In the present study we investigated whether hypocapnia that accompanies hypoxic hyperventilation might affect the biphasic, stimulatory/depressant, ventilatory response to hypoxia. The experiments were carried out in anesthetized, vagotomized, spontaneously breathing, and poikilocapnic rats. The animals were subjected to acute steady-state hypoxia consisting of 12% $O_2$ in $N_2$ in inspiratory mixture. Ventilation and its frequency and volume components were assessed from the integrated electromyographic activity of the diaphragm. We found that despite the development of significant hypocapnia, the hypoxic ventilatory response consisted of rapid stimulation followed by a gradual decline. The frequency component contributed more to the ventilatory increase than that of volume. The results indicate that the hypoxic ventilatory profile in the anesthetized poikilocapnic rat resembles that known to be present during isocapnia. We conclude that hypocapnia neither hampers the hypoxic ventilatory reactivity nor alters the biphasic hypoxic ventilatory profile. These observations may aid planning experimental rat model studies.

**Key words:** acute hypoxia, carotid body, hypoxic ventilatory response, poikilocapnia, ventilatory profile

**INTRODUCTION**

Breathing depends on the central and peripheral neuroregulatory mechanisms. Hypoxia appears to activate both peripheral and central chemoreceptors (1, 2, 3). The ventilatory response to hypoxia may thus be used as a functional test for neural respiratory mechanisms. The response to a steady-state type of hypoxic
exposure encompasses both the stimulatory phase, transmitted by peripheral chemoreceptors, and the depressant phase, generated by a hypoxemic brain (4, 5). The two phases are apparent in conscious animals, but are even better expressed in the state of anesthesia (6).

The ventilatory response to hypoxia is usually investigated in controlled isocapnic conditions. Such conditions, however, do not take place in clinical hypoxic situations, such as critical care settings, in which the initial hypoxic hyperventilation leads to CO$_2$ washout. Hypocapnia can limit ventilatory stimulation (7), which can accelerate the hypoxic ventilatory decline, particularly in the state of anesthesia. Anesthesia is a respiratory depressant that slows breathing (8). It affects neuronal membrane excitability of neurons that generate the respiratory pattern, interacts in the reciprocal synaptic inhibition and stimulation of functionally antithetic neuronal pools, and interferes with the central neurotransmitters involved in respiratory regulation (9, 10). In the present study we thought it worthwhile to examine the ventilatory responses to inhalation of a hypoxic gas in the anesthetized, spontaneously breathing, and poikilocapnic rats. We wished to determine whether such responses differed from those known for the isocapnic condition. We found that the poikilocapnic hypoxic ventilatory profile resembles that present during isocapnia. Therefore, hypocapnia does not seem to be a major factor hampering the hypoxic ventilatory reactivity. These observations may be useful for planning experimental studies in the rat model.

MATERIAL AND METHODS

Data describing the time course of hypoxic ventilatory profiles were collected from 24 rats of either sex, weighing 269.8 ±10.2 g (SE). The animals were anesthetized with α-chloralose and urethane (35 mg and 800 mg/kg, ip), placed supine, tracheostomized, vagotomized, and spontaneously breathing. The experiment was conducted within an hour from induction of anesthesia. Since the anesthesia used was long-acting, no supplementary doses were required. Stability of the anesthesia level was monitored by applying noxious stimuli, such as pinching a paw. No hind limb withdrawal reflex or blood pressure response was noted. Rectal temperature was kept at 38°C with an external heating pad. The protocol of the study was approved by a local Ethics Committee.

The rats breathed through a two-way valve. The protocol consisted of a period of stabilization and measurement of ventilation on air and then exposing the rat to a hypoxic gas mixture introduced into the inlet port of the valve. The mixture contained 12% O$_2$ (balance N$_2$). The test lasted 2.5 min and CO$_2$ was allowed to run free. Ventilation was assessed from the electromyographic (EMG) activity of the diaphragm. This activity was recorded with two unipolar electrodes inserted into the costal diaphragm. The EMG signal was amplified and band-pass filtered at 50-5000 Hz, rectified and integrated with a time constant of 70 ms to obtain moving time averages (Digitimer-Neurolog System, Welwyn Garden City, UK). Expiratory gas was sampled from the trachea for the end-tidal PCO$_2$ measurement with an infrared analyzer (Gambr Engström AB, Sweden). All these signals along with arterial blood pressure were recorded on a polygraph (Honeywell-Omnilight 8M36, NEC San-ei Instruments, Tokyo, Japan). In addition, arterial blood samples were withdrawn for measurements of blood gas content and pH during change of inspired gas (AVL Compact 2 Blood Gas assembly, Graz, Austria).
Ventilatory variables included the breathing frequency (f, breaths/min), estimated from the number of respiratory cycle volleys of diaphragm activity, the volume of breathing (V, arbitrary units), indexed from the peak height of integrated diaphragm activity, and the minute diaphragm output calculated as the product of peak height and frequency, taken as an index of minute ventilation (MV). Data are given as mean ±SE percentage changes from the baseline level, presented as 100%. The results were analyzed using one-way analysis of variance followed by the Scheffe test for repeated measures. Significance was accepted at P<0.05.

RESULTS

The average hypoxic profiles of the minute ventilatory output and its constituent frequency and volume components are depicted in Fig. 1. The ventilatory response was a biphasic response. Ventilation increased briskly and peaked at 0.5 min. The mean minute diaphragm output reached 157.3 ±6.0% of the baseline level. High ventilation started then a steady decline toward the baseline, falling, on the average, to 133.5 ±5.3% of baseline at 2.5 min. Thus, ventilation receded 42% from its peak increase. Both frequency and volume components contributed to the ventilatory increase in hypoxia, but the frequency contribution predominated. Frequency increased at 0.5 min to 136.1 ±4.3% of baseline compared with the 117.1 ±2.2% increase in diaphragmatic amplitude. Once increased, frequency was held at a relatively steady level throughout the hypoxic exposure, decreasing to a mere 129.7 ±3.9% of baseline at test end, which made the ventilatory roll-off gradual. The roll-off was mostly driven by a decline in the volume component that returned to a near baseline level. Changes in minute ventilation and frequency were significantly different from the baseline level at all time points of the hypoxic course and the ending value for ventilation at 2.5 min was different from those at 0.5 min through 1.5 min (P<0.007). Changes in volume were significant for up to 2.0 min into hypoxia and the test ending volume value at 2.5 min was different from those at 0.5 min through 1.5 min (P<0.006 for both; one-way ANOVA and Sheffe's test).

Respiratory changes during hypoxia were accompanied by arterial blood-gas and blood pressure changes. The mean values of arterial PO$_2$, PCO$_2$, and pH are presented in Table 1. At baseline, the PaO$_2$ amounted to 89.9 ± 1.8 mmHg and decreased due to hypoxia down to 47.5 ±1.0 mmHg. Since the hypoxic tests were poikilocapnic, hypoxic hyperventilation was accompanied by hypocapnia and a corresponding alkaline shift in pH. All these changes were highly significant (P<10$^{-7}$; paired t-test). The mean arterial blood pressure decreased from the mean of 12.5 ± 0.4 kPa at baseline to 10.2 ± 0.4 kPa during the peak and down to 6.6 ± 0.4 kPa during the nadir of the respiratory hypoxic changes. These values differed from one another (P<3x10$^{-5}$).

![Fig. 1. Hypoxic response profiles of minute ventilation (MV, closed circles), frequency (f, target circles), and volume (V, open circles) components. Hypoxia was steady-state, poikilocapnic. Values are mean ±SE percentage changes from the baseline level marked at 100% with a dotted line. See text for description and statistical comparison.](image)
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<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Hypoxia</th>
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<tbody>
<tr>
<td>PaO$_2$ (mmHg)</td>
<td>89.9 ±1.8</td>
<td>47.5 ±1.0</td>
</tr>
<tr>
<td>PaCO$_2$ (mmHg)</td>
<td>37.3 ±1.1</td>
<td>27.1 ±0.9</td>
</tr>
<tr>
<td>pHa</td>
<td>7.31 ±0.01</td>
<td>7.37 ±0.01</td>
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</table>

Values are means ±SD. *Significantly different from the baseline value at P<10$^{-7}$.

**DISCUSSION**

The purpose of this study was to characterize the ventilatory response to acute poikilocapnic hypoxia in the anesthetized spontaneously breathing rat. The results indicate that the response is biphasic, consisting of the initial stimulation followed by ventilatory fall-off. The response was primarily driven and upheld by the stimulation of breathing frequency with a smaller contribution from the volume component. Frequency stimulation is a feature of carotid body action, which mediates the ventilatory response to reductions in arterial oxygen tension in both unanesthetized humans (12) and anesthetized rodents (11). Even though in the present study the PaCO$_2$ was not kept constant, and as expected substantially decreased due to the initial hyperventilation, the response profile resembles that typically found in anesthetized rodents when isocapnia is maintained (13). This indicates that the hypoxic stimulus has priority at the chemoreceptor site and concurrent hypocapnia does not dramatically mitigate the response.

Neural structures that regulate respiration are sensitive to anesthetic agents. A mixture of α-chloralose and urethane is often used for experimental animals, as it is thought to impair respiration the least. Urethane, a major component of the mixture, has been found to affect the capability of the plasma membrane of respiratory neurons to generate depolarizing potentials by interfering with the main central neurotransmitter systems, such as the stimulatory glutamatergic or
nitrergic pathway and the inhibitory GABAergic one (10). This interference, however, does not focus on any single neurotransmitter. The delicate neurotransmitter balance is unchanged under this anesthetic mixture, which makes the potential depressant effect less likely (10).

Our rats were breathing spontaneously, so that the ventilatory responses were set by the peripheral and central regulatory mechanisms. The rats were vagotomized to avoid interferences from the possible stimulatory effect of hypocapnia on lung stretch receptors (14) or from hypoxia-induced changes in the end-expiratory lung volume (15), which can influence breathing pattern. Hypoxic exposure caused the arterial blood pressure to drop considerably, particularly in the latter depressant phase of the ventilatory response. Carotid chemoreceptor afferent discharge, which is excited by hypoxia and mediates the ventilatory response, is fairly insensitive to blood pressure decline at least down to the level of 6.58 kPa mmHg (16). The drop of blood pressure observed toward the end of hypoxic exposure in the present experiments was approaching that level, which could have had some effect on the chemoreceptor discharge and ventilation. However, arterial blood pressure decline, which is a feature of hypoxia, basically does not interfere with the ventilatory profile of acute hypoxic responses (17). The responses generated in the present study were thus mostly dependent on the nature of the chemosensing mechanisms and likely represented the attainable limit of ventilation in a given O\textsubscript{2}-CO\textsubscript{2} setting. We conclude that ventilatory stimulation and depression during systemic hypoxia are distinct phases in anesthetized naturally poikilocapnic rats and the expression of the two phases does not necessarily require permissive maintenance of the isocapnic condition.

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REFERENCES


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