Carotid body denervation eliminates apnea in response to transient hypocapnia

HIDEAKI NAKAYAMA,1 CURTIS A. SMITH,1 JOSHUA R. RODMAN,1 JAMES B. SKATRUD,2 AND JEROME A. DEMPSEY1
1John Rankin Laboratory of Pulmonary Medicine, and 2Departments of Population Health Sciences and Medicine, University of Wisconsin School of Medicine, Madison, Wisconsin 53726
Submitted 5 August 2002; accepted in final form 12 September 2002

WE WERE CONCERNED IN this study with the role of the carotid chemoreceptors as a cause of hypocapnia-induced central apnea and periodic breathing (PB) in sleep. There is evidence available to support peripheral or central CO2 chemoreceptors as key mediators of this response. A case for carotid chemoreceptors may be made because central apneas often occur within 10–15 s of a transient ventilatory overshoot in sleep (7, 27, 43). The functional response time of the central chemoreceptors appears to be on the order of 25–30 s (10), which would be too slow to account for apnea in response to transient hypocapnia. Furthermore, Bowes et al. (9) showed that a single, passive augmented breath resulted in significant expiratory time (TE) prolongation in vagally blocked awake dogs and that this TE prolongation was dependent on the presence of carotid chemoreceptors. Others have shown in anesthetized animals that carotid sinus nerve activity and ventilation are very sensitive to local changes in PCO2 confined to the carotid body (CB) (19, 26) and that congestive heart failure (CHF) patients with and without Cheyne-Stokes respiration (CSR) can be distinguished on the basis of their ventilatory response to transient CO2, a test that presumably reflects the responsiveness of the carotid chemoreceptors (43).

On the other hand, medullary chemoreceptors are also highly sensitive to H+ concentration changes in their environment, as shown by the ventilatory responses to ventricular-cisternal perfusion in awake goats (18, 36) and to focal acidosis of various chemosensitive areas of the medulla (32). Marked ventilatory depression and even apnea are also caused by ablation of medullary chemosensitive regions in anesthetized cats and goats (20, 40). Furthermore, some studies estimate the time delay from lung to medullary surface to be sufficiently short so as to approximate the delay from ventilatory overshoot to apnea (1, 16, 25). Millhorn et al. (29) have also shown in anesthetized cats that periodic oscillations in phrenic nerve activity corresponded in time and amplitude with those in pH on the medullary surface. PB in CHF patients has also been shown to correspond with an enhanced ventilatory response to hyperoxic CO2, a test that presumably reflects the sensitivity of the medullary chemoreceptors exclusively (24, 45). Finally, our own studies using extracorporeal perfusion of the isolated carotid chemoreceptor in the sleeping dog showed that a progressive CB hypocapnia led to immediate and progressive reductions in tidal volume (Vt) and minute ventilation but that step reductions in CB PCO2 of more than 10 Torr or increases in CB PO2 of >500 Torr did not affect breath timing or cause apnea (42).

Our present approach was to study the intact and CB denervated dog under conditions that mimicked the occurrence of central sleep apnea and some types of PB commonly seen in sleeping humans. Accordingly, we...
studied the animals in non-rapid eye movement sleep and used mechanical ventilation in the pressure support mode (PSV) to cause transient increases in VT and reductions in arterial PCO2 (PETO2). It had already been established that similar approaches would produce apnea in the intact sleeping dog (30) and in humans (28, 48) once end-tidal PCO2 (PETCO2) was reduced below the apneic threshold.

METHODS

Studies were performed over several days during non-rapid eye movement sleep on four unanesthetized, CB-denerverated, female mixed-breed dogs (20–25 kg). These dogs were a subset of six dogs reported on in a previous publication when they were neurally intact (30). The dogs were trained to sleep in an air-conditioned (19–22°C), sound-attenuated chamber. They were unrestrained and allowed to pick their own sleeping position. Throughout all experiments, the dogs’ behaviors were monitored by an investigator seated within the chamber and also by closed-circuit television. The Animal Care and Use Committee of the University of Wisconsin approved the surgical and experimental protocols for this study.

Chronic instrumentation and CB denerervation. These dogs were chronically instrumented for a prior study (30). Details of this chronically instrumented dog model are described in detail elsewhere (12, 30, 39). Briefly, the dogs were prepared with a chronic tracheostomy and indwelling electromyogram (EMG) electrodes in the crural diaphragm, a five-lead electroencephalogram montage, and an arterial catheter.

A relatively minor additional surgical procedure was required to achieve CB denervation (CBX). The carotid sinus region was exposed bilaterally, and all tissues surrounding the arteries of the region were removed over a distance of 1–2 cm. General anesthesia and strict sterile surgical techniques were used. Dogs recovered for at least 1 wk before any studies were performed. CBX was confirmed before each experiment by a lack of a significant ventilatory response to intravenous bolus injections of 20–40 mg/kg of sodium cyanide (NaCN). When the dogs were intact, identical doses of NaCN resulted in a transient two- to threefold increase in ventilation.

Experimental setup and measurements. Dogs breathed via a capped endotracheal tube (10.0 mm outer diameter; Shiley, Irvine, CA) that was inserted into the chronic tracheostomy. Airflow was measured via a heated pneumotachograph system (model 3700, Hans Rudolph, Kansas City, MO; model MP-45-14-871, Validyne, Northridge, CA) connected to the endotracheal tube. The pneumotachograph was calibrated before each study with four known flows. Tracheal pressure was measured at a port in the endotracheal tube that was connected to a pressure transducer (model MP-45-14-871, Validyne, Northridge, CA) connected to the endotracheal tube. The pneumotachograph was calibrated before each study with four known flows. Tracheal pressure was measured at a port in the endotracheal tube that was connected to a pressure transducer (model MP-45-14-871, Validyne). The pressure transducer was calibrated before each study by applying six known pressures. Airway PO2 and PCO2 were monitored by a mass spectrometer (model MGA-1100, Perkin-Elmer, Norwalk, CT) through a second port in the endotracheal tube. Three to six 1-ml arterial samples were obtained at the start of each experiment from the aortic catheter and analyzed for pH, PO2, and PCO2 on a blood-gas analyzer (model ABL-505, Radiometer, Copenhagen, Denmark). 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 for both body temperature and systematic errors revealed by tonometry. The inspiratory and expiratory tubes of the ventilator were connected to the pneumotachograph by using a Y connector. A silent balloon valve was placed between the pneumotachograph and the Y connector such that the dog could breathe spontaneously from room air or be abruptly switched to PSV by inflation of the balloon. All signals were digitized (128-Hz sampling frequency) and stored on the hard disk of a personal computer for subsequent analysis. Key signals were also recorded continuously on a polygraph (Gould ES 2000 or AstroMed K2G). All ventilatory data were analyzed on a breath-by-breath basis by means of custom analysis software developed in our laboratory.

Use of PSV to characterize the time course of Tc responses to hypocapnia. Dogs breathed room air spontaneously through the open port in the balloon valve (see above). The mechanical ventilator (Veolar, Hamilton Medical) was set in pressure support mode, and the trigger sensitivity was set as low as possible (approximately –2 cmH2O). When the balloon was inflated and the low resistance shunt to the room sealed, the ventilator delivered preset levels of inspiratory pressure support whenever the trigger threshold was reached. The expiratory positive airway pressure was set at 0 cmH2O. Thus the dog set its own breathing frequency, and increased levels of pressure support resulted in increased VT and decreased PECO2. As assessed from the diaphragmatic electromyogram (EMGdia), 95–100% of the increase in VT during PSV occurred during neural inspiratory time. Each level of pressure support was maintained for 2 min, and then the balloon was deflated and the dog was allowed to breathe spontaneously again (Fig. 1). At least 2 min elapsed before another PSV trial was performed. PSV was begun at a pressure of 3–5 cmH2O and increased in steps of 1–2 cmH2O in each succeeding trial until apnea and/or PB was achieved (range of 3–35 cmH2O). Trials in which there was a state change or sigh were excluded from analysis (see Ref. 30 for details).

Characterizing PETCO2 thresholds for Tc prolongation/apnea in the intact and CBX dog. Tc was measured from the end of the inspiratory flow to the onset of the next EMGdia burst. In the intact dog, PB was identified visually by the presence of at least three cycles of hyperpnea and apnea, as judged by EMGdia, with a consistent cycle length, and the apnea lengths had to be at least 3 standard deviations greater than the mean baseline spontaneous Tc. It is important to note that the apneic threshold was taken to be the PETCO2 observed in the breath immediately preceding the initial apneic period only.

After CBX, PB was never observed, so it was not straightforward to establish a PETCO2 threshold for apnea. Our method in the CBX dogs was to choose the longest Tc in the 10th to 60th seconds of each PSV trial. We reasoned that apneas that occurred before 10 s of PSV would not be chemoreceptor mediated because CB were lacking. We then associated the chosen Tc with the lowest PETCO2 observed from 0 to the 50th seconds of PSV; i.e., the lowest PETCO2 had to precede the longest Tc by at least 10 s. Our reasoning here was that any PETCO2 within 10 s of the chosen Tc could not have been sensed by the central chemoreceptors (see RESULTS); any breath before the 10-s cutoff could have been sensed by the central chemoreceptors, and the lowest PETCO2, therefore, probably had the greatest influence. Thus each trial provided one Tc value and a corresponding PETCO2 value. For each dog, we then regressed the change in (Δ) Tc (i.e., longest Tc – spontaneous Tc) on the ΔPETCO2 (10–13 trials, i.e., 10–13 points per dog). Using each dog’s regression equation, we then calculated the ΔPETCO2 that would have been required to produce an increase in Tc that was the same as the increase observed at the apneic threshold when that dog was intact. It is important to note that calculated ΔPETCO2,
was always within the range of the regression data; we did not extrapolate from the regression line.

**ΔpH calculations.** The Siggaard-Anderson nomogram was used to estimate changes in pH that accompanied changes in the measured ΔPETCO₂ values between spontaneous breathing and the apneic threshold. For changes in arterial pH, we assumed that the dogs’ blood buffer slopes were parallel to the normal human blood buffer slope and passed through the measured eupneic PaCO₂-pH point determined for each dog on each day. Changes in cerebrospinal fluid (CSF) pH for a given change in ΔPETCO₂ between eupnea and apnea were estimated by assuming no change in CSF HCO₃⁻ concentration ([HCO₃⁻]). We also assumed that CSF [HCO₃⁻] was equal to the measured eupneic arterial [HCO₃⁻] measured on that day, that CSF PCO₂ was equal to PETCO₂ + 6 Torr (41), and that ΔPETCO₂ equaled the change in CSF PCO₂.

**Statistics.** Significance of group mean data was determined with a paired t-test. Differences were considered significant at *P* < 0.05.

**RESULTS**

**CB denervation.** CB denervation resulted in hypoventilation and respiratory acidosis in all dogs, although the changes in breathing frequency and VT employed varied between dogs (Table 1). The absence of significant CO₂ retention post-CBX in dog Ni is noteworthy. Despite the small CO₂ retention, this dog was clearly denervated because she showed no ventilatory response to doses of NaCN that promoted vigorous hyperpnea when the dog was intact.

**Time course of response to PSV: intact vs. CBX.** Figure 1A shows a typical intact PSV trial at a level of pressure support (15 cmH₂O) that produced sufficient hypocapnia to elicit PB. In this dog, 3 trials at PSV levels of 15–20 cmH₂O elicited PB, whereas 11 trials at PSV levels of 5–19 cmH₂O did not. PSV was initiated...
at the asterisk, and VT was immediately doubled. About 10–11 s after the second PSV breath, a clear apnea occurred. This was terminated by a spontaneous inspiratory effort that triggered a preset pressure support breath with a VT that remained twofold greater than during spontaneous breathing. PB continued for the remainder of the trial, which consisted of cycles of two augmented VT separated by a near-normal TE and followed by an apnea (average cycle length = 19.1 s). PETCO2 preceding the first apnea was taken to be the apneic threshold for this trial. Also note that diaphragm EMG was reduced to 72% of the spontaneous control value on the first PSV breath and remained reduced for the duration of the trial. This abrupt transition from eupnea to substantial Te prolongation and a PB pattern was a consistent feature of achieving the apneic threshold during progressive hypocapnia via PSV in the intact dog (see additional examples in Ref. 30).

Figure 1B shows that, in the absence of CB, similar levels of PSV, increased VT, and hypocapnia in the same dog shown in Fig. 1A also caused a reduced EMGdia beginning with the first PSV cycle, but Te was not consistently prolonged (i.e., >3 standard deviations longer than control Te) until the eighth cycle (i.e., after 33.7 s of PSV). Thereafter, Te remained longer than spontaneous baseline, but PB did not occur.

Figure 2 illustrates the mean time course data for each of the four dogs. On average, 10.7 ± 2.8 s (range = 6.0–13.3 s) were required in the intact dogs before apnea occurred (typically on the second PSV breath). In contrast, 32.8 ± 5.2 s (range = 25–39 s; significantly different from intact, P < 0.001) beyond the onset of PSV and the concomitant reduction in PETCO2 were required in the CBX dogs before clear, hypocapnia-induced prolongations in Te were observed. By 40–60 s of PSV, Te was similar to the prolonged Te observed during PB when the dogs were intact.

PB: intact vs. CBX. Persistent PB occurred in response to PSV-induced hypocapnia in intact dogs. This PB typically consisted of doublets followed by apnea and with each periodic cycle averaging 19.4 ± 2 s. This pattern persisted throughout the trial (Figs. 1 and 2). All PSV breaths from each trial in which PB occurred were plotted as a histogram. Two clear peaks in Te emerged: one peak averaging slightly longer than spontaneous eupneic Te and the other centered on apnea duration. This distribution of Te is predictable from a breathing pattern consisting almost entirely of doublets (Fig. 1). In contrast, although PSV-induced hypocapnia in the same dogs after CBX resulted in Te prolongations over time, PB was never observed (Figs. 1 and 2). When viewed as a histogram, these data present a single broad peak at a mean Te about midway between the two Te peaks of the intact dog (Fig. 3).

Central vs. peripheral chemoreceptor sensitivity to hypocapnia. When the dogs were intact, apnea occurred (Te = 8.4 ± 1 s) after the second ventilator breath (10–11 s of PSV); this required a decrease in PETCO2 of 5.1 ± 0.4 Torr. After CBX, as shown above (Figs. 1 and 2), PB never occurred in response to PSV-induced hypocapnia. However, given time, Te was always prolonged in the CBX dogs, and this allowed us to calculate an apneic threshold (see METHODS). After CBX, an apnea equivalent in length to that seen intact (8.4 ± 1 s) required a twofold decrease in PETCO2 of 10.1 ± 2.1 Torr (range = 8.2–12.9 Torr; significantly different from intact, P < 0.02), relative to intact, and this apnea occurred after ~33 s of PSV (Fig. 4). These differences in ΔPETCO2 between intact and CBX coincided with changes in the slopes of the ventilatory responses below eupnea, i.e., from apnea to eupnea (ΔVT/ΔPETCO2, where VT is inspiratory minute ventilation, in l·min−1·Torr−1). For dogs G, J, N, and Ni, respectively, the intact slopes were 0.74, 0.75, 0.59, and 0.38; slopes after CBX were 0.28, 0.26, 0.21, and 0.24, respectively.

When the dogs were intact, apnea required an increase in estimated pHα of 0.036 ± 0.005 and estimated CSF pH of 0.052 ± 0.005. After CBX, an apnea equivalent to that seen in intact animals required increases in pHα of 0.068 ± 0.016 (significantly different from intact dogs, P < 0.03) and estimated CSF pH of 0.094 (significantly different from intact, P < 0.02). Thus, with CBX, baseline PCO2 was increased and pH was reduced in both arterial blood and (presumably) CSF. The changes in PCO2 and pH required in the CBX dog to cause the same apneic length as was observed when the dog was intact both increased about twofold.

### Table 1. Mean eupneic values during spontaneous breathing in NREM sleep before and after CBX

<table>
<thead>
<tr>
<th>Dog</th>
<th>T1, s</th>
<th>Te, s</th>
<th>VT, liter</th>
<th>fbreaths/min</th>
<th>pHα</th>
<th>PCO2, Torr</th>
<th>PO2, Torr</th>
<th>[HCO3]−, mEq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>G intact</td>
<td>1.5 ± 0.2</td>
<td>4.5 ± 0.8</td>
<td>0.32 ± 0.03</td>
<td>10.4 ± 1.7</td>
<td>7.394 ± 0.008</td>
<td>35.7 ± 0.9</td>
<td>98 ± 3</td>
<td>21.3 ± 0.7</td>
</tr>
<tr>
<td>G CBX</td>
<td>1.6 ± 0.2</td>
<td>5.9 ± 0.9</td>
<td>0.28 ± 0.04</td>
<td>8.2 ± 1.1</td>
<td>7.340 ± 0.007</td>
<td>46.6 ± 2.6</td>
<td>88 ± 7</td>
<td>24.6 ± 1.4</td>
</tr>
<tr>
<td>J intact</td>
<td>1.4 ± 0.2</td>
<td>4.0 ± 0.9</td>
<td>0.29 ± 0.02</td>
<td>11.6 ± 2.6</td>
<td>7.369 ± 0.004</td>
<td>39.9 ± 0.1</td>
<td>110 ± 3</td>
<td>22.2 ± 0.1</td>
</tr>
<tr>
<td>J CBX</td>
<td>1.3 ± 0.1</td>
<td>3.1 ± 0.7</td>
<td>0.19 ± 0.04</td>
<td>14.2 ± 2.5</td>
<td>7.340 ± 0.011</td>
<td>51.1 ± 1.1</td>
<td>85 ± 6</td>
<td>26.8 ± 1.3</td>
</tr>
<tr>
<td>N intact</td>
<td>1.4 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>0.22 ± 0.03</td>
<td>14.2 ± 0.9</td>
<td>7.356 ± 0.025</td>
<td>36.5 ± 0.5</td>
<td>97 ± 2</td>
<td>21.3 ± 1.0</td>
</tr>
<tr>
<td>N CBX</td>
<td>1.4 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>0.18 ± 0.03</td>
<td>15.4 ± 4.7</td>
<td>7.354 ± 0.002</td>
<td>45.9 ± 0.8</td>
<td>88 ± 3</td>
<td>24.9 ± 0.2</td>
</tr>
<tr>
<td>Ni intact</td>
<td>1.5 ± 0.3</td>
<td>3.4 ± 0.6</td>
<td>0.16 ± 0.04</td>
<td>12.8 ± 1.2</td>
<td>7.359 ± 0.019</td>
<td>37.9 ± 0.3</td>
<td>111 ± 3</td>
<td>21.8 ± 0.9</td>
</tr>
<tr>
<td>Ni CBX</td>
<td>1.3 ± 0.1</td>
<td>3.7 ± 0.5</td>
<td>0.17 ± 0.02</td>
<td>12.2 ± 1.0</td>
<td>7.341 ± 0.013</td>
<td>45.3 ± 1.8</td>
<td>95 ± 9</td>
<td>23.9 ± 0.7</td>
</tr>
<tr>
<td>Intact mean</td>
<td>1.5 ± 0.1</td>
<td>3.7 ± 0.6</td>
<td>0.25 ± 0.06</td>
<td>12.3 ± 1.5</td>
<td>7.377 ± 0.014</td>
<td>38.0 ± 1.9</td>
<td>104 ± 7</td>
<td>21.7 ± 0.4</td>
</tr>
<tr>
<td>CBX mean</td>
<td>1.4 ± 0.1</td>
<td>3.9 ± 1.2</td>
<td>0.21 ± 0.04</td>
<td>12.5 ± 2.7</td>
<td>7.343 ± 0.006*</td>
<td>47.2 ± 2.3*</td>
<td>89 ± 4*</td>
<td>25.1 ± 1.1*</td>
</tr>
</tbody>
</table>

Values are means ± SD. NREM, non-rapid eye movement; CBX, carotid body denervation; T1, inspiratory time; Te, expiratory time; VT, tidal volume; fβ, breathing frequency; PCO2, arterial PO2; PO2, arterial PO2 [HCO3]−, HCO3 concentration; G, J, N, Ni, dog designation. *Significantly different from intact mean (P < 0.05).
Hyperoxia. In three of the four dogs, we completed several trials of PSV under hyperoxic conditions (PET\textsubscript{CO\textsubscript{2}} of \(~300\text{--}350\) Torr) when the dogs were still neurally intact. Steady-state hyperoxia had no consistent effect on spontaneous breathing pattern or PET\textsubscript{CO\textsubscript{2}}. As shown in Fig. 5, hyperoxia did not prevent rapid development of apneas in response to PSV-induced hypocapnia, as prolonged TE (>3 SD than control mean) occurred by the second or third PSV breath. However, as shown in the histogram in Fig. 3, relative to normoxia, 1) the average apneic lengths in hyperoxia were shorter (6.0 ± 1.6 vs. 8.4 ± 1 s), 2) PB was transient and not tightly organized in doublets as seen in intact dogs, and 3) periodicity ceased after \(~40\) s of PSV, although TE was prolonged (see Fig. 5). Thus the effects of hyperoxia on hypocapnia-induced PB were very similar to those following CBX except for the rapidity with which TE was prolonged after the ventilatory overshoot.

Neuromechanical effects of PSV. PSV in both intact and CBX dogs had an immediate effect on TE and EMG\textsubscript{dia}. In the intact dog, the first breath of PSV prolonged TE to 121 ± 16\% of spontaneous eupnea and reduced diaphragm EMG to 78 ± 8\% of the spontaneously breathing, eupneic values. These prolongations of TE on the first breath of PSV (+0.6 ± 0.4 s) were only
a small fraction of the apnea length observed after the second or third breath (4.7 ± 1.1 s). After CBX, the first breath of PSV also prolonged T_E to 122 ± 16% (not significantly different from intact) and reduced EMGdia to 85 ± 10% (not significantly different from intact) of the spontaneously breathing, eupneic values (Table 2).

**DISCUSSION**

Our study used intact and CBX sleeping dogs subjected to varying levels of pressure support ventilation to address the role of the carotid chemoreceptors in hypocapnic-induced apnea and PB. By contrasting the time course of responses of intact vs. CBX animals to PSV, we determined that the presence of carotid chemoreceptors was required to produce the apnea normally caused by a transient hyperventilation and hypocapnia and that these receptors were also required for the PB pattern produced by PSV. Hyperoxia in the intact animal did not (unlike CBX) prevent the immediate apnea in response to transient hyperventilation but (like CBX) prevented prolonged apneas and a distinct periodic pattern. Finally, the level of hypocapnia required to cause apnea in the CBX animal was found to be about two times that required for apneas of similar length in the intact animal. Implications of these findings to human sleep apnea are discussed.

**Critical role of carotid chemoreceptors in hypocapnic-induced apnea.** Our data in sleeping dogs point strongly to an obligatory role for carotid chemoreceptors in causing the central apneas that commonly occur after transient ventilatory overshoots, which drive PaCO2 below the apneic threshold. First, apneas occurred within 10–12 s or two breaths of the onset of PSV and the transient rise in VT and fall in PETCO2. More to the point, denervation of the carotid chemoreceptors prevented the occurrence of apnea within its normal short latency after the ventilatory overshoot. Apneas still occurred in the CBX animal but not until ~33 s after the ventilatory overshoot, and PB never occurred. So, carotid chemoreceptors appear to be obligatory in causing the apneas during the time period in which they commonly occur as a result of brief, transient, ventilatory overshoots. This means that hypocapnia-induced apnea required an ~5 Torr reduction in PaCO2, relative to eupnea, at the carotid chemoreceptor.

**Is CB hypocapnia sufficient by itself to cause apnea?** On the one hand, there is ample evidence demonstrating substantial sensitivity of the carotid chemoreceptors to changes in pH and CO2 (see Introduction). On the other hand, there are also several lines of evidence...
to suggest that additional mechanisms beyond just CB hypocapnia, per se, contribute to the apnea after transient hyperventilation.

First, our own previous data in intact sleeping dogs showed that progressive step changes in hypocapnia of CB PCO$_2$ of $-3$ to $-15$ Torr applied via extracorporeal perfusion to the isolated carotid chemoreceptor caused an immediate (within 5 s) dose-dependent reduction in VT and minute ventilation; however, apnea or substantial changes in breath timing did not occur. Second, even extreme hyperoxia (CB PCO$_2$ $> 500$ Torr) applied to the isolated carotid chemoreceptor did not cause apnea (42). So, how can the carotid chemoreceptors be required for apneas that occur after a transient ventilatory overshoot but not cause apnea when subjected to CB-specific transient hypocapnia (or even hyperoxia)?

One key difference between the studies is that the transient hypocapnia in the present study was produced by an increased VT (via PSV), whereas the hypocapnia presented to the isolated CB via extracorporeal perfusion was not accompanied by a ventilatory overshoot. The increase in VT may exert two additional types of potential inhibitory effects on respiratory motor output. First, within-breath oscillations in PaCO$_2$ and pH would be enhanced with an augmented VT, which in turn would be sensed by carotid chemoreceptors and therefore affect respiratory motor output, depending on when in the respiratory cycle the peak or nadir of the PCO$_2$ oscillation arrived at the CB, i.e., a “gating” effect on respiratory motor output (14, 17). For example, to cause TE prolongation, the trough of the PaCO$_2$ (and alkaline peak of arterial blood pH) would have to arrive at the carotid chemoreceptors during early expiration. On the other hand, even substantial oscillations in arterial pH caused only 30% prolongations of TE in anesthetized dogs (14) and removal of all normal oscillations in PaCO$_2$ in anesthetized (44) or awake (42) animals did not influence ventilatory output significantly, at least under conditions of resting CO$_2$ production.

Second, the feedback effects of increased VT and lung stretch also would have accompanied ventilatory overshoot but not specific CB hypocapnia via CB perfusion. Certainly, lung stretch by itself could not have caused apnea because apnea did not occur immediately after the ventilatory overshoot in the CBX animal (see Fig. 2) nor did apnea occur when hypocapnia was prevented [via increased inspired CO$_2$ fraction (FICO$_2$)] during the

Table 2. Mean T$_i$, T$_e$, and EMGdia of the first breath of all PSV trials when dogs were intact and after CBX

<table>
<thead>
<tr>
<th>Dog</th>
<th>Intact T$_i$, % of spontaneous</th>
<th>CBX T$_i$, % of spontaneous</th>
<th>Intact T$_e$, % of spontaneous</th>
<th>CBX T$_e$, % of spontaneous</th>
<th>Intact EMGdia, % of spontaneous MEA</th>
<th>CBX EMGdia, % of spontaneous MEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>94 ± 15</td>
<td>103 ± 14</td>
<td>102 ± 22</td>
<td>95 ± 15</td>
<td>76 ± 16*</td>
<td>70 ± 18*</td>
</tr>
<tr>
<td>J</td>
<td>104 ± 12</td>
<td>112 ± 15*</td>
<td>117 ± 16*</td>
<td>127 ± 32*</td>
<td>74 ± 14*</td>
<td>83 ± 14*</td>
</tr>
<tr>
<td>N</td>
<td>111 ± 10*</td>
<td>133 ± 20*</td>
<td>117 ± 30*</td>
<td>138 ± 20*</td>
<td>69 ± 17*</td>
<td>98 ± 20</td>
</tr>
<tr>
<td>Ni</td>
<td>119 ± 18*</td>
<td>115 ± 14*</td>
<td>146 ± 31*</td>
<td>128 ± 32*</td>
<td>91 ± 17*</td>
<td>89 ± 15*</td>
</tr>
<tr>
<td>Mean</td>
<td>107 ± 9</td>
<td>116 ± 11</td>
<td>121 ± 16</td>
<td>122 ± 16</td>
<td>78 ± 8</td>
<td>85 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE. PSV, pressure support ventilation; MEA, mean electrical activity.
PSV-induced ventilatory overshoot (30). However, during the ventilatory overshoot, rapid decreases in PaCO₂ and carotid sinus nerve activity occur coincident with lung stretch. Bajic et al. (5), using recordings from medullary respiratory neurons, showed that these combined carotid chemoreceptor-mechanoreceptor inputs have a clear additive interaction at the level of the central respiratory controller. Perhaps, then, this interactive effect was required to cause apnea. This is a hypothesis that may be tested by determining the effects of vagal blockade on the response to transient hyperventilation.

Our findings do not rule out a significant contribution from central chemoreceptors to the hypocapnic-induced apnea in the intact animal. The recent work of Okada et al. (35), Ballantyne and Scheid (6), and Richardson et al. (37), for example, supports the idea of a perivascular location for many chemosensitive medullary neurons. Older studies have shown, on the basis of medullary surface pH measurements, that pH can change very rapidly, approximating the delay from ventilatory overshoot to apnea (1, 16, 25). Taken together, these data are consistent with rapidly responding central chemoreceptors. That said, it is important to recognize that most evidence suggests that the functional (i.e., respiratory motor output) response of the central chemoreceptors alone is relatively slow (25–30 vs. 10–15 s after ventilatory overshoot in an intact animal (Refs. 9, 10, present study)). These data all come from studies that utilized CBX. However, whatever mechanisms may be responsible for the slow central response time in CBX animals, CB are required to produce apneas in response to transient hypocapnia. So, although we believe the most straightforward interpretation of available data indicates that central chemoreceptors respond to hypocapnia relatively slowly, we acknowledge the possibility that in the intact animal the sensitivity and/or response time of central chemoreceptors might be significantly enhanced by tonic or phasic input from the carotid chemoreceptors and thereby contribute significantly to the observed apnea (see Relative CO₂ sensitivities of the peripheral and central chemoreceptors below).

In summary, our past and present data in intact and CBX sleeping dogs, respectively, demonstrate that, although carotid chemoreceptors are essential to explain apneas caused by the hypocapnia normally resulting from transient ventilatory overshoots, the effects of CB hypocapnia, per se, are not sufficient to cause these apneas. Additional interactive effects with carotid chemoreception appear to be necessary to explain the apneas in the intact animal.

Relative CO₂ sensitivities of the peripheral and central chemoreceptors. The degree of hypocapnia (or change in pH) required in the CBX animal (and therefore presumably of the medullary chemoreceptors) was about twice that in the intact animal to achieve the same apnea length. We note that absolute values for PETCO₂ at the apneic threshold were relatively hypercapnic after CBX, because the eupneic PETCO₂ was elevated. However, it is not this absolute value but rather the magnitude of the change in PETCO₂ that is required to produce apnea that defines the sensitivity of the CO₂ response below eupnea (see Potential limitations of the experimental model below). Although our ability to quantify the apneic threshold (i.e., the ΔPETCO₂ from eupnea to apnea) in the CBX dog in the absence of PB was not as precise as in the intact dog, our approach did provide a conservative estimate of the differences in apneic threshold between intact and CBX conditions (see METHODS).

These differences in ΔPETCO₂ (eupnea – apnea) and in ΔV/ΔPETCO₂ imply that the carotid chemoreceptor sensitivity to hypocapnia (at least the sensitivity that was elicited by transient changes in PCO₂ resulting from a ventilatory overshoot) is substantially greater than that of the central chemoreceptors. Cross et al. (14) and Borison et al. (8) also showed little change in breath timing in response to increases in CO₂ and reductions in pH in CBX vs. intact anesthetized animals. Furthermore, in our studies in the isolated, intact, perfused CB, sustained CB hypocapnia alone caused hypoventilation that persisted for >2 min, even in the face of substantial systemic (PaCO₂ of +5–7 Torr) and presumably central nervous system hypercapnia (42). Only when CB hypocapnia was suddenly removed was the ventilatory response to systemic hypercapnia manifested in a marked ventilatory overshoot. Apparently then, the inhibitory effects of a persistent CB hypocapnia masked the excitatory influence of a substantial brain extracellular fluid acidosis.

On the other hand, CBX preparations, as presently used by us and others (8), removes the normal tonic input to the medullary pattern generator. Nattie (31) and Cohen (13) recently emphasized the importance of maintaining tonic inputs intact when quantification of chemoreceptor drives was attempted. We also showed qualitatively different ventilatory responses to systemic hypoxia in CBX animals vs. animals with the isolated CB intact and maintained normoxic and normocapnic via extracorporeal perfusion (15). Accordingly, our present estimates of central chemoreceptor sensitivity to hypocapnia in the CBX animals may underestimate the actual gain and thus its relative importance to apnea and PB.

In summary, we propose that both past and present findings point to a relatively high sensitivity to hypocapnia at the level of the carotid chemoreceptors that may exceed that of the medullary chemoreceptors. To test this hypothesis, additional experiments using the intact, isolated CB perfusion model are needed to quantify the sensitivity of the central chemoreceptors to systemic hypocapnia in the presence of normal tonic input from the carotid chemoreceptors.

Potential limitations of the experimental model. We used PSV to produce graded increases in Vt and thereby graded decreases in PETCO₂. This method readily produces PB in normal, intact human subjects (29, 31, 50), and the ΔPETCO₂ (eupnea – apnea) at which PB occurs is quite reproducible. However, this method also causes some neuromechanical inhibition of ventilatory drive with the primary effect
being a reduction in the amplitude of the EMGdia and minor effects on breath timing (see Table 2). That these effects observed on the first cycle of PSV (Fig. 1, A and B) are not attributable to chemoreceptor inhibition is shown by the almost identical changes obtained in intact vs. CBX dogs (see Table 2). Similar, relatively small changes in breath timing and EMG amplitude occurred throughout 1–2 min of PSV that was applied to the intact animal when FICO2 was raised to prevent PETCO2 below baseline normocapnic values (30). These data support the contention that the apneas and PB produced by the PSV method are attributable to the accompanying hypcapnia rather than to any mechanical effects of PSV.

Our use of CBX had two major relevant side effects: 1) removal of tonic sensory input to the medullary central pattern generator, which we discussed above (Relative CO2 sensitivities of the peripheral and central chemoreceptors); and 2) resultant hypoventilation and CO2 retention. The change in the background drive to breathe and in Paco2 is an important determinant of the sensitivity to imposed hypcapnia, as judged by the ΔPETCO2 (eupnea – apnea) required to produce apnea (or hypopnea). For example, an increase in the drive to breathe and a decrease in eupneic Paco2 via metabolic acidosis or pharmacological CB stimulation in the intact animal was shown to increase the ΔPETCO2 between eupnea and the apneic threshold [as was determined with PSV used in the same way as in the present study (30)]. Because CBX decreased the background drive and increased eupneic Paco2, we need to ask what effect we would expect this change in baseline conditions to have on the sensitivity to hypcapnia. Specifically, might this change in background conditions alone explain some or even all of the apparent CO2 insensitivity, i.e., increased ΔPETCO2, in the CBX animal? Previous work used metabolic alkalosis to reduce the background drive and increase Paco2 in the intact animal, and this resulted in a significant decrease in the ΔPETCO2 (and ΔpH), showing an enhanced sensitivity to hypcapnia (30). Thus the reduction in background drive and the increase in absolute Paco2 in the CBX animal by themselves do not account for the substantial increase in ΔPETCO2 between eupnea and the apneic threshold (Fig. 4).

Relevance to human sleep apnea and periodicity. Our data in the sleeping dog showing the critical importance of the carotid chemoreceptors in causing apnea after ventilatory overshoots and subsequent PB has considerable relevance to mechanisms for apnea and PB in the sleeping human, including the CSR of CHF (11, 34), idiopathic central sleep apnea (4, 23, 33, 47, 48), and hypoxia-induced PB (7).

First, the timing of central apneas in humans is consistent with a pivotal role for carotid chemoreceptors. In CHF patients with CSR, the time from the breath with the nadir PETCO2 during the ventilatory overshoot until the onset of the resulting apnea approximates the lung-to-CB delay (27). Furthermore, when PSV was used in sleeping humans to increase VT sufficiently to reduce PETCO2 below the apneic threshold, two PSV breaths (or 10–12 s) were sufficient to cause apnea (46). Finally, increased FlCO2 in sleeping humans with PB was shown to eliminate apnea within 10–15 s of the onset of its application (3, 7, 47).

Second, the magnitude of the ventilatory responsiveness to transient (single breath) increases in FlCO2 was shown to correlate positively with the presence of PB in CHF and in idiopathic central sleep apnea (43). The fact that the degree of peripheral chemosensitivity (as was estimated from the transient hypcapnia test) accounted for <50% of the variance in the apnea hypopnea index among all patients (43) may be reflective of the importance of both the chemoreceptor-driven propensity to ventilatory overshoot as well as the relative sensitivity of the hypcapnia-induced apneic threshold in causing central sleep apnea.

Third, we found that, unlike CBX, hyperoxia did not prevent significant Tε prolongation immediately after transient ventilatory overshoots. These findings are consistent with recordings of carotid sinus nerve activity in the anesthetized cat (26) and the effects of CBX in the awake cat and dog (22, 38), which showed that CB chemoreceptors remained sensitive to CO2 even in the presence of hyperoxia. On the other hand, with hyperoxia, hypcapnia-induced apneas were significantly shorter than in normoxic hypcapnia, and, like CBX, clear PB was not sustained. The findings are analogous to the effects of supplemental oxygen treatment in patients with central apnea and CSR, in whom the number of apneas and hypopneas was significantly reduced but not eliminated (2, 21, 27).

We thank Kathleen S. Henderson for many contributions to this study.

This research was supported in part by National Heart, Lung, and Blood Institute Grants HL-50531 and HL-62561 and Veterans AdministrationMerit Review.

Present address of H. Nakayama: Division of Respiratory Medicine, Niigata University Graduate School of Medical and Dental Sciences, 1-757, Asahimachi-dori, Niigata 951-8510, Japan.

REFERENCES


J Appl Physiol • VOL 94 • JANUARY 2003 • www.jap.org