Ventilatory responses to specific CNS hypoxia in sleeping dogs

AIDAN K. CURRAN, JOSHUA R. RODMAN, PETER R. EASTWOOD, KATHLEEN S. HENDERSON, JEROME A. DEMPSEY, AND CURTIS A. SMITH
The John Rankin Laboratory of Pulmonary Medicine, Department of Preventive Medicine, University of Wisconsin, Madison, Wisconsin 53705

Curran, A., Rodman, J., Eastwood, K., Henderson, K., Dempsey, J., and Smith, C. Ventilatory responses to specific CNS hypoxia in sleeping dogs. J. Appl. Physiol. 88: 1840–1852, 2000.—Our study was concerned with the effect of brain hypoxia on cardiorespiratory control in the sleeping dog. Eleven unanesthetized dogs were studied; seven were prepared for vascular isolation and extracorporeal perfusion of the carotid body to assess the effects of systemic [and, therefore, central nervous system (CNS)] hypoxia (arterial PaO₂ = 52, 45, and 38 Torr) in the presence of a normocapnic, normoxic, and normohydric carotid body during non-rapid eye movement sleep. A lack of ventilatory response to systemic boluses of sodium cyanide during carotid body perfusion demonstrated isolation of the perfused carotid body and lack of other significant peripheral chemosensitivity. Four additional dogs were carotid body denervated and exposed to whole body hypoxia for comparison. In the sleeping dog with an intact and perfused carotid body exposed to specific CNS hypoxia, we found the following. 1) CNS hypoxia for 5–25 min resulted in modest but significant hyperventilation and hypocapnia (minute ventilation increased 29 ± 7% at arterial PaO₂ = 38 Torr); carotid body-denervated dogs showed no ventilatory response to hypoxia. 2) The hyperventilation was caused by increased breathing frequency. 3) The hyperventilatory response developed rapidly (<30 s). 4) Most dogs maintained hyperventilation for up to 25 min of hypoxic exposure. 5) There were no significant changes in blood pressure or heart rate. We conclude that specific CNS hypoxia, in the presence of an intact carotid body maintained normoxic and normocapnic, does not depress and usually stimulates breathing during non-rapid eye movement sleep. The rapidity of the response suggests a chemoreflex mediated by hypoxia-sensitive respiratory-related neurons in the CNS.

carotid body; hypoxic depression; chemoreceptors; hypocapnia

OUR STUDY WAS CONCERNED with the effect of brain hypoxia on cardiorespiratory control in the sleeping dog. The effects of central nervous system (CNS) hypoxia are controversial; some of this controversy may result because the effects of CNS hypoxia appear to be critically dependent on the experimental preparation used. Hypoxic depression of ventilation has been clearly demonstrated in anesthetized animals that have been carotid body denervated, have been made hypoxicemic with CO (27, 28, 32), or have specific pontomedullary hypoxia (51). In contrast, when hypoxia was applied to carotid body-denervated awake animals, most studies reported that ventilation was unchanged or increased rather than depressed (4–6, 8, 13, 20, 22, 25, 35, 45). However, Miller and Tenney (29) did observe alveolar hypoventilation in response to hypoxia in unanesthetized, carotid body-denervated cats. In awake goats with carotid bodies maintained normoxic and normocapnic by means of extracorporeal perfusion, a marked ventilatory stimulation was observed in response to specific CNS normocapnic hypoxia (11).

On the basis of the limited data available in physiological preparations, the effects of CNS hypoxia on ventilatory control appear to be critically dependent on such key factors as state of consciousness, whether the carotid chemoreceptors are intact, and the duration of hypoxia (3). Our studies provide new information about the controversial problem of CNS hypoxic effects on ventilatory control, because 1) we used a preparation with an intact, as opposed to denervated, carotid body, 2) we studied the effects of CNS hypoxia in non-rapid eye movement (REM) sleep, and 3) we examined the cardiorespiratory responses to CNS hypoxia over a wide range of systemic hypoxia [arterial PaO₂ (Pao₂) = 35–55 Torr] and duration of hypoxic exposure (seconds to minutes).

METHODS

Seven mixed-breed female dogs (20–25 kg) were used in the study. Our protocol and methods were approved by the Animal Care and Use Committee of the University of Wisconsin, Madison.

Chronic Instrumentation

Two surgical sessions were required, separated by ≥2 wk. All surgery was performed under general anesthesia (~1% halothane in O2) with use of sterile technique. Appropriate pre- and postoperative medications were administered (antibiotics and analgesics).

In the first surgical session, we implanted a catheter in the abdominal aorta via a small branch of the right femoral artery. Fine-wire electrodes were sewn into the crural diaphragm via a small thoracotomy at T8–9. A five-lead electroencephalogram (EEG)/electrooculogram montage was installed subcutaneously over the cranium and near each orbit. All catheters and electrode leads were tunneled subcutaneously.
and exteriorized near the scapulae. Arterial catheters were filled with 10,000 U/ml heparin solution when not in use.

In the second session, we prepared one group of dogs for carotid body perfusion (Fig. 1) (48). We confirmed vascular isolation of the carotid sinus region by manually occluding the common carotid artery and external carotid artery and withdrawing blood via the cannulated lingual artery. The carotid sinus region would visibly collapse and remain collapsed if vascular isolation was successful. Catheters were tunneled subcutaneously and exteriorized in the dorsal aspect of the neck. Catheters were filled with 10,000 U/ml (arterial) or 1,000 U/ml (venous) heparin solution when not in use. The animals were allowed ≥24 h of recovery before studies began. No studies were performed until body temperature and breathing frequency were within normal limits for a given dog.

In a second group of dogs, we performed bilateral carotid body denervation by isolating both carotid sinus regions and stripping away the surrounding tissue. Successful denervation was confirmed after recovery (1–2 days post-denervation) by means of bolus injections of sodium cyanide.

**Carotid Body Perfusion**

Dogs lay unrestrained on a bed in an air-conditioned, sound-attenuated chamber. The extracorporeal perfusion circuit was primed with ~800 ml of saline, 120 ml of autologous blood, and 5,000 U of heparin (derived from beef lung; supplemented with 2,500 U/h). Pco₂, Po₂, and pH in the perfusion circuit were matched to a given dog’s eupneic values by adjustment of the gas concentrations supplying the circuit and by addition of NaHCO₃. The carotid sinus region was perfused at flow rates <100 ml/min, which raised the pressure in the sinus region by 5–10 Torr. Before data acquisition, a 30-min period of normal perfusion of the carotid sinus region was used to ensure uniformity between systemic and extracorporeal circuit blood.

**Measurements**

Ventilatory variables were obtained via a tight-fitting facemask connected to a heated pneumotachograph. Crural diaphragm electromyogram (EMG) signals were amplified and recorded as raw signals and as a moving time average (rectified; 100-ms time constant; CWE). One-milliliter arterial and circuit blood samples were obtained at regular intervals from the aortic catheter or circuit and analyzed for pH, Pco₂, and Po₂ on a blood-gas analyzer (model ABL-300, Radiometer). The blood-gas analyzer was validated daily with dog blood tonometered with three different combinations of Po₂ and Pco₂ covering the range encountered in the experiments. Samples were corrected to the dog’s rectal temperature and also for any tonometer corrections. Except during blood sampling, blood pressure was recorded continuously from the aortic catheter. Airway Po₂ and Pco₂ were recorded continuously from the facemask and analyzed by a mass spectrometer (model MGA-1100, Perkin-Elmer).

**Protocol**

Normocapnic, normoxic, and normohydric carotid body perfusion was maintained throughout each trial, thus maintaining normal conditions at the carotid body, despite systemic (“CNS hypoxia”) hypoxia. Each trial was performed during stable non-REM sleep (occasional brief state changes were encountered, but data from these periods were not used), and there were 6–14 trials per dog. Each carotid body perfusion trial consisted of a 5- to 10-min control period followed by 5–25 min of systemic hypoxia at one of three levels: mild (PaO₂ > 52 Torr), moderate (PaO₂ > 45 Torr), and severe (PaO₂ ≤ 38 Torr). Each dog underwent a similar protocol before the carotid sinus isolation surgery to provide the “whole body” hypoxic response data reported here (i.e., both carotid bodies and CNS were hypoxic).
The carotid body-denervated dogs were exposed to three levels of hypoxia similar to those described above for 5–10 min each during periods of wakefulness or non-REM sleep. At least 15 min elapsed between trials.

Data Analysis

Mean data were collected from 1-min segments of data for a given condition and time point. Statistical comparisons were made with Friedman's repeated-measures test on ranks combined with Dunnett's test for multiple comparisons with control. Changes were considered significant if \( P < 0.05 \).

RESULTS

Eupneic Control

Eupneic, air-breathing control measurements were obtained in each dog in two different conditions: 1) before surgical preparation for carotid body perfusion (i.e., both carotid bodies were intact) and 2) after the dogs had been surgically prepared for carotid body perfusion (i.e., with unilateral carotid body denervation and perfusion catheters in place; see Methods). In the second condition the dogs were carotid body perfused for \( \geq 5 \) min with blood that closely approximated their normal, non-carotid body-perfused eupneic values while breathing room air (carotid body \( \text{PCO}_2 = 38.4 \pm 1.3 \text{Torr} \), carotid body \( \text{PO}_2 = 99.7 \pm 3.9 \text{Torr} \), carotid body \( \text{pH} = 7.386 \pm 0.02 \)). In both conditions, measurements were obtained after \( \geq 5 \) min of air breathing during non-REM sleep before each trial of systemic hypoxia induced via reduced inspired \( \text{O}_2 \) fraction. In both types of eupnea, typical canine arterial blood gases and \( \text{pH} \) and stable ventilatory patterns were observed (Table 1), although the postoperative controls showed a mild hyperpnea due to increased breathing frequency and tidal volume (VT). All dogs manifested this mild hyperpnea (but not hyperventilation) postoperatively, probably because of the slightly elevated body temperatures (0.2 ± 0.2°C) encountered in the postoperative period. We showed previously (47) that carotid body perfusion per se has no effect on ventilation when perfusate blood gases and \( \text{pH} \) values are matched to eupneic values.

Transient Ventilatory Responses to Hypoxia

Whole body hypoxia. We use data from our most severe level of hypoxia (\( \text{PAO}_2 = 37.5 \pm 0.8 \text{Torr} \)) to illustrate the transition from air breathing to hypoxia (Fig. 2). All dogs responded rapidly to hypoxia; inspired minute ventilation (\( \dot{V} \text{I} \)) and VT increased significantly and end-tidal \( \text{PCO}_2 \) (\( \text{PETCO}_2 \)) decreased significantly by 20–40 s of hypoxic exposure \( (P < 0.05) \). Breathing frequency also tended to increase slightly, particularly after \( \sim 40 \) s of hypoxic exposure, but did not achieve statistical significance. Occasional brief episodes of changes in sleep state occurred in some dogs during these trials; data from these periods were not used.

The time course of the ventilatory responses obtained during the transitions from air breathing to mild or moderate hypoxia was similar to that obtained with severe hypoxia, but the responses were smaller in magnitude and usually did not achieve statistical significance within the first 2 min of hypoxic exposure. We illustrate this point with the \( \text{PETCO}_2 \) data for these conditions in Fig. 3.

CNS hypoxia. We use a polygraph record (Fig. 4) and data from our most severe level of hypoxia (\( \text{PAO}_2 = 37.9 \pm 2.7 \text{Torr} \); Fig. 2) to illustrate the transition from air breathing to hypoxia. All dogs responded rapidly to hypoxia; \( \text{PETCO}_2 \) decreased significantly by 20–40 s of hypoxic exposure, and breathing frequency increased significantly by 40–60 s of hypoxic exposure. Breath-by-breath \( \dot{V} \text{I} \) was clearly tending to increase after 20–40 s of exposure to hypoxia in most dogs but did not quite achieve statistical significance \( (P < 0.072) \). Unlike the situation in whole body hypoxia, the early increase in ventilation was mediated exclusively by increased breathing frequency, inasmuch as there was no significant change or even a discernible trend in VT for at least the first 2 min of hypoxic exposure in all dogs.

<table>
<thead>
<tr>
<th>Table 1. Control values for whole body and CNS hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><strong>Whole body hypoxia</strong></td>
</tr>
<tr>
<td>Premild hypoxia control</td>
</tr>
<tr>
<td>± 0.007</td>
</tr>
<tr>
<td>Premoderate hypoxia control</td>
</tr>
<tr>
<td>± 0.007</td>
</tr>
<tr>
<td>Presevere hypoxia control</td>
</tr>
<tr>
<td>± 0.007</td>
</tr>
<tr>
<td><strong>CNS hypoxia</strong></td>
</tr>
<tr>
<td>Premild hypoxia control</td>
</tr>
<tr>
<td>± 0.015</td>
</tr>
<tr>
<td>Premoderate hypoxia control</td>
</tr>
<tr>
<td>± 0.012</td>
</tr>
<tr>
<td>Presevere hypoxia control</td>
</tr>
<tr>
<td>± 0.010</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of dogs. CNS, central nervous system; \( \text{PaO}_2 \), arterial \( \text{PO}_2 \); \( \text{PaCO}_2 \), arterial \( \text{PCO}_2 \); [\( \text{HCO}_3^- \)], \( \text{HCO}_3^- \) concentration; \( \text{T}_1 \), inspiratory duration; \( \text{T}_e \), expiratory duration; \( \dot{V} \), breathing frequency; \( \dot{V} \text{I} \), inspired minute ventilation; \( \dot{V} \text{I} / \text{Ti} \), inspiratory duty cycle; VT, tidal volume; \( \text{Ti} / \text{TT} \), respiratory cycle duration.
Occasional brief episodes of changes in sleep state occurred in some dogs during these trials; data from these periods were not used.

The time course of the ventilatory responses obtained during the transitions from air breathing to mild or moderate hypoxia was similar to the observations obtained with severe hypoxia, but the responses were smaller in magnitude and usually did not achieve statistical significance within the first 2 min of hypoxic exposure. We illustrate this point with the PETCO2 data for these conditions in Fig. 3.

Steady-State Responses (5 min)

Whole body hypoxia. Figure 5 summarizes the minute-by-minute ventilatory responses to 5 min of whole body hypoxia. Whole body hypoxia increased ventilation in a graded way as the severity of hypoxia increased, reaching a steady state by the 2nd min of hypoxic exposure. This increased Vt was due to increases in breathing frequency and Vr and inspiratory duty cycle (Vr/Ti) as inspiratory and expiratory time (Ti and Te, respectively) decreased. The rate of rise of the moving time

Fig. 2. Transient responses to severe whole body hypoxia (A) and severe central nervous system (CNS) hypoxia (B) (arterial Po2 (Pao2) = 38 ± 0.8 and 38 ± 1.2 Torr, respectively) during non-rapid eye movement (non-REM) sleep. Each line represents breath-by-breath data from 1 dog. Time 0 (vertical dashed line), point at which end-tidal PO2 fell below 60 Torr. Horizontal dashed line, no change from normoxic control values. Gaps in lines represent excluded data due to brief state changes. Note rapid onset of ventilatory response in whole body and CNS hypoxia. During first 120 s, ventilatory response to CNS hypoxia is mediated entirely by increased breathing frequency (f). PETCO2, end-tidal PCO2; Vt, inspired minute ventilation; Vr, tidal volume.
average of the crural diaphragm EMG increased in about two-thirds of all trials. The increased ventilation was a true hyperventilation, inasmuch as PETCO2 decreased progressively in all dogs as PaO2 decreased.

CNS hypoxia. Figure 5 summarizes the minute-by-minute ventilatory responses to 5 min of CNS hypoxia. CNS hypoxia increased ventilation in a graded way as the severity of hypoxia increased, although the magnitude of the changes was smaller than that observed during whole body hypoxia. The time course of response was similar, reaching a steady state by the 2nd min of hypoxic exposure. This increased Vl was attributable

Fig. 3. Transient whole body (A and C) and CNS (B and D) ΔPETCO2 responses to mild and moderate hypoxia at PaO2 = 52 ± 0.6 and 45 ± 0.7 Torr (A and C, respectively) and 52 ± 1 and 44 ± 0.6 Torr (B and D, respectively) during non-REM sleep. Each line represents breath-by-breath data from 1 dog. Time 0, point at which PETO2 fell below 60 Torr. Horizontal dashed line, no change. Although responses are smaller in magnitude than those of severe hypoxia, time course of hyperventilation is similar.

Fig. 4. Polygraph record of air breathing-to-hypoxia transition in a dog in which carotid bodies were maintained normocapnic, normoxic, and normohydric via perfusion. Relative to control, between 20 and 40 s of CNS hypoxic exposure (starting from point at which PETO2 ≤ 60 Torr), breathing frequency increased 2 breaths/min, PETCO2 decreased 2 Torr, heart rate increased 11 beats/min, and mean arterial blood pressure increased 6 Torr. EMGd, diaphragmatic electromyogram; BP, blood pressure; EOG, electrooculogram; EEG, electroencephalogram.
Fig. 5. Minute-by-minute means of PETCO₂, V̇i, V̇r, inspiratory duty cycle (V̇r/TI), and breathing frequency for mild (PaO₂ = 52 ± 0.6 Torr) and severe (PaO₂ = 38 ± 0.8 Torr) whole body hypoxia (A; n = 7) and mild (PaO₂ = 52 ± 1 Torr) and severe (PaO₂ = 38 ± 1 Torr) CNS hypoxia (B; n = 5). Moderate hypoxia is not shown because some 2- to 4-min points could not be used due to brief EEG arousals. Hyperventilatory responses to whole body hypoxia were progressive with severity of hypoxia and due to increased V̇r and breathing frequency; responses to CNS hypoxia, although similar in time course, were smaller in magnitude and due entirely to increased breathing frequency. *Significantly different from control (P < 0.05).
entirely to increases in breathing frequency mostly as a result of decreases in $T_e$ and lesser decreases in $T_i$. $V_t$ was unchanged or tended to decrease, so neither $V_t/T_i$ nor the rate of rise of the crural diaphragm EMG changed significantly. The increased ventilation was a true hyperventilation, inasmuch as $P_{ET}$ decreased progressively with severity of hypoxia in all dogs.

Prolonged Hypoxia (10–25 min)

Whole body hypoxia. Four of the seven dogs were exposed to our most severe level of whole body hypoxia ($P_{A,O_2} = 37.5 \pm 0.8$ Torr) for up to 15–25 min. Occasional brief EEG arousals occurred, but the steady-state data shown in Fig. 6 were obtained in stable non-REM sleep. Arterial $PCO_2$ ($P_{A,CO_2}$) tended to reach a plateau or continued to decrease slightly after 5 min of hypoxia. $V_t$ and breathing frequency were more variable, but the net result was $V_i$ that reached a plateau by 5 min of hypoxic exposure or decreased slightly relative to the 5-min point.

CNS hypoxia. The same four dogs were exposed to our most severe level of whole body hypoxia ($P_{A,O_2} =$...
37.9 ± 1.2 Torr) for up to 15–25 min while the carotid bodies were maintained normoxic, normocapnic, and normohydric via perfusion. Occasional brief EEG arousals occurred, but the steady-state data shown in Fig. 6 were obtained in stable non-REM sleep. PaCO₂ decreased in three of the four dogs and reached plateaus by 5 min of hypoxic exposure. One dog progressively retained CO₂ between 5 and 15 min of hypoxic exposure. PaCO₂ changes were generally reflected in changes in V̇I. Vt and breathing frequency were more variable, but increased breathing frequency was usually the dominant component of the increased V̇I.

Blood Pressure and Heart Rate Responses to Whole Body and CNS Hypoxia

Whole body hypoxia. Figure 7 shows minute-by-minute means of mean arterial blood pressure and heart rate during exposure to our most severe level of hypoxia (PaO₂ = 37.5 ± 0.8 Torr). Mean arterial blood pressure and heart rate increased progressively up to 4 min of hypoxic exposure.

CNS hypoxia. Figure 7 shows minute-by-minute means of mean arterial blood pressure and heart rate during exposure to our most severe level of hypoxia (PaO₂ = 38 ± 0.8 and 38.9 ± 1.2 Torr). The control values were elevated relative to whole body controls. There were no significant changes in mean arterial blood pressure or heart rate in the first 3 min of hypoxic exposure, although there was a slight trend toward increased heart rate.

Hypoxia and Carotid Body Denervation

During non-REM sleep or wakefulness in four unanesthetized, carotid body-denervated dogs, there was no overall ventilatory response (i.e., no change in PaCO₂ or V̇I) to a wide range of hypoxia (PaO₂ = 30–55 Torr; Fig. 8). However, in all four dogs, breathing frequency
DISCUSSION

In summary, we have found that 5–25 min of exposure to specific CNS hypoxia (PaO2 = 35–55 Torr) during non-REM sleep in dogs did not depress ventilation; rather, there was significant hyperventilation. The hyperventilation was mediated entirely by increased breathing frequency, unlike the larger hyperventilation observed during whole body hypoxia, which was mediated by increases in breathing frequency and VT. Initiation of the ventilatory response to CNS hypoxia was rapid: the time course was comparable to that observed during whole body hypoxia. Most dogs maintained hyperventilation for up to 15–25 min of CNS hypoxia; only one of the four dogs studied over the longer term manifested hypoxic ventilatory depression. Blood pressure and heart rate did not change significantly in response to CNS hypoxia.

We conclude that specific CNS hypoxia, when the carotid bodies are intact and normoxic and normocapnic, usually stimulates breathing. The rapidity of the response suggests a chemoreflex mediated by hypoxia-sensitive respiratory-related neurons in the CNS.

Limitations of the Study

Adequacy of carotid body isolation. A crucial assumption in our study is that the remaining carotid body is completely isolated from the systemic circulation when it is perfused and, therefore, cannot contribute to the observed responses to arterial hypoxemia. A corollary of this assumption is that the intact aortic bodies (or any other putative peripheral chemosensors) have a negligible effect on ventilation and blood pressure in the range of hypoxia we used. We believe these are reasonable assumptions, because large boluses of intravenous sodium cyanide, known to elicit ventilatory responses much larger than any we observed during CNS hypoxia, failed to produce any ventilatory or blood pressure responses when given while the carotid body was perfused (see METHODS). In addition, carotid body-denervated dogs (with intact aortic chemoreceptors) showed no ventilatory responses to the same doses of sodium cyanide, which would seem to rule out a significant contribution from aortic chemoreceptors. Also, the ventilatory responses observed during CNS hypoxia were qualitatively different from those observed during whole body hypoxia (increased breathing frequency only vs. mostly increased VT), suggesting that different sets of receptors were stimulated.

Is brain blood flow compromised during carotid body perfusion? Our technique requires temporary occlusion of the external carotid artery and ligation of several arterial branches in the carotid sinus region. It is well known that the normal response of brain vasculature to hypoxia is a vasodilatation leading to increased cerebral blood flow, which in turn results in CNS alkalosis and, presumably, reduced stimulation of medullary chemoreceptors. In fact, this is one of the mechanisms proposed to explain ventilatory depression in response to CNS hypoxia (31). We presume that this increased cerebral blood flow also occurred in our studies of CNS hypoxia, at least when PaO2 was <50 Torr, but we cannot be sure that this increase in cerebral blood flow does indeed occur in our preparation with potentially compromised cerebral blood flow. If this mechanism of increased cerebral blood flow/CNS hypocapnia was reduced in our preparation, then the net effect of any direct hypoxic CNS stimulation and inhibition due to hypocapnia would be biased against ventilatory depression. However, the absence of increased cerebral blood flow would not explain the ventilatory stimulation we showed with CNS hypoxia. We do not believe that brain blood flow is compromised in our preparation because of the extensive collateral circulation in the canine brain (12) and the fact that basilar artery flow in the dog has been shown to increase more than threefold with acute occlusion of both common carotid arteries (44). However, there is also evidence to suggest that the external carotid artery carries a significant amount of the total cerebral blood flow, possibly as much as 40% of the amount supplied by the internal carotid artery (23).

To our knowledge, the functional significance of ipsilateral occlusion of the internal and external carotids is unknown.

Poikilocapnia. We let PaCO2 change spontaneously with breathing; usually this resulted in hypocapnia. Consequently, we did not examine a pure effect of CNS hypoxia but, rather, the combination of hypoxia and hypocapnia. This implies that we probably underestimated the ventilatory response to CNS hypoxia, and this is supported by contrasting the present findings with the 70% increase in ventilation in the carotid body-perfused awake goat exposed to CNS hypoxia but with systemic isocapnia maintained (11). We used poikilocapnia, because we wished to avoid the problem of even very small errors in PaCO2, while we were attempting to maintain isocapnia, which might have caused central chemoreceptor-induced ventilatory stimulation. This approach also allowed us to determine the precise onset and time course of the ventilatory response from the initiation of systemic hypoxia. Nevertheless, we recognize that our ventilatory responses beyond the first few breaths of hypoxia represent the net effect of hypoxic stimulation and hypocapnic inhibition. It would have been ideal to use isocapnic and hypocapnic CNS hypoxia. However, our preparation had a finite life, permitting only a limited number of trials, so we chose to do repeated poikilocapnic trials across a range of PaO2.

Comparison With Other Models Used to Study Ventilatory Effects of Central Hypoxia

Previous studies have used three basic approaches to address the question of ventilatory responses to CNS hypoxia. Although they were useful, each approach had certain limitations that we hoped to avoid.

Anesthetized models. Depressive effects of CNS hypoxia on ventilation are seen consistently in anesthetized preparations, usually with CO-induced hypoxemia and with PaCO2 and blood pressure controlled (27,
tages of our preparation compared with other prepara-

than depression. Animals generally causes ventilatory stimulation rather 

CNS hypoxia in unanesthetized, carotid body-intact 

present study are consistent with this idea, i.e., that 

termed potentiation after abrupt removal of hypoxic 

systemic circulation (including the brain) was made 

able to keep the carotid bodies intact but isolated from 

the systemic circulation. 

Intact but vascularity isolated and perfused carotid 

bodies. Intact, awake goats with extracorporeal perfu-

sion of the vascularity isolated carotid body, in which 

brain and carotid body O2, CO2, and pH were controlled 

independently, manifested clear hyperpnea when the 

systemic circulation (including the brain) was made 

hypoxic (isocapnia maintained) and the carotid body 

was maintained normoxic and normocapnic (11). Not 

only was there a hyperpnea, but there was no effect of 

CNS hypoxia on the ventilatory manifestation of short-

term potentiation after abrupt removal of hypoxic 

carotid chemoreceptor stimulation, an effect that had 

been thought to be quite labile to CNS hypoxia (2, 14). 

Despite the fact that the foregoing study was done 

during wakefulness and isocapnia, the results of the 

present study are consistent with this idea, i.e., that 

CNS hypoxia in unanesthetized, carotid body-intact 

animals generally causes ventilatory stimulation rather 

than depression.

In summary, we believe there are three major advan-

tions reported in the literature. The first advantage is 
lack of anesthesia. Anesthetized preparations consist-
tently depress ventilation in response to CNS hypoxia; 
unanesthetized preparations do not. Another advan-
tage is intact carotid bodies. Preparations with one 

intact carotid body maintain some low level of tonic 

chemoreceptor input to the respiratory controller, and 

the intact connections appear to prevent central remod-
eling that may occur in response to denervation. Fi-

ally, we believe that sleep is an advantage, in that 

non-REM sleep eliminates behavioral responses unre-

lated to the ventilatory effects of CNS hypoxia. Fur-

more, given the marked qualitative differences in the 

response to CNS hypoxia between sleep and anesthe-
sia, it is clear that extrapolations from the anesthetized 
to the unanesthetized but sleeping animal are unwar-

CNS Hypoxia: Implications for Respiratory 

and Cardiovascular Control in Sleep 

and in Sustained Hypoxia

Non-REM sleep is a physiological state in which 
baseline respiratory motor output is reduced, as are the 
ventilatory responses to hypoxia and hypercapnia. Fur-

thermore, ventilatory depression and even apnea can 
occur in non-REM sleep with even very small decreases 
in PaCO2 (40). It has also been suggested that the 
periodic breathing during sleep in hypoxia might be 
attributed in part to the depressive effects of CNS 
hypoxia, especially on the ventilatory manifestation of 
short-term potentiation (2, 14), a known stabilizer of 
breathing. Our data do not confirm this concept, be-
cause ventilation increased with CNS hypoxia, and this 
increase was sustained for many minutes, even in the 
presence of mild systemic hypocapnia during non-REM 
sleep. Rather, as in the awake, carotid body-perfused 
goat (11), our data would suggest that CNS and carotid 
body hypoxic stimulation contribute to the elevated 
ventilation normally attending hypoxia during sleep 
(or wakefulness). Our studies were not sufficiently 
comprehensive to permit a quantitative comparison of 
CNS hypoxia effects between wakefulness and sleep. 
The mechanisms mediating the increased blood pressure 
and heart rate observed in response to hypoxia in the 
intact animal are complex (7). The pressor response is 
attributed to increased sympathetic vasoconstrictor 
effects, which in turn are known to be mediated by 
carotid body stimulation (7) and by CNS hypoxia (49). 
In CNS hypoxia in our sleeping dogs, we observed a 
slight tendency for blood pressure and heart rate to 
increase, but this response was highly variable. We 
found this surprising in view of the clear sympathetic 
excitation elicited by brain hypoxia in the anesthetized 
rat (48). It is also important to emphasize that we 
observed no tendency toward depression of blood pres-
ure and heart rate with CNS hypoxia. We did not 
measure blood flow or vascular resistance, and these 
would be important to know to demonstrate whether 
our unanesthetized, intact preparation showed a symp-
pathetically mediated vasoconstriction similar to that 
seen with CNS hypoxia in anesthetized rats (42).
A time-dependent ventilatory response to hypoxia has been described whereby the response peaks in the first few minutes and then gradually declines toward control values (24). Furthermore, the ventilatory response to acute hypoxia does not return for a substantial time period after the sustained hypoxic exposure (24). An effect of sustained hypoxia causing CNS depression of ventilation has been proposed to explain this hypoxic ventilatory decline ("roll-off") (31). However, our data in CNS hypoxia sustained for up to 25 min showed a persistent hyperventilatory response in three of the four dogs tested, even in the face of a reduction in $P_{aO_2}$ (Fig. 6). These data in sleep confirm the persistent hyperventilation seen over several minutes of sustained CNS hypoxia in the awake goat with intact perfused carotid bodies that were maintained normoxic and normocapnic (11, 46). Accordingly, we favor the explanation that the source of hypoxic ventilatory decline in prolonged hypoxia may be the time-dependent central inhibitory effects of sustained carotid sinus nerve stimulation rather than CNS hypoxic depression of ventilation per se (3). Indirect evidence in support of this claim is the absence of any time-dependent hypoxic ventilatory decline in carotid body-denervated animals (25) and the increased release of dopamine in the nucleus tractus solitarius with sustained stimulation of carotid sinus nerve afferents (15). However, a ventilatory depression was observed during long-term hypoxic exposure in one dog in the present study. An effect of the duration of CNS hypoxic exposure and/or a threshold for CNS hypoxic depression (which might vary between individuals and species) cannot be excluded.

Mechanism of Ventilatory Stimulation by CNS Hypoxia

Hypoxia-sensitive CNS neurons require tonic carotid body afferent input. The hyperventilation observed in response to CNS hypoxia in this study and the rapid time course of response suggest a central $O_2$-sensitive chemosensor-like mechanism. Because the carotid bodies cannot be directly involved, this must mean that CNS neurons were responding to the hypoxia to mediate the ventilatory and cardiovascular responses. Given the different responses to CNS hypoxia between intact and carotid body-denervated animals, we speculate that tonic, low-level carotid body afferent input to the CNS is required for the CNS-mediated hyperventilatory response to hypoxia. One approach to test this hypothesis would be to assess central hypoxic responses while carotid chemoreceptor output was minimized with a hyperoxic local perfusion.

A role for sympathetic efferents? In our experimental model with an intact carotid body, it is important to note that sympathetic efferent activity stimulated by CNS hypoxia (48) could potentially modulate carotid body sensitivity by altering blood flow through the carotid body or by direct effects on the carotid chemoreceptor type I cells. This might explain an increase in ventilation, even in the face of a carotid body maintained normoxic and normocapnic via perfusion. However, the role of carotid sinus nerve sympathetic efferents, if any, is controversial. Electrical stimulation of sympathetic fibers inhibits (36) or augments (30, 36) carotid body output. Some found an augmentation of the carotid body hypoxic response when sympathetics to the carotid body were cut (16, 21, 41), whereas others found no significant effect on the ventilatory responses to acute or chronic hypoxia in awake (43) and anesthetized (9, 26) preparations. Furthermore, we observed that CNS hypoxia caused hyperventilation exclusively by increased breathing frequency, whereas hypoxic carotid body stimulation increased $V_t$ and breathing frequency (11, 46). Regardless of whether sympathetics have a role in the response to specific CNS hypoxia, it seems clear to us that the hypoxia is sensed initially by the CNS.

A role for CNS lactacidosis? Another possible mechanism of the ventilatory response to CNS hypoxia is the elaboration of lactic acid by the brain. It is known that acidification occurs on the medullary surface when systemic hypoxia is induced (18, 32). Arguing against this mechanism is the fact that brain lactacidosis would presumably also occur in the carotid body-denervated preparation, yet no hyperventilatory response was observed under these conditions. Also, the time course of acidification does not correlate with the ventilatory measurements observed in the present study; medullary surface acidification required 1.5–2 min for the initial response, whereas ventilation in the present study began to change within 20 s of hypoxic exposure (32, 52).

It is also not clear whether CNS lactacidosis is in fact a ventilatory stimulus. Systemic pretreatment with dichloroacetate (DCA), a blocker of lactic acid production, enhanced the acute hyperventilatory response to hypoxia (1) in awake goats. Similar findings were obtained in the anesthetized cat (33); systemic DCA treatment prevented the hypoxia-mediated fall in medullary surface pH, yet phrenic activity was maintained until extremely low arterial $O_2$ contents were reached. However, when applied topically to the surface of the ventrolateral medulla in anesthetized cats, DCA again prevented the hypoxia-mediated fall in medullary surface pH, but the time course and magnitude of the decrease in phrenic activity were essentially identical to the pretreatment response (33). Taken together, these data suggest that hypoxia-induced CNS lactacidosis is depressant to ventilation or has no role in ventilatory control.

A role for higher centers? The fact that the hyperventilatory response to CNS hypoxia in our animals consisted of an increase in breathing frequency with maintained $V_t$ might implicate an indirect role for supramedullary structures, as proposed some time ago by Tenney and colleagues (29, 38, 50). These authors also observed a tachypneic response to various levels of hypoxia in awake carotid body-denervated cats; however, unlike the hyperventilatory response in our carotid body-intact dogs, they showed a reduced $V_t$ and $V_l$ with $CO_2$ retention. Their subsequent studies in decerebrate and decorticate animals led them to postu-
late that this tachypneic response was due to CNS hypoxic depression of cortical structures, which in turn would remove the inhibitory influence normally exerted by the cortex on "rate-facilitating" neurons in the diencephalon, thereby facilitating the hypoxia-induced increase in breathing frequency. This mechanism might be especially important in non-REM sleep, during which higher CNS structures may be more susceptible to depression by hypoxia.

Our data do not permit us to say whether the hyperventilation we observed is due to a true chemoreflex or a hitherto unappreciated nonspecific effect of CNS hypoxia. In any case, it has been demonstrated that many respiratory- or cardiovascular-related neurons in the medulla or hypothalamus depolarize in response to hypoxia (10, 17, 19, 34, 42, 48, 49). The fact that these neurons do depolarize in response to physiologic levels of hypoxia suggests a potential means of transduction of CNS hypoxia to enhanced neural respiratory motor output. On the other hand, many of these same studies have shown that other medullary neurons hyperpolarize in response to hypoxia. If indeed there is a direct effect of hypoxia on these medullary or hypothalamic neurons to cause the observed hyperventilatory response to CNS hypoxia, then we must conclude that the net effect is weighted in favor of excitation, even in the sleeping state, where we would expect the baseline (normoxic) activity of medullary neurons to be depressed relative to the state of wakefulness (37).

The technical assistance of Maria Zayas and Andrew Neeb is gratefully acknowledged.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-50531 and HL-07654.

Address for reprint requests and other correspondence: C. A. Smith, 504 N. Walnut St., Madison, WI 53705-2368 (E-mail: casmith4@facstaff.wisc.edu).

Received 7 June 1999; accepted in final form 11 December 1999.

REFERENCES