Time-dependent modulation of carotid body afferent activity during and after intermittent hypoxia

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Cummings, Kevin J., and Richard J. A. Wilson. Time-dependent modulation of carotid body afferent activity during and after intermittent hypoxia. Am J Physiol Regul Integr Comp Physiol 288: R1571–R1580, 2005. First published January 27, 2005; doi:10.1152/ajpregu.00788.2004.—The ventilatory response to several minutes of hypoxia consists of various time-dependent phenomena, some of which occur during hypoxia (e.g., short-term depression), whereas others appear on return to normoxia (e.g., posthypoxic frequency decline). Additional phenomena can be elicited by acute, intermittent hypoxia (e.g., progressive augmentation, long-term facilitation). Current data suggest that these phenomena originate centrally. We tested the hypothesis that carotid body afferent activity undergoes time-dependent modulation, consistent with a direct role in these ventilatory phenomena. Using an in vitro rat carotid body preparation, we found that 1) afferent activity declined during the first 5 min of severe (40 Torr PO2), moderate (60 Torr PO2), or mild (80 Torr PO2) hypoxia; 2) after return to normoxia (100 Torr PO2) and after several minutes of moderate or severe hypoxia, afferent activity was transiently reduced compared with prehypoxic levels; and 3) with successive 5-min bouts of mild, moderate, or severe hypoxia, afferent activity during bouts increased progressively. We call these phenomena sensory hypoxic decline, sensory posthypoxic decline, and sensory long-term facilitation. These results suggest that time-dependent changes in carotid body activity, at least under some circumstances, may act in tandem with central mechanisms previously implicated in ventilatory LTF.

The purpose of this study was to investigate whether the responses of the carotid body to bouts of hypoxia include time-dependent changes in activity consistent with playing a direct and more general role in shaping the hypoxic ventilatory response. Using an arterially perfused in vitro rat carotid body preparation, we examined afferent activity in the carotid sinus nerve (CSN) in response to multiple bouts of hypoxia and hypercapnia. We report that, during and after several minutes of moderate or severe hypoxia, carotid body afferent activity declines in a manner resembling hypoxic ventilatory phenomena. Hypercapnia failed to produce equivalent phenomena. However, bouts of either 50 Torr hypercapnia or hypocapnic hypoxia resulted in sensory long-term facilitation (sLTF). These results suggest that time-dependent changes in carotid body activity, at least under some circumstances, may act in tandem with central mechanisms previously implicated in ventilatory LTF.

MATERIALS AND METHODS

Male Sprague-Dawley rats (n = 78; 150–250 g, 4–6 wk old) were used for experiments. Experimental procedures were approved by the University of Calgary Animal Care Committee and were in accordance with national guidelines.

Arterially Perfused, In Vitro Carotid Body Preparation

Rats were treated with heparin (1,000 U/animal ip) 30 min before dissection. Immediately before dissection, animals were anesthetized...
deeply in halothane until respiration ceased and animals failed to respond to noxious paw pinch. Animals were then transected below the diaphragm, and the head, neck, and thorax were immersed in ice-cold saline consisting of (in mM) 115 NaCl, 4 KCl, 1 MgSO4, 24 NaHCO3, 1.25 NaH2PO4, 2.0 CaCl2, 10 d-glucose, and 12 sucrose. The saline had a PO2 of 100 Torr and a PCO2 of 40 Torr. Preparations were decerebrated at the precollicular level, and the right common carotid artery was exposed, cannulated, and perfused with ice-cold saline with the use of a peristaltic pump. Typically, perfusion commenced within 5 min from the time of midbody transection. Once the carotid was perfused, the right carotid sinus region was cleared of surrounding tissue, and the CSN was identified. The CSN was carefully desheathed with fine dissection scissors and resected immediately proximal to where it joins the glossopharyngeal nerve (CNIX). The internal and external branches of the carotid artery were cut distal to the carotid body, and the preparation was transferred to a recording chamber (perfusion was briefly disrupted during transfer).

Perfusate used for recording was identical to that used in the dissection and was recirculated from the cut ends of the internal and external carotid arteries into a 175-ml tonometer. Before reentering the carotid artery, the perfusate was reequilibrated, passed through a 45-μm filter (Millipore, Billerica, MA), and warmed with a heat exchanger (total volume: ~200 ml) (Fig. 1A.). Over the first 90 min in the recording chamber, the temperature of the perfusate was increased gradually to 36–37°C. Carotid body flow rate, which we determined by measuring the effluent buffer leaving the chamber, was maintained at 15 ml/min. This flow rate was sufficient to generate a pressure of ~100 mmHg at the tip of the cannula, which is close to the mean arterial pressure of rats. Adequate perfusion of the carotid body was confirmed in three preparations by the addition of fast-blue dye to the perfusion buffer and observing homogenous staining of the carotid body.

We monitored afferent CSN activity (Fig. 1, Ba and Bb) using a suction electrode, a differential AC amplifier (0.3–1 kHz; model EX1, ADI Instruments, Colorado Springs, CO), and a secondary amplifier (0.1–1 kHz; model AM502, Tektronix, Beaverton, OR). Recordings were monitored on an oscilloscope, rectified, and stored on a computer at 2 kHz using an AD board (model TL2, Axon Instruments, Union City, CA) and data-acquisition software (Axotape2; Axon Instruments).

The preparation was left for 90 min to recover before experimental protocols were initiated, allowing time to achieve a stable level of

![Fig. 1. In vitro carotid body (CB) preparation. A: schematic. B: method of analyses: example of raw data (Ba), which was then rectified (Bb) and 1-min bin averaged (Bc). Horizontal bar in Bu and filled vertical bars in Bc indicate exposure to chemochallenge (in this case, a single bout of 60 Torr hypoxia). C: stability over time. Preparations were perfused for 100 min with perfusate having a PO2 of 100 Torr and a PCO2 of 40 Torr. Extracellular recordings from individual preparations were rectified, divided into 1-min bins, and integrated. Graph shows mean (±SE) data from 7 preparations, normalized to the average activity in the first 5 min. D: time to equilibrate perfusion system. Perfusate PO2 was measured while changing PO2 from 100 to 60 Torr (indicated by the solid bars), using a polargraphic Clark-style oxygen electrode, with the tip of the cannula used to perfuse the common carotid artery. Traces from 3 runs were divided into 5-s bins and averaged. CSN, carotid sinus nerve.](http://ajpregu.physiology.org/doi/10.1152/ajpregu.00472.2004)
normoxic, normocapnic afferent activity. Before the protocol was initiated, flow was transiently reduced. Most preparations responded with an increase in afferent activity. An increase in activity was considered indicative of a suitable nerve recording for experimentation, with chemoreceptor afferent response (drop in tissue Po2 from reduced flow) dominating afferent baroreceptor activity (which would be expected to decrease as pressure falls). A small number of preparations failed to show an increase in afferent activity during reduced flow and were not used for experimentation.

Experimental Protocols

In most experiments, preparations were challenged by changing the Po2 and/or PCO2 of the gas mixture equilibrating the perfusate. We produced gas mixtures using computer-controlled mass flow controllers, monitored using CO2 and O2 gas analyzers (models CA-2A and PA1B, respectively, Sable Systems, Las Vegas, NV). The time constant to equilibrate the normoxic perfusate at the end of the cannula with 60 Torr Po2 was 20.4 ± 0.2 s; the time constant for equilibration in the reverse direction was 20.0 ± 0.3 s (Fig. 1D, n = 3) (measured using a Clark-style Po2 electrode and polarographic amplifier; model 1900, AM Systems, Carlsborg, WA). Because of the dilution effect of recirculating perfusate, the perfusate leaving the cannula was slightly more hyperoxic than the equilibrating gas (e.g., the perfusate at the tip of the cannula had a Po2 of 63 Torr when the perfusate in the tonometer was bubbled with a gas having a Po2 of 60 Torr).

In experiments involving 1-min challenges, where a greater temporal resolution was desirable, we employed two tonometers containing perfusate equilibrated with different gas mixtures. Computer-controlled pinch valves were used to govern which source of perfusate was used. During switch over, perfusate was momentarily drawn from the time-constant for switched delivery was ~1 s.

Data Analysis

Data were analyzed offline. We reduced the data 100-fold using the MinMax routine in Clampfit (Axon Industries), thus creating more manageable files while preserving the salient features of the original data. Rectified activities over user-defined time bins were then summed with software custom written in VEE (Fig. 1B; Agilent Technologies, Palo Alto, CA) by R. J. A. Wilson. Results were normalized with the average value of baseline activity (Fig. 1Bc). Baseline was defined as the five 1-min time bins preceding the first hypoxic or hypercapnic challenge. Binned activity at different time points (one factor) and effects of perfusate gas partial pressures (e.g., level of hypoxia, second factor) were compared by two-way repeated-measures ANOVA. When differences in activity reached significance (P < 0.05), we performed pairwise comparisons using Tukey’s post hoc test. We performed between-subject comparisons (e.g., four 5-min bouts of hypoxia vs. one 20-min bout) using a two-tailed t-test. P values are presented in the body of the text and in the figure legends; these are accompanied by footnoted references containing the full description of the statistical analyses.

Definition of Terms Used to Describe Gases

Unless stated otherwise, we refer to perfusate equilibrated with 100 Torr Po2 as normoxic and that equilibrated with 80, 60, and 40 Torr Po2 as mildly, moderately, and severely hypoxic, respectively. Extreme hypercapnia, moderate hypercapnia, normocapnia, and hypocapnia are used to refer to perfusate equilibrated with 60, 50, 40, and 30 Torr PCO2, respectively.

RESULTS

Time control experiments in which the in vitro preparation was perfused with normoxic, normocapnic saline demonstrated that after 1.5 h of recovery, CSN activity was stable over the following 1 h 40 min (Fig. 1C). With these time controls as a backdrop, we then investigated the effect of hypoxic and hypercapnic stimuli on CSN activity.

Response During Different Levels of Hypoxia

As expected, the activity of the CSN increased rapidly in response to hypoxic challenges (Fig. 2, A–C; 80 Torr Po2, n = 8; 60 Torr Po2, n = 10; 40 Torr Po2, n = 7). During the first challenge, both the peak response (i.e., the maximum CSN activity reached in any 1 min during the challenge compared with baseline activity) and overall response (i.e., the CSN activity over the duration of challenge normalized to the activity during the 1 min immediately preceding the challenge) were significantly augmented as the level of hypoxia was increased (Fig. 2, D and E; peak: P = 0.015, overall: P = 0.028). Irrespective of the degree of hypoxia, minute 2 of the first challenge contained the highest level of integrated activity. Despite continued hypoxic stimulation, activity then fell by ~7.5% over the next 3 min (Fig. 2, A–C; P < 0.001). We will refer to this phenomenon as sensory hypoxic decline. Sensory hypoxic decline was independent of the level of hypoxia (P = 0.066) and did not occur during bouts 2–4 (see Fig. 2, B and C).

Over subsequent bouts, the CSN response depended on both the intensity of hypoxia and the number of bouts. That is, the decline in CSN peak response with successive bouts was more marked with severe hypoxia than with mild hypoxia (Fig. 2D; P = 0.002, P = 0.007). CSN overall response, on the other hand, increased with successive bouts (sensory progressive augmentation, P < 0.001), with no significant difference between severe and mild hypoxia.

Sensory progressive augmentation was associated with a progressive decline in normoxic CSN activity between the bouts of hypoxia (sensory posthypoxic decline). Posthypoxic activity reached a minimum within 2 min after each bout of hypoxia, with the level being dependent on the degree of hypoxia (P < 0.005). At 2 min after the last bout of severe hypoxia, normoxic activity was reduced to 72% of the prechallenge level; after a series of bouts of mild hypoxia, however, there was no demonstrable difference between the postchallenge minimum and the prechallenge baseline. Moderate hypoxia produced a posthypoxic minimum that was not significantly different from that produced by severe hypoxia. A single bout of either moderate or severe hypoxia produced near-maximal posthypoxic sensory decline. (Fig. 2F). The depres-

1 Figure 2, D and E; 1-factor ANOVA, level of hypoxia effect: F = 5.17, P = 0.015 (peak); F = 4.21, P = 0.028 (overall).
2 Figure 2, A–C; 2-factor repeated-measures ANOVA, time effect: F = 9.99, P < 0.001.
3 Two-factor repeated-measures ANOVA, level of hypoxia × time interaction: F = 2.06, P = 0.066.
4 Two-factor repeated-measures ANOVA, hypoxic level × bout interaction: F = 3.96, P = 0.002 (peak); F = 2.11, P = 0.064 (overall).
5 Two-factor repeated-measures ANOVA, bout effect: F = 4.46, P = 0.007 (peak); F = 7.56, P < 0.001 (overall).
6 Figure 2F; 2-factor repeated-measures ANOVA, level of hypoxia effect: F = 6.83, P = 0.005; bout effect: F = 10.178, P < 0.001.
7 Tukey’s post hoc, comparing baseline activity with activity after the first bout: q = 4.379, P = 0.029 (moderate); q = 4.517, P = 0.022 (severe). Tukey’s post hoc, comparing posthypoxic activity after the first and fourth bouts: q = 2.55, P = 0.47 (moderate); q = 2.16, P = 0.65 (severe).
Fig. 2. CSN response to four 5-min bouts of mild (A; 80 Torr PO2), moderate (B; 60 Torr PO2), and severe (C; 40 Torr PO2) hypoxia during normocapnia. Challenges are indicated by solid bars. During the remainder of the protocol, the perfusate was equilibrated with 100 Torr PO2 and 40 Torr PCO2. Data were normalized to the average bin activity in the 5 min preceding the first challenge. Effects of PO2 on peak response and overall response, as well as the posthypoxic activity, are summarized in D, E, and F, respectively. Baseline refers to the activity in the last minute of normoxia preceding the first challenge. Peak response (D) refers to the maximum CSN activity reached in any 1 min during the challenge compared with the baseline activity. Overall response (E) refers to the increase in activity over the duration of each challenge normalized (*) to that in 1 min preceding each challenge. Posthypoxic activity (F) was assessed using the minute having the lowest level of activity following each challenge, relative to baseline activity. Wash refers to activity 40 min into the normoxic washout period following challenges. The sensory hypoxic decline apparent during the first challenge was significant in A, B, and C (P < 0.0015). The sensory progressive augmentation, i.e., the increased CSN response with each bout, was significant in E (bout effect: P < 0.0015). The sensory posthypoxic decline in F was significant (bout effect: P < 0.0015) and was of greater magnitude with more severe levels of hypoxia (P = 0.0055). Broken bars late in runs in A indicate moderate hypoxic challenges, performed to confirm CSN viability at the end of the experiment.

Effect of Hypoxic Duration on Sensory Posthypoxic Decline

To determine how CSN activity was affected by the duration of hypoxia, we exposed carotid body preparations to different length bouts of moderate hypoxia. In one set of experiments, we used a single 20-min bout of 60 Torr hypoxia (Fig. 3A, n = 8). Two minutes after the end of the bout, normoxic activity was reduced to 83% of the prechallenge level (P = 0.0169). This degree of suppression was not significantly different from the magnitude of sensory posthypoxic decline after four 5-min bouts (P = 0.23810). After the 20-min bout of hypoxia, activity returned to prechallenge levels within 5 min11, faster than the recovery after four 5-min bouts (compare Fig. 3A and B).

In a second set of experiments, we exposed the carotid body to twenty 1-min bouts at 2-min intervals (Fig. 3C, n = 9). We chose 2 min for the intervening washouts because, in the 5-min and 20-min bout experiments, this was the time taken for the majority of sensory posthypoxic decline to develop. However, with 1-min bouts, no sensory posthypoxic decline was observed (P > 0.0512). The apparent gradual increase in activity after the last 1-min bout was not significantly different from baseline (P = 0.1113), suggesting hypoxia alone was not sufficient to induce sLTF.

In summary, 1) CSN activity declines during 5 min of severe, moderate, or mild hypoxia (sensory hypoxic decline); 2) exposure of the carotid body to several minutes of moderate or severe hypoxia leads to a transient reduction in normoxic afferent activity after the bout (sensory posthypoxic decline); and 3) overall afferent responses increase progressively between successive 5-min bouts of mild, moderate, or severe hypoxia (sensory progressive augmentation).

Effect of Hypercapnia

To determine whether the time-dependent responses of the carotid body were specific to hypoxia, we exposed preparations to four 5-min bouts of normoxia with either moderate (Fig. 4A; n = 7) or extreme (Fig. 4B; n = 8) hypercapnia. In both cases, the speed of the hypercapnic response was faster compared

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8 Tukey’s post hoc, by 24th min, activity compared with baseline, P > 0.05;
Tukey’s post hoc, by 22nd min, activity compared with baseline, P > 0.05.
9 One-factor repeated-measures ANOVA, time effect: F = 5.62, P = 0.016.
10 Between-subject t-test, challenge duration: t = −1.23, P = 0.238.
11 Tukey’s post hoc, by 5th min, activity compared with baseline, P > 0.05.
12 One-factor repeated-measures ANOVA, time effect: F = 3.10, P = 0.073.
13 One-factor repeated-measures ANOVA, baseline compared with the maximum activity during washout: P = 0.11.
with hypoxic responses, with the hypercapnic response approaching its maximum within the first minute after the onset of stimulation.

Both the peak and overall CSN responses to extreme hypercapnia were significantly greater than the responses to moderate hypercapnia (compare Fig. 4, C and D; $P = 0.007^{14}$). Despite a substantial response to extreme hypercapnia (40% above baseline), there was no indication of a decline in activity, akin to sensory hypoxic decline, toward baseline during maintained stimulation (Fig. 4B). In addition, although there appeared to be a mild, transient decline in activity after the first bout of extreme hypercapnia, activity was not significantly different from baseline activity ($P = 0.66^{15}$). On the contrary, normoxic-normocapnic activity following successive bouts of both moderate and extreme hypercapnia increased progressively (Fig. 4E; $P < 0.001^{16}$). For example, normoxic-normocapnic activity at the end of a 1-h washout period was elevated by 26% over baseline activity after extreme hypercapnic bouts ($P < 0.001^{17}$). This elevated activity appears to be akin to the hypoxic sLTF described by Peng et al. (22).

In summary, 1) the response of the carotid body to hypercapnia is faster than the hypoxic response; 2) three of the time-dependent phenomena that we observed with hypoxia (sensory hypoxic decline, sensory posthypoxic decline, and sensory progressive augmentation) are not mimicked by hypercapnia; and 3) after four bouts of moderate hypercapnia, a long-term increase in CSN activity was observed (sLTF).

**Interaction Between Hypoxia and CO₂**

To determine how the level of CO₂ during a stimulus affected the time-dependent responses to hypoxia, we repeated the four 5-min bout protocol, combining moderate hypoxia with either hypocapnia (30 Torr PCO₂) (Fig. 5A; $n = 6$) or hypercapnia (60 Torr PCO₂) (Fig. 5C; $n = 8$). Although the responses to hypoxia with concurrent normocapnia and hypercapnia were similar, hypocapnia appeared to reduce both the peak and overall responses (Fig. 5, D and E). However, whereas these differences may have been apparent from the means, and expected given previously published data (15, 26), when we included hypocapnic, normocapnic, and hypercapnic hypoxia datasets for statistical comparison, the level of PCO₂ had no significant effect on the peak or the overall responses to hypoxia over the four bouts ($P = 0.38$, $P = 0.15^{18}$). If we considered just the hypoxic hypoxia and hypercapnic hypoxia, excluding the normocapnic hypoxia dataset from the analysis, only the difference in overall response reached significance (Fig. 5E; $P = 0.015^{19}$). If only the responses to the first bout are considered, the peak responses are significantly different, only if the data are first transformed by raising to the power of two ($P = 0.024^{20}$). Together, the analyses indicated that the effect of PCO₂ on the peak and overall hypoxic response of our preparation, at least over the range that we tested, is rather weak.

Similarly, PCO₂ has little or no effect on sensory progressive augmentation (i.e., the increase in overall response with re-

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14 Two-factor repeated-measures ANOVA, PCO₂ level effect: $F = 7.63$, $P = 0.016$ (peak); $F = 10.46$, $P = 0.007$ (overall).

15 Figure 4B; Tukey’s post hoc comparing baseline with minute 2 after first bout: $q = 2.12$, $P = 0.66$.

16 Figure 2E; 2-factor repeated-measures ANOVA, bout effect: $F = 12.88$, $P < 0.001$.

17 Tukey’s post hoc comparing baseline with 60th min of washout: $q = 8.08$, $P < 0.001$.

18 Two-factor repeated-measures ANOVA, effect of PCO₂: $F = 1.02$, $P = 0.38$ (peak); $F = 2.04$, $P = 0.15$ (overall).

19 Two-factor repeated-measures ANOVA, hypocapnic hypoxia vs. hypercapnic hypoxia only: $F = 7.96$, $P = 0.015$ (overall); $F = 2.92$, $P = 0.11$ (peak).

20 Kruskal-Wallis 1-way ANOVA on ranks, PCO₂ effect, peak response: $H = 7.48$, $P = 0.024$; 1-factor repeated-measures ANOVA, effect of PCO₂, overall response: $F = 1.17$, $P = 0.33$. 

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peated hypoxic bouts): although sensory progressive augmentation occurs during hypoxia with all three levels of concurrent PCO₂ (Fig. 5E; \( P < 0.001^{23} \)), its magnitude over the four bouts was insensitive to PCO₂ level (\( P = 0.07^{22} \)).

However, PCO₂ level did affect sensory hypoxic decline (\( P = 0.009^{23} \)). Sensory hypoxic decline was present during normocapnic and hypercapnic hypoxia (\( P < 0.001^{24} \)) but was absent during hypoxic hypcapnic challenges (\( P = 0.93^{23} \)).

PCO₂ also affected sensory posthypoxic decline (\( P = 0.029^{26} \)). Sensory posthypoxic decline occurred after bouts of hypercapnic hypoxia and normocapnic hypoxia (\( P < 0.001^{27} \)) but not after bouts of hypocapnic hypoxia\(^{28} \). We note that, although concurrent hypocapnia abolishes the sensory hypoxic decline and sensory posthypoxic decline produced by moderate or severe hypoxia, four hypocapnic hypoxic bouts give rise to sLTF (\( P = 0.008^{29} \)).

In summary, the level of PCO₂ has little or no influence on the peak and overall responses that normally occur during a bout of moderate hypoxia, nor does the level of PCO₂ affect the progressive augmentation that results from consecutive bouts. However, the level of PCO₂ does influence other time-dependent phenomena. On the one hand, hypocapnia curtails the sensory hypoxic decline and sensory posthypoxic decline that normally make up the responses to moderate or severe hypoxia. On the other hand, hypocapnic hypoxia produces sLTF, which was not seen with moderate hypoxia and higher levels of PCO₂.

**DISCUSSION**

The mammalian response to hypoxia is dynamic, in both the short and long term, and although central mechanisms have
been proposed for many of the time-dependent phenomena (24), the possibility exists that there are underlying redundant, peripheral mechanisms for some or all of them. In the present study, we observed time-dependent hypoxic sensory responses of the rat carotid body that resemble time-dependent hypoxic ventilatory responses: sensory progressive augmentation, sensory hypoxic decline and sensory posthypoxic decline. Our results add to findings by Peng et al. (22), who suggested a plasticity in the response of the carotid body to intermittent stimulation. Together, our data led us to speculate that the carotid body plays a role in certain time-dependent respiratory phenomena, such as short-term depression, PHFD, progressive augmentation, and LTF.

Critique of Methods

Data acquisition and analyses. Many studies investigating carotid body responses to hypoxia and/or hypercapnia have examined action potential spike frequencies from single, multifiber, or whole-nerve preparations, with a temporal resolution of seconds. Our goal, however, was to assess the changes in overall CSN afferent activity over a time scale equivalent to that of ventilatory phenomena. Therefore, we chose not to analyze the spike frequency of individual units, preferring instead to rectify the recordings made from the whole nerve and average the activity over 1-min bins. A major advantage of our approach is that it allows quantification of the overall afferent information originating from the peripheral chemoreceptor, including both changes in mean frequency and recruitment of different amplitude units. However, a disadvantage is that it does not allow us to resolve the relative contribution of changes in frequency and recruitment to time-dependent phenomena.

Delivery of perfusate. Many other studies have used either superfusion or perfusion at relatively low pressures. Although we did not account for the effect of surrounding tissue on carotid body PO2, we delivered recirculated perfusate through the common carotid artery at a pressure close to that which occurs in vivo. Superfusion delivers perfusate ectopically and may lead to unexpected tissue PO2 and PCO2 levels at the site of chemoreception, accurate predictions of which are confounded by the very high metabolic rate of the carotid body. As noted by Roy et al. (26), arterially perfused preparations respond to PO2 over the physiological range, in contrast to superfused preparations whose dynamic range is above that found in situ (23). In our preparation, afferent CSN activity was inversely proportional to perfusion pressure. An increase in afferent activity with a reduction in perfusion pressure suggests that afferent baroreceptor activity contributed negligibly to our...
signal, but it also suggests that the use of a lower perfusion pressure may increase baseline activity, which, in turn, may be sufficient to “drown out” time-dependent effects.

Although recirculation of the perfusate could have affected CSN activity over time, we note that time controls do not appear to be affected, and all time-dependent responses are transient, with activity returning to baseline.

Oxygenation and stimuli. During dissection, we minimized the period of ischemia to ~4 min and left the preparation to recover for 1.5 h before beginning experiments. During dissection, recovery, and baseline recordings and between chemosensory challenges, the preparation received normoxic (PO2 100 Torr), normocapnic (PCO2 40 Torr) perfusate. We also used hypoxic and hypercapnic challenges that were within physiological bounds. This is in contrast to many studies that used J) hypoxic perfusate during both dissections and baseline recordings and/or 2) more extreme challenges (e.g., anoxia).

Age of Rats Used

The magnitudes of both the hypoxic ventilatory response and LTF of breathing after exposure to chronic intermittent hypoxia have been shown to be greater in 1-mo-old rats vs. 2-mo-old rats (18). Therefore, although we limited the age of rats used in the study to between 4 and 6 wk, the possibility exists that dynamics of the CSN responses were variable between animals. However, we are reporting data that has stood up to stringent statistical analyses (2-factor ANOVA with Tukey’s post hoc tests). Therefore, the effects of hypoxia and hypercapnia on CSN activity over time were relatively consistent between animals, overcoming variability caused by developmental factors.

Comparison with Chemosensitivity of Other In Vitro and In Situ Carotid Preparations

Dynamic ranges of both hypoxic and hypercapnic responses that we observed appear similar to both the in vitro rat preparation of Roy et al. (26) and the in situ rat preparation of Vidruk et al. (31). Furthermore, although concurrent hypoxia (30 Torr PO2) blunts the hypoxic response compared with hypercapnic hypoxia, our preparation shows no additive or multiplicative interaction between moderate hypoxia and severe hypercapnia (60 Torr PCO2). The lack of interaction between severe hypercapnia and hypoxia is similar to the results of experiments that used both whole nerve and single fiber recordings (15, 26). Similarly, our data suggest that 1-min bouts of hypoxia were insufficient to elicit sensory posthypoxic decline and that maximal sensory posthypoxic decline induced by longer stimuli occurred 2 min after returning to normoxia. This is consistent with in vitro experiments performed by Roy et al. (26) and in vitro and in vivo experiments performed by Peng et al. (22), who observed no time-dependent hypoxic effects. Their protocols consisted of very short periods of stimulation (1–2 min for Roy et al. and 15 s for Peng et al.). In addition, the in vitro preparation of Peng et al. was superfused at 1–2 ml/min, likely resulting in a relatively lower tissue PO2 and higher CSN activity compared with our preparation, thus perhaps drowning out time-dependent effects.

Some studies have revealed a certain degree of carotid body adaptation to hypoxia. However, these studies used superfusion and extreme hypoxia or anoxia (6, 13). Li et al. (16), who used an anesthetized rabbit preparation, showed adaptation to severe levels of hypoxia (~25–30 Torr PO2), beginning after 5 min of exposure and continuing for 1 h of continuous stimulation (16). After 30 min of extreme hypoxia, activity declined by 20%, but no details were provided regarding the response of the preparation on its return to normoxia. Therefore, whether the reported decline in afferent activity was the result of neuronal damage is unclear.

Carotid Body Contribution to the Dynamics of Ventilatory Responses to Hypoxia

Although the carotid bodies in awake animals provide the majority of the initial hypoxic drive to breathe, previous data supporting the role of carotid body activity in shaping the subsequent dynamics of the hypoxic ventilatory response are ambiguous. Current evidence suggests that central mechanisms mediate a considerable proportion of the awake hypoxic ventilatory phenomena. Recently, for example, Coles et al. (5) studied the role of the carotid body in the hypoxic ventilatory response of conscious rats. During an acute bout of hypoxia, CSN-denervated rats demonstrated a slight residual augmentation of respiration, minuscule compared with that of control animals, and no significant decline in ventilatory frequency during hypoxia. However, despite the lack of a robust ventilatory hypoxic facilitation, following the termination of hypoxia, ventilatory frequency declined abruptly to below prehypoxic levels, i.e., PHFD. These results suggest that neither carotid body input nor an augmentation of respiration is necessary for PHFD and suggest that the central effects of hypoxia, including the inhibition of respiratory-modulated neurons, may be sufficient to induce PHFD. There are several other studies demonstrating hypoxic-respiratory depression in the absence of peripheral chemoreceptor input in anesthetized animals (19, 20). Together, these studies demonstrate the sufficiency of central mechanisms but do not exclude a role for the carotid body.

Some data from the anesthetized cat are incongruous with a role for the carotid body. Recordings from the CSN have demonstrated that activity remains elevated during hypoxia, even in circumstances sufficient to induce HVD (2, 32). However, in the awake state, the peripheral chemoreceptor appears to play a more important role (11, 25). For example, in awake cats challenged with an arterial PO2 of 40–77 Torr, chemodenervation eliminates both HVD and PHFD (17). Similar results have been found in carotid body-resectioned humans (14). Furthermore, Hayashi et al. (9) provided convincing evidence demonstrating the importance of carotid body input in producing time-dependent hypoxic-like responses, using a carotid denervated, artificially ventilated anesthetized rat preparation. Specifically, they demonstrated that, during electrical stimulation of the CSN, the frequency of neuronal ventilation was first increased but subsequently reduced (short-term depression), which transiently fell below baseline after termination of the stimulus (PHFD).

The Effects of PCO2 on the Response Dynamics of the Carotid Body

In this study, intermittent hypocapnic hypoxia led to two qualitatively different response dynamics compared with normocapnic and hypercapnic hypoxia: hypocapnic hypoxia failed
to produce sensory hypoxic decline and sensory posthypoxic decline but led to sLTF.

Our demonstration of sLTF with intermittent moderate hypercapnia and intermittent hypocapnic hypoxia adds to recent evidence suggesting a role for the carotid body in the long-term ventilatory changes after exposure to chronic intermittent hypoxia (22). The appearance of LTF may be related to the strength of the hypercapnic stimulation. In our study, it appears that sLTF is more likely with mild CSN stimulations; for example, sLTF was elicited by both moderate hypercapnia and hypocapnic, moderate hypoxia but not with severe hypercapnia or normocapnic, moderate hypoxia. Interestingly, Peng et al. (22) investigated the capability of the carotid body to generate sLTF in response to hypoxic bouts with a background arterial PCO2 of ~32 Torr. Our findings suggest that hypoxia may be necessary for the manifestation of hypoxia-induced sLTF. However, given that four bouts of normoxic hypoxia were also capable of producing sLTF, we note that hypoxia per se may not be necessary for sLTF. In anesthetized, vagotomized rats, Bach and Mitchell (1) demonstrated that bouts of hypercapnia can elicit long-term depression. However, they attribute this to a central mechanism involving α2-adrenergic receptors and report that blocking these receptors reverses hypercapnia-induced LTF, attributed to peripheral chemoreceptor activation.

The absence of sensory hypoxic decline and sensory posthypoxic decline with bouts of hypocapnic hypoxia indicate that the cellular mechanisms responsible may require a minimum level of PCO2 (i.e., a direct PCO2-dependent mechanism) and/or may result from the reduced hypoxic response in the presence of hypocapnia (i.e., an indirect mechanism in which hypocapnia blunts the magnitude of the hypoxic response). In the case of sensory posthypoxic decline, a magnitude-dependent mechanism appears most likely since, with concurrent normocapnia, the magnitude of sensory posthypoxic decline depends on the level of hypoxic stimulation. For sensory hypoxic decline, we favor a direct PCO2-dependent mechanism because (1) sensory hypoxic decline is independent of the level of hypoxia (with concurrent normocapnia) and (2) sensory hypoxic decline is independent of the total response magnitude; that is, the CSN response to hypocapnic, moderate hypoxia (no sensory hypoxic decline) was of a similar magnitude to that caused by normocapnic, mild hypoxia (which induced sensory hypoxic decline; compare Figs. 5A and 2A). Accordingly, sensory hypoxic decline appears to differ in this respect from HVD in humans, which is directly proportional to the magnitude of the acute hypoxic response (7, 8).

In conclusion, the response to hypoxia includes initial facilitation (mediated primarily by the peripheral chemoreceptor afferents) followed by a number of hypoxic ventilatory responses that occur during or after several minutes of stimulation. In intact animals, these time-dependent phenomena have been attributed to a number of previously described mechanisms, including (1) hypoxia acting centrally, depressing neuronal activity directly (10, 12), and (2) the activation of central modulatory pathways by the hypoxia-mediated barrage of carotid body afferents. These modulatory pathways may be excitatory (as in progressive augmentation and LTF) or inhibitory/defacilitatory (as in HVD and PHFD) and may continue to be active after hypoxia is terminated (as in PHFD and LTF). Species, state of consciousness, and the level and duration of hypoxia may partially explain the dichotomous results of experiments investigating the contribution of these mechanisms to hypoxic ventilatory phenomena. However, we favor the more parsimonious explanation that the integrative time-dependent ventilatory response to hypoxia originates from multiple, redundant mechanisms, with no one mechanism being absolutely necessary. Our data suggest that time-dependent changes in the activity of carotid body afferents should be added to the list of mechanisms, as postulated by Robbins (25) and expanded on by Honda et al. (11).

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