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Biphasic Ventilatory Response to Hypoxia in Unanesthetized Rats

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Summary

To determine the role of postinspiratory inspiratory activity of the diaphragm in the biphasic ventilatory response to hypoxia in unanesthetized rats, we examined diaphragmatic activity at its peak (D_I), at the end of expiration (D_E), and ventilation in adult unanesthetized rats during poikilocapnic hypoxia (10 % O_2) sustained for 20 min. Hypoxia induced an initial increase in ventilation followed by a consistent decline. Tidal volume (V_T), frequency of breathing (f_R), D_I and D_E at first increased, then V_T and D_E decreased, while f_R and D_I remained enhanced. Phasic activation of the diaphragm ($D_I - D_E$) increased significantly at 10, 15 and 20 min of hypoxia. These results indicate that 1) the ventilatory response of unanesthetized rats to sustained hypoxia has a typical biphasic character and 2) the increased end-expiratory activity of the diaphragm limits its phasic inspiratory activation, but this increase cannot explain the secondary decline in tidal volume and ventilation.

Key words

Ventilation • Biphasic response to hypoxia • Diaphragmatic activity • Pattern of breathing

Introduction

The ventilatory response to sustained hypoxia starts typically with an immediate and brief increase in ventilation followed by its decline, often called "roll-off". Such a decline is more pronounced in newborns (Long and Lawson 1984, LaFramboise and Woodrum 1985, Bonora *et al*. 1992) than in adult humans (Easton *et al*. 1986, Easton and Anthonisen 1988, Georgopoulos *et al*. 1989) or other species (Vízek *et al*. 1987, Tatsumi *et al*. 1992, Gershan *et al*. 1994). In newborns, the ventilation decreases close to its control levels, while it is reduced in adults by approximately 20 % of its peak value.

The initial ventilatory increase is a result of the stimulation of peripheral chemoreceptors. However, the cause of the subsequent reduction in ventilation is still not clear. The decrease of $PaCO_2$, resulting from the primary increase in ventilation, cannot be the only cause of the roll-off since the decline in ventilation has also been found during isocapnic hypoxia (Long and Lawson 1984, Easton *et al.* 1986, Vízek *et al.* 1987, Easton and Anthonisen 1988, Georgopoulos *et al*. 1989). A wide range of other mechanisms, including a decrease in the metabolic rate (Gershan *et al*. 1994), an increase in inhibitory neuromodulators, such as adenosine (Easton and Anthonisen 1988, Georgopoulos *et al*. 1989), GABA (Weil 1994) or endorphins (Long and Lawson 1984), or a

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decrease in the efficiency of the ventilatory pump (LaFramboise and Woodrum 1985) has been studied to explain the roll-off. However, none of them has been fully accepted. LaFramboise and Woodrum (1985) based their assumption of a decrease in the efficiency of the ventilatory pump on the fact that the reduction in tidal volume occurs despite constantly elevated peak activity of the diaphragm. The decrease in tidal volume without any change in diaphragmatic peak activity could, however, result from a reduction of the phasic activation of the diaphragm caused by an increase in its tonic activity (activity at the end of expiration). To test whether the hypoxia-induced increase of diaphragmatic postinspiratory inspiratory activity (Bonora *et al*. 1992) participates in the roll-off phenomenon, we studied the time-course of the ventilatory and diaphragmatic changes, including their possible relationships, during sustained hypoxia in unanesthetized rats.

Methods

Our studies were performed on 12 adult male Wistar rats with an average body weight of 331.8±9.4 g (S.E.M.)

Preparation

The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg). A midline abdominal incision was made and two electrodes (multistrand teflon coated-wires) were implanted into the rostral part of the right hemi-diaphragm. A third electrode (used as ground) was tied into the neck muscles. All the wires were tunneled subcutaneously and exteriorized at the back of the neck. 10 ml of glucose (5 %) was administered (s.c.) and the animals were allowed to recover for at least 3 days.

Measurements

Ventilation (V_E) was measured in a plethysmograph using the barometric method described by Chapin (1954) and Bartlett and Tenney (1970). The pressure signal due to breathing was monitored by a differential pressure transducer (Elema-Schonander EMT 32). The diaphragm EMG signal was recorded by connecting the bared terminal part of the wires to the amplifier (input impedance 250 MΩ, common mode rejection ratio 55 dB, noise level 0.7 μV). The signal was filtered (30-1000 Hz) and integrated by a computer program with a time constant of 100 ms (moving time average). Raw and integrated diaphragmatic signals were displayed and recorded by a computer program together with the respiratory signal. Values of tidal volume (V_T), respiratory frequency (f_R), ventilation (V_E), instantaneous values of integrated diaphragmatic EMG at the peak, which corresponds to the end of inspiration (D_I), and at the trough, which corresponds to the end of expiration (D_E) were measured during control breathing of air, during breathing of a hypoxic gas mixture (10 % O_2 in N_2) and during recovery from hypoxia.

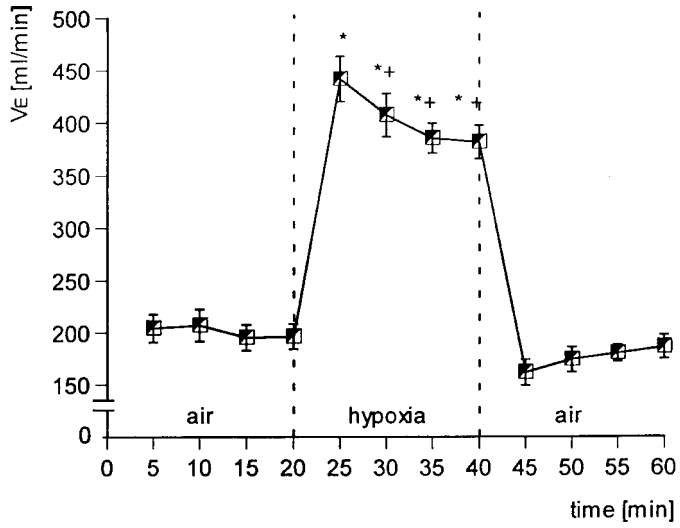

Fig. 1. *Mean values (± S.E.M.) of minute ventilation (V_E) in response to hypoxia (10 % O_2). * $p<0.05$ from normoxic values at 5-20 min, $^+$ $p<0.05$ from hypoxic value at 25 min (at 5th min of hypoxia).*

Protocol

The animals were placed in a plethysmograph. When breathing became stabilized, ventilation and diaphragmatic EMG activity were recorded during breathing air (F_IO_2 = 0.21) after 5, 10, 15 and 20 min, in hypoxia (F_IO_2 = 0.10) again after 5, 10, 15 and 20 min, and finally in air after 5, 10, 15 and 20 min of recovery from hypoxia.

Data analysis and statistics

Each variable of ventilation and diaphragmatic activity was averaged over six consecutive respiratory cycles. The results are presented as means ± S.E.M. Statistical analysis was done using ANOVA with P values adjusted for multiple comparisons by Fisher's PLSD test. Differences were considered significant when $P<0.05$.

Results

As shown in Figure 1, 5 min after the onset of hypoxia (10 % O_2) VE increased to 227 % of its control value. This increase was followed by a partial decline in ventilation to 208 % of the control value at 10 min and to 197 % and 195 % at 15 and 20 min of hypoxia, respectively. The increase in ventilation was due to an increase of fR to 172 % and VT to 133 % of the control value (Fig. 2). The secondary decline in ventilation was mainly due to a decrease of VT, while fR remained relatively stable.

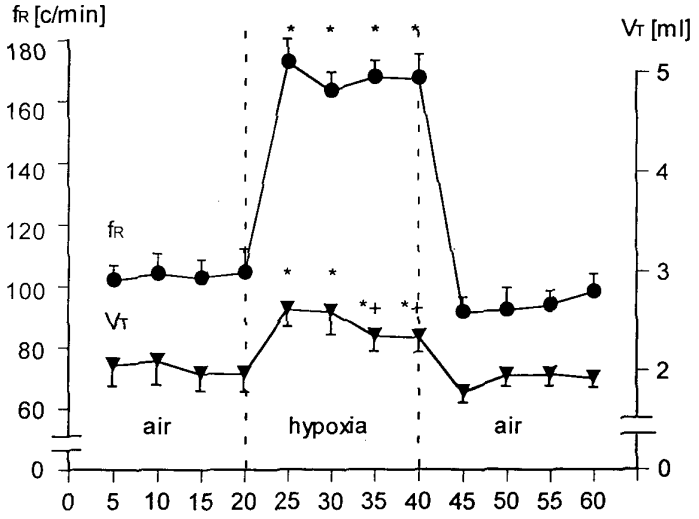

Fig. 2. *Mean values (± S.E.M.) of respiratory frequency (f_R) and tidal volume (V_T) in response to hypoxia (10 % O_2). * $p<0.05$ from normoxic values at 5-20 min, $^+$ $p<0.05$ from hypoxic value at 25 min.*

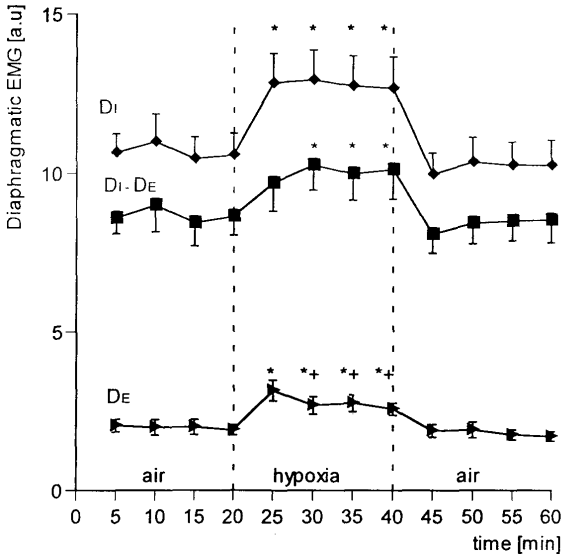

Fig. 3. *Mean values (± S.E.M.) of integrated diaphragmatic EMG at the peak (D_I), the trough (D_E) and the difference between them ($D_I - D_E$) in response to hypoxia (10 % O_2). * $p<0.05$ from normoxic values at 5-20 min, $^+$ $p<0.05$ from hypoxic value at 25 min.*

The changes in diaphragmatic activity are presented in Figure 3. Whereas the D_I increased during the first minutes of exposure to hypoxia and did not change throughout the exposure, the D_E increased more at 5 min of the exposure than at 10, 15 and 20 min. Because of the changes in D_E, $D_I - D_E$ were increased significantly only at 10, 15 and 20 min of hypoxia.

Figure 4 compares the relative changes in D_E and $D_I - D_E$ during the response to hypoxia to those of V_T. This figure shows that the decrease in V_T between 10th and 15th min of exposure was accompanied neither by an increase in D_E nor a decrease in $D_I - D_E$. Changes in V_T also did not correlate with changes of D_E ($r_{x,y}$=0.16, P=0.2).

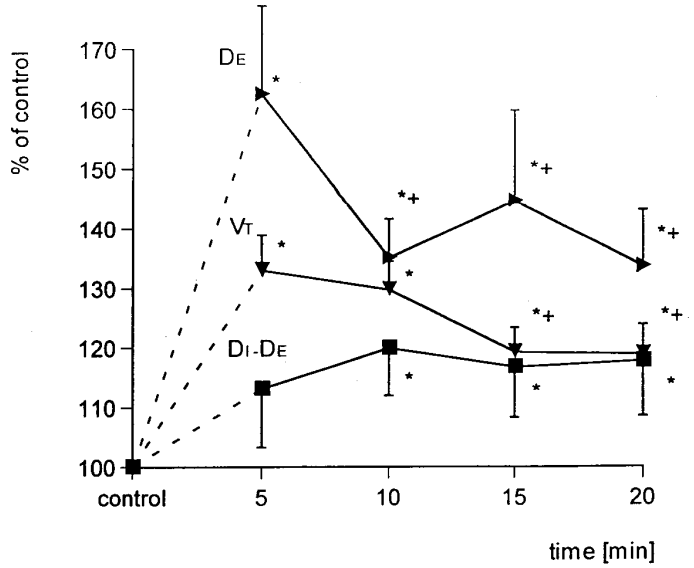

Fig. 4. *Relative changes (% of mean control normoxic value ± S.E.M.) of the diaphragmatic activity at the end of expiration (D_E), phasic activation of the diaphragm ($D_I - D_E$) and tidal volume (V_T) during hypoxia (10 % O_2).*

Discussion

The present study was designed to extend the previous findings (Vízek *et al.* 1987, Bonora *et al.* 1992) by determining the relationship between the changes in ventilation and both inspiratory and postinspiratory diaphragmatic activity during sustained hypoxia in unanesthetized rats. It has been showed that the exposure to 10 % O_2 in adult unanesthetized rats induces an initial increase in ventilation followed by its partial decline – the ventilatory roll-off. It also showed that poikilocapnic hypoxia increased the diaphragmatic activity at the end of expiration (D_E). The increased D_E attenuated the phasic activation of the diaphragm, however, the phasic change of the diaphragmatic activity ($D_I - D_E$) increased during the roll-off because of a secondary decline in D_E. This result suggests that the changes in D_E alone could not directly cause the roll-off phenomenon.

Our study was performed on unanesthetized rats and as far as we know this is the first study showing the roll-off phenomenon in awake rats. Although the secondary decline in ventilation during hypoxia was highly significant in our group of rats, the decline was lower than 10 % of the peak value in 5 out of 12 rats. Mean decrease in ventilation was 14 % of the peak value, which is in the range also reported in other studies (11-30 %) performed either during isocapnic (Easton *et al.* 1986, Vízek *et al.* 1987, Easton and Anthonisen 1988, Georgopoulos *et al.* 1989) or poikilocapnic hypoxia (Vízek *et al.* 1987, Gershan *et al.* 1994). The decline was significant after 10 min of hypoxia, similarly as reported in humans (Easton *et al.* 1986, Easton and Anthonisen 1988), but later than in anesthetized rats (Vízek and Bonora 1998). We used poikilocapnic hypoxia since isocapnic conditions would not represent a normal physiological response to hypoxia. Although we did not measure $PaCO_2$, the large increase in ventilation was

probably accompanied by hypocapnia. This hypocapnia should contribute to the biphasic ventilatory response by decreasing the chemical drive, although it cannot be the only cause, since ventilation also declines during isocapnic hypoxia (Long and Lawson 1984, Easton *et al*. 1986, Vízek *et al*. 1987, Georgopoulos *et al*. 1989). The fact that we did not see a substantial secondary decline in ventilation in all our rats and that the ventilatory decline did not correlate with the initial change of V_E and therefore with the decrease in $PaCO_2$ also supports the assumption that the expected decrease in $PaCO_2$ is not likely the only cause of the roll-off.

The first part of our study confirmed that hypoxia induced an increase in D_E as reported in other studies (Bonora *et al.* 1992, Sherrey *et al*. 1988, Smith *et al*. 1989, Bonora and Boulé 1994, Bonora and Vízek 1995). The increase in frequency and thus the shortening of expiration may affect the end-expiratory activity of the diaphragm. However, it could not be the only cause of increased D_E since the changes in f_R and D_E did not correlate. We have also shown previously that similar changes in f_R during hypercapnia and hypoxia were accompanied by an increase in D_E during hypoxia only (Bonora and Vízek 1995).

The secondary decline in ventilation in our rats was mainly due to a decrease in V_T as is commonly reported in adults (Easton and Anthonisen 1988, Georgopoulos *et al*. 1989). However, the major finding of the present study concerns the concomitant changes in diaphragmatic activity. The increase in D_I during the initial increase in ventilation was counteracted by a similar increase in D_E and therefore the phasic activation of the diaphragm did not change. This suggests that other inspiratory muscles also participate, or that they completely ensure the increase of V_T. During the decline, the changes of V_T were not parallel to those of $D_I - D_E$. Between the 5th and 15th min of hypoxia, V_T decreased, while $D_I - D_E$ did not change significantly. Thus, the changes in V_T could not be explained by the changes in $D_I - D_E$. Hypocapnia could also play a role in this phenomenon by increasing D_E, since the diaphragm was found to be more active during expiration in hypocapnic than in normocapnic hypoxia (Sherrey *et al*. 1988, Bonora and Boulé 1994). However, hypoxia *per se* is also involved in this mechanism, since the increase in D_E induced by hypocapnic hypoxia was not completely suppressed in normocapnic hypoxia (Smith *et al*. 1989, Bonora and Boulé 1994). Moreover, D_E also increased during CO hypoxemia while end-tidal P_{CO_2} (P_{ETCO2}) did not change, and decreased in hyperoxia while P_{ETCO2} was slightly decreased (Bonora and Boulé 1994).

In conclusion, we found that 1) the ventilatory response of unanesthetized rats to sustained hypoxia is typicaly biphasic in character, and 2) in unanesthetized rats hypoxia induces an increase in the diaphragmatic activity at the end of expiration. This increased end-expiratory activity attenuated the phasic activation of the diaphragm throughout the biphasic ventilatory response to hypoxia. The secondary decline in V_T in unanesthetized rats cannot be explained by a decrease of the phasic activation of the diaphragm.

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