Ventilatory response of the cat to hypoxia in sleep and wakefulness

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Ventilatory response of the cat to hypoxia in sleep and wakefulness. J Appl Physiol 95: 545–554, 2003. First published April 25, 2003; 10.1152/japplphysiol.01051.2002.—This study characterized ventilation, the airflow waveform, and diaphragmatic activity in response to hypoxia in the intact adult cat during sleep and wakefulness. Exposure to hypoxia for up to 3 h caused sustained hyperventilation during both wakefulness and sleep. Hyperventilation resulted from significant increases in minute ventilation due to increases in both tidal volume and frequency. Diaphragmatic activity changed significantly from augmenting activity with little postinspiratory-inspiratory activity (PIIA) in normoxia to augmenting activity with increased PIIA in hypoxia. The increase in PIIA was least in rapid eye movement sleep. These changes in diaphragmatic activity were associated with changes in airflow waveforms in inspiration and expiration. We conclude that the ventilatory response to hypoxia involves a change in the output of the central pattern generator and that the change is dependent on the state of consciousness.

Our concern in this study was the pattern of breathing of intact, unanesthetized cats exposed to hypoxia. Although we were interested in overall ventilation, an interest sparked by disagreements among published studies (1, 14, 22, 27), our primary interest was in the form of the individual breaths. For example, we wanted to know whether inspiratory airflow and diaphragmatic activity had augmenting forms in hypoxia as they do in normoxia. Furthermore, we wanted to know how state of consciousness affected diaphragmatic activity and thus airflow waveforms of inspiration and expiration. This information is essential for understanding changes in output of the respiratory central pattern generator in response to hypoxia. Therefore, we analyzed ventilation, diaphragmatic activity, and the form of inspiratory and expiratory airflow during normoxia and hypocapnic hypoxia in sleep and wakefulness. The results show that the airflow waveform is indeed altered in hypoxia, as is the pattern of diaphragmatic activity. They show also that these changes are superimposed on state-specific breathing patterns.

Materials and methods

Subjects. Five adult cats (3.2–5.3 kg) were prepared for recordings of electroencephalographic (EEG), pontogeniculoccipital (PGO), and diaphragmatic electromyographic (EMG) activity. Tracheal fistulas were created, and headcaps containing a connector for electrodes were attached to the animals’ skulls. The headcap contained also standoffs that were used to immobilize the animal’s head during recordings. The animals recovered from surgery for 1 mo before experimentation. After recovery, they were adapted to the experimental apparatus. The Animal Care and Use Committee of Texas Tech University School of Medicine approved all surgical and experimental procedures.

Surgical procedures. The animals were initially anesthetized with acepromazine maleate (2.5 mg im) and ketamine (17 mg/kg im). Surgery was performed under antiseptic conditions. A midline incision was made from below the cricoid cartilage to just above the suprasternal notch, and the sternothyroid, sternohyoid, and sternomastoid muscles were retracted to expose the trachea. The trachea was opened longitudinally, and the cut edges of the rings were sewn to the skin margins on the corresponding side to create a fistula. Anesthesia was then maintained by administration of 1–2% halothane in O2 through the trachea.

The animal was placed in a supine position, and an incision was made caudal to the costal margin from the xiphoid process to the midaxillary line. Four EMG electrodes (Teflon-coated multistranded stainless steel wires; Cooner AS 632) were implanted within crural and semitendinous regions of the right diaphragm. The electrodes were placed as medially as possible to avoid intercostal muscle activity contamination. The EMG wires were run subcutaneously to the back of the neck, where they were routed to the skull.

The animal was placed in a stereotaxic frame, and a midline incision exposed the dorsal skull. EEG electrodes [4–40 stainless steel screws with multistranded Cooner (AS 632) stainless steel leads] were screwed into the skull over medial occipital and parietal cortices bilaterally. PGO electrodes were constructed from stainless steel insulated wires (0.006-in. diameter) twisted into a tripolar electrode. The...
Fig. 1. Breathing during non-rapid eye movement (NREM) sleep in normoxia and hypocapnic hypoxia. A: raw traces of airflow, tidal CO₂%, EEG, and pontogeniculo-occipital (PGO) waves during a 45-min exposure to hypoxia in 1 cat. Arrow indicates the onset of hypoxia. a.u., Arbitrary units; *, augmented breaths. B: breath-by-breath representation of data in A (augmented and abnormal breaths removed). Dashed lines indicate mean values in normoxia control. VT, tidal volume; f, frequency of breathing; VE, minute ventilation; TTOT, total breath duration; TI, inspiratory time; TE, expiratory time.
insulation was stripped from the tips for a distance of $\sim0.5$ mm, and the tips were separated by $0.5 \text{–} 1.0$ mm vertically.

Two tripolar electrodes, one in each hemisphere of the brain, were implanted stereotaxically at coordinates $A6.4$, $L10$, and $H2.5$. These are the coordinates of the fibers of the optic tract where they enter the lateral geniculate body of the thalamus. PGO wave recordings are optimal when the electrode tip in these fibers is referred to an electrode in the overlying lateral geniculate body.

EEG and PGO electrodes and 4-40 anchor screws were cemented to the skull. A prefabricated headcap containing standoffs for immobilization of the head was fixed to the skull with dental cement. Gold cinch pins were crimped to the ends of the diaphragmatic, EEG, and PGO electrode wires and were inserted into a connector block. The connector block was then attached to the headcap.

**Recording procedures and exposure to hypoxia.** On nights before recording sessions, the animals were housed in a cold ($0^\circ \text{C}$) environment to prevent rapid eye movement (REM) sleep and therefore to consolidate REM sleep the following day. During recordings, the trachea was intubated with a 4.0-mm endotracheal tube that was attached to a Validyne pneumotachograph. Total dead space of the tracheal tube and pneumotachograph was 8 ml, which is approximately equal to the dead space of the upper airway. Pressure levels in the tube were measured by using a volumetric pressure transducer. Tidal $O_2$ and $CO_2$ were measured with an $O_2$ analyzer (Beckman OM-11) and infrared $CO_2$ analyzer (Beckman LB-2). Tidal $O_2$ and $CO_2$ percentages; EEG, EMG, and PGO activity; airflow; and intratracheal pressures were recorded on paper (Astro-Med 9500) and on magnetic tape. Diaphragmatic activity was amplified with a Grass p511 amplifier set to pass frequencies from 0.3 to 30 kHz. Control conditions were obtained with animals breathing room air in Lubbock, Texas (altitude 1,000 m). Once control conditions were obtained, animals breathed a hypoxic gas mixture (inspired $O_2$ fraction $\sim10\% \text{ in } N_2$; alveolar $Po_2 = 63$ Torr).

In this study, $CO_2$ levels were always allowed to decrease as a function of ventilation (hypocapnic hypoxia). Three cats breathed hypoxic gas for periods up to 3 h, and two cats breathed hypoxic gas for a time sufficient to record all three states of consciousness. Recording sessions lasted $\sim4$ h. Non-rapid eye movement (NREM) and REM sleep and wakefulness were defined on the basis of standard EEG criteria (17).

**Data analysis.** Diaphragmatic activity, PGO activity, EEG activity, and airflow were digitized from analog tape records.

### Table 1. Respiratory parameters during wakefulness in normoxia and 60, 120, and 180 min of hypoxia

<table>
<thead>
<tr>
<th>Animal</th>
<th>Treatment</th>
<th>Time, min</th>
<th>Breaths</th>
<th>$V_t$, ml</th>
<th>$f$, min$^{-1}$</th>
<th>$V_e$, ml/min</th>
<th>$T_r$, s</th>
<th>$T_i$, s</th>
<th>$T_e$, s</th>
<th>End-tidal $CO_2$, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDM</td>
<td>Normoxia</td>
<td>0</td>
<td>13</td>
<td>23.2 ± 0.7</td>
<td>26.2 ± 0.6</td>
<td>610 ± 23</td>
<td>2.30 ± 0.05</td>
<td>0.85 ± 0.02</td>
<td>1.45 ± 0.03</td>
<td>30.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
<td>60</td>
<td>14</td>
<td>28.4 ± 0.4*</td>
<td>33.0 ± 0.5*</td>
<td>936 ± 21*</td>
<td>1.82 ± 0.03*</td>
<td>0.78 ± 0.01*</td>
<td>1.05 ± 0.02*</td>
<td>21.3 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
<td>120</td>
<td>31</td>
<td>29.1 ± 0.5*</td>
<td>33.7 ± 0.4*</td>
<td>979 ± 12*</td>
<td>1.78 ± 0.02*</td>
<td>0.78 ± 0.01*</td>
<td>1.00 ± 0.02*</td>
<td>19.9 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>Hypoxia</td>
<td>180</td>
<td>35</td>
<td>24.9 ± 0.2*</td>
<td>36.2 ± 0.5*</td>
<td>951 ± 15*</td>
<td>1.58 ± 0.02*</td>
<td>0.69 ± 0.01*</td>
<td>0.89 ± 0.01*</td>
<td>19.4 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. $V_t$, tidal volume; $f$, frequency of breathing; $V_e$, minute ventilation; $T_r$, breath duration; $T_i$, inspiratory duration; $T_e$, expiratory duration. *$P < 0.05$, 1-way ANOVA with Dunnett’s correction for multiple comparisons.
at 1,000 samples/s by use of a National Instruments data acquisition board. For breath-by-breath analysis, tidal volume (VT), inspiratory duration (TI), and breath duration (TT) were derived from the airflow signal by use of custom software. Expiratory duration (TE), frequency of breathing (f), and minute ventilation (VE) were calculated from the derived parameters. Diaphragmatic activity was transformed to a rectified signal.

For qualitative analysis of airflow waveforms and diaphragmatic activity, the respiratory cycles were divided into 1,000 bins, and the data for multiple breaths were averaged over the bins. In wakefulness and NREM sleep, single or multiple (n ≤ 3) episodes of consecutive normal breaths (not interrupted by augmented breaths and abnormalities in the airflow trace caused by swallows, movement, coughs, or vocalizations) in the same state were selected without regard to breath duration. In REM sleep, all the breaths for the REM period were analyzed.

For quantitative analysis of diaphragmatic EMG, averaged activity was divided into inspiration (I), the first half of expiration (E1), and the second half of expiration (E2). Activity (arbitrary units) in each division (I, E1, and E2) was summed and divided by the time of the corresponding division to give activity/s. Baseline noise (activity/s) was subtracted from that value. Because activity varied among animals, data were normalized in each cat by expressing the activity as a ratio of the total mean diaphragmatic activity per second during NREM sleep in normoxia.

Airflow waveform was quantitatively characterized by breath by using an integral of the deviation of the actual waveform from a pair of fixed waveform templates: the triangular and square waveform. The triangular waveform template was constructed as two right triangles: one positive right triangle (inspiration) and one negative right triangle (expiration) with the right angles positioned at the transition from inspiration to expiration. The square waveform template consisted of two square waves: one positive (inspiration) and one negative (expiration). The template dimensions were adjusted to the parameters of the actual waveforms. The duration of TI or TE for the template was determined by the TI or TE of the actual waveform. The areas (VT) of the actual inspiratory and expiratory airflows were normalized to a value of 1. The amplitudes of the template waveforms for inspiration and expiration were selected to make the areas of the templates also equal to 1. The integral of the absolute value of the difference between the template waveforms and the actual waveforms was calculated to obtain breath-by-breath values. Thus, the more closely a waveform resembled the template waveform, the smaller the calculated value. Square-wave deviation and triangular-wave deviation were calculated independently for inspiration and expiration, which yielded four numbers for each breath. The inspiratory and expiratory values for square-wave deviations and for triangular deviations were averaged over multiple breaths, and mean values were used for analysis. Equations for and a graphical representation of this method for examining airflow waveforms are in the APPENDIX.

Fig. 3. Respiratory parameters during W and NREM and REM sleep in normoxia and hypocapnic hypoxia. Data are from 5 cats. Number of breaths analyzed: W 21% O2 (348), 10% O2 (410); NREM 21% O2 (514), 10% O2 (767); REM 21% O2 (886), 10% O2 (877). Values are means ± SE. *Significance (P < 0.05), paired t-test.

Fig. 4. Airflow waveform and diaphragmatic activity (EMG DIA) in normoxia (A) and hypocapnic hypoxia (B) during NREM sleep.
RESULTS

Results reported here were obtained from five adult cats during 51 recording sessions.

Hypoxia-induced hyperventilation in wakefulness and sleep. Hypoxia caused a sustained hyperventilation in quiet wakefulness and NREM and REM sleep (Figs. 1, 2, and 3). We did not observe a single instance of ventilatory depression in any of the cats in any of the sessions. Hyperventilation was continuous, and end-tidal CO$_2$ levels continued to decrease throughout the recording period (Table 1, Fig. 1). Respiratory parameters during wakefulness for a 3-h period of hypoxia in one animal (EDM) are reported in Table 1. During this 3-h recording period, VT, f, and V$\dot{E}$ were significantly increased, and T$_I$, T$_E$, T$_T$, and end-tidal CO$_2$ were significantly decreased (Table 1).

The pattern of breathing depended on the state of consciousness in both normoxia and hypoxia (Figs. 2 and 3). In NREM sleep, compared with wakefulness, f was low, VT was large, and there was little variability. During REM sleep, compared with NREM sleep, f increased, VT decreased, variability increased, and end-tidal CO$_2$ decreased.

In hypoxia, state-specific patterns were maintained on a background of hyperventilation. The hyperventilation was caused by a significant increase in V$\dot{E}$ in all states (Fig. 3). The increase in V$\dot{E}$ was a result of a significant increase in VT. The f was significantly increased only in NREM sleep (Fig. 3). Changes in VT were caused by increased initial airflow rates (Figs. 4–7), increased peak airflow rates (Figs. 1, 2, and 4–7), and increased average airflow that resulted from a change in the airflow waveform (Figs. 4–8).

Airflow waveform in wakefulness and sleep. Airflow waveform was analyzed in all five cats. In general, the typical triangular waveforms seen during normoxia (Fig. 4 and 5A) became, within a few breaths after the onset of hypoxia, more like a square waveform (Figs. 4–6). An example of the breath-by-breath results obtained for one cat in NREM sleep in hypoxia and normoxia is presented in Fig. 7. In hypoxia in all states, the waveforms in both inspiration and expiration deviated significantly from the corresponding triangular template (Figs. 7 and 8). The expiratory waveforms more closely resembled a square-wave template, as did the inspiratory waveform in wakefulness (Figs. 7 and 8). However, in NREM and REM sleep the inspiratory airflow waveform deviation from a square-wave template was not more than the deviation in normoxia. This indicates that peak flows in hypoxia occurred early in inspiration rather than late. This can be seen also in Figs. 4–6. Similarly, in hypoxia, peak expiratory flow, although generally more constant, tended to occur at the end of expiration. The exception to this was seen in REM sleep when peak expiratory flows occurred at the onset of expiration, just as in normoxia (Fig. 6). Peak flow rates in inspiration and expiration were greater in hypoxia than in normoxia (Figs. 4–6).

Diaphragmatic activity during hyperventilation. Diaphragmatic activity was examined in four cats. Diaphragmatic activity increased in response to hypoxia, and the augmenting profile of diaphragmatic activity seen in normoxia was unchanged (Figs. 4–6). Quantitative analysis revealed that diaphragmatic activity during inspiratory airflow was significantly increased in all states (Fig. 9). During the first part of expiration...
PIIA was significantly increased in all three states, although the increase was least in REM sleep (Figs. 6 and 9). In late expiration (E2), diaphragmatic activity was significantly increased in wakefulness and NREM sleep (Figs. 6 and 9).

Periodic breathing. Periodic breathing in response to hypoxia was infrequent and of short duration (<2 min) and did not occur in all sessions. However, periodic breathing commonly occurred when cats returned to breathing room air after exposure to hypoxia (Fig. 10).

DISCUSSION

Breathing significantly increased in response to hypoxia in both sleep and wakefulness. The increase in breathing was a true hyperventilation with a sustained reduction in end-tidal CO2. This hyperventilation was associated with significant changes in both the airflow waveform and diaphragmatic activity. Sustained periodic breathing occurred infrequently in hypoxia but was commonly seen as a posthypoxic response.

Augmentation vs. depression. Ventilation increases in response to hypoxia (9, 16, 20). However, recent work in awake cats and humans shows that a depression of ventilation occurs secondarily if exposure to hypoxia is prolonged (27, 28). This depression has received considerable attention, especially from those interested in the effects of hypoxia on the central nervous system (3). However, the conditions necessary for the occurrence of the depression are not known. Nor is it known whether this depression occurs because of peripheral or central mechanisms. Robbins (21) has suggested that different mechanisms may be responsible for the depression of ventilation under different conditions (e.g., peripheral mechanisms in awake animals and central mechanisms in anesthetized preparations). Our data showed only augmentation of ventilation during hypoxia. We do not know why we did not see a secondary depression.

Chemosensitivity and breathing during sleep. The ventilatory response to progressive hypoxia during NREM sleep is generally assumed to be less than that during wakefulness. In REM sleep, ventilation in response to hypoxia has been reported to be either decreased or not significantly different compared with NREM sleep (2, 6–8, 12, 18). Breathing irregularities observed in REM sleep reportedly persist in hypoxia (2, 18). In our study, VE was significantly increased and end-tidal CO2 was significantly decreased in hypoxia in all states, and state-specific patterns persisted. Thus, in REM sleep compared with NREM sleep, VR values were lower, frequencies were higher, and end-tidal CO2 levels were lower in hypoxia, just as they were in normoxia. These results do not support claims of impaired chemosensitivity to oxygen in sleep.

Airflow waveform and diaphragmatic activity. There were major changes in the airflow waveform in hypoxia. The inspiratory pattern consisted of a generally square waveform with peak flows at the onset of the phase, which is the opposite of that seen in normoxia. The expiratory pattern was also reversed from the normoxic pattern, with peak airflow at the end of expiration rather than the beginning of that phase. This was true of expiration in wakefulness and NREM sleep, but not REM sleep, in which expiratory flows were maximal at the onset of expiration. Similar changes in airflow waveforms have been reported during exercise and in response to changes in dead space and airway resistance (10, 11, 13, 19). Hypoxia causes an increase in airway resistance, but the ventilatory response to hypocapnic hypoxia apparently overrides the constricting effect of low O2 (25).
Fig. 7. Airflow waveform deviation. A: averaged airflow waveforms during NREM sleep in 1 cat. Number of breaths analyzed: 21% O₂ (80); 10% O₂ (222). Dashed line is zero flow. B: breath-by-breath plot of waveform factors (see APPENDIX) data in A.

Fig. 8. Analysis of airflow waveforms in normoxia and hypocapnic hypoxia. A: deviation from triangular waveform. B: deviation from square waveform. Data are from 5 cats. Numbers of breaths are listed in legend for Fig. 3. Values are means ± SE. *Significance (P < 0.05), paired t-test.

Fig. 9. Diaphragmatic activity during normoxia and hypocapnic hypoxia. Data are from 4 cats. Number of breaths analyzed: Wakefulness 21% O₂ (308), 10% O₂ (378); NREM 21% O₂ (431), 10% O₂ (696); REM 21% O₂ (773), 10% O₂ (539). Values are means ± SE. I, inspiration; E1, first half of expiration; E2, second half of expiration. *Significance (P < 0.05), paired t-test.
Changes in the pattern of diaphragmatic activity could also cause changes in airflow waveforms. We found that in hypoxia diaphragmatic activity began early during expiratory airflow, had a rapid rate of rise, and was increased during inspiration and early expiration, which confirms the work of others (4, 5, 15, 23, 24). The increased rate of rise of diaphragmatic activity beginning during expiratory airflow could cause the early peak in inspiratory airflow rates. Augmenting and intense diaphragmatic activity could produce a constant airflow inspiratory waveform, particularly if breathing is occurring at higher lung volumes (5, 24).

In expiration, increased PIIA could slow expiratory airflow in early expiration, and the absence of this braking activity in late expiration, perhaps in association with active expiratory efforts, could allow greater flows at that time (5, 26). Together, these events could produce an expiratory airflow waveform that tends to be square with peak flows at end expiration. In REM sleep, when PIIA is least, Tₐ is decreased, the expiratory waveform is correspondingly less square, and peak expiratory airflow occurs during early expiration.

Airflow waveforms have been the subject of modeling and experimental studies that have demonstrated that square inspiratory waveforms and waveforms with peak flows in early inspiration require less work for achieving a given alveolar ventilation than sinusoidal waveforms (11, 29). Furthermore, square waveforms during active expiration and waveforms with peak flows in early expiration during passive expiration are mechanically more economical than the corresponding sinusoidal wave (29). Similarly, an increased end-expi-

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Fig. 10. Periodic breathing as a posthypoxic response. Arrow indicates return to breathing room air.

Fig. 11. Airflow waveform factors. Heavy lines are actual waveforms, and light lines are triangular (top) and square (bottom) waveform templates in normoxia (A) and hypoxia (B). Shaded areas in inspiration and expiration represent the corresponding factors for $F_Z^I$, $P_Z^E$, $P_I^E$, and $F_I^{E'}$, respectively. C: data represented by the pairs $(F_Z, P_I)$ can only reside within the shaded area. The same dependency holds for the pairs $(F_Z^E, P_I^E)$ (not shown).
atory lung volume may conserve oxygen stores in hypoxia, and braking of expiratory airflow as the result of increased PIIA may allow more time for gas exchange (24, 29). Thus it may be that the airflow waveforms that we observed in hypoxia require less mechanical work than the waveforms seen during normoxia. However, we have no data to support or refute this idea.

APPENDIX

Quantitative Characterization of the Airflow Waveform

Changes in airflow waveform were measured by using four numerical factors, $F^i$, $F^e$, $F^i_t$, and $F^e_t$. They measure deviation (Fig. 11, A and B) of an actual airflow waveform from Z- and H-templates. The latter represent, respectively, the triangular waveform template and the square waveform template respiratory patterns. Mathematically, the factors are defined by the equations

$$F^i = \left(1/V_i\right) \int_0^{T_i} \left(f(t) - \frac{2V_i}{T_i} \cdot t\right) \, dt;$$

$$F^e = \left(1/V_e\right) \int_0^{T_e} \left(f(t) - \frac{2V_e}{T_e} \cdot (T_e - t)\right) \, dt;$$

$$F^i_t = \left(1/V_i\right) \int_0^{T_i} \left(f(t) - \frac{V_i}{T_i}\right) \, dt;$$

$$F^e_t = \left(1/V_e\right) \int_0^{T_e} \left(f(t) - \frac{V_e}{T_e}\right) \, dt;$$

where $f(t)$ is the airflow, $T_i$ and $T_e$ are duration of inspiration and expiration, and $V_i$ and $V_e$ are tidal volumes of inspiratory and expiratory waves, i.e.

$$V_i = \int_0^{T_i} f(t) \, dt, \quad V_e = \int_0^{T_e} f(t) \, dt.$$

The values of these factors are bound to the interval from 0 to 2. For a given breath, the values of the factors are not independent (Fig. 11C).

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DISCLOSURES

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