentation in central neural drive was observed during the late ventilatory decline in the infant monkey when an adverse alteration in diaphragmatic force-length configuration associated with an increase in func-

tional residual capacity presumably occurred. This finding may reflect the observation that infants, in contrast to adults, adopt a breathing strategy to limit the extent of change in absolute lung volume, rather than to defend ventilation, at increased lung volume (28).

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