Effect of central CO\textsubscript{2} drive on lung inflation responses of expiratory bulbospinal neurons in dogs

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Effect of central CO\textsubscript{2} drive on lung inflation responses of expiratory bulbospinal neurons in dogs. Am J Physiol Regulatory Integrative Comp Physiol 279: R1606–R1618, 2000.—The purpose of these studies is to better understand the nature of the reflex interactions that control the discharge patterns of caudal medullary, expiratory (E) bulbospinal neurons. We examined the effect of central chemodrive inputs measured as arterial CO \textsubscript{2} tension (PaCO\textsubscript{2}) during hypoxia on the excitatory and inhibitory components of the lung inflation responses of these neurons in thiopental sodium-anesthetized, paralyzed dogs. Data from slow ramp inflation and deflation test patterns, which were separated by several control inflation cycles, were used to produce plots of neuronal discharge frequency (F\textsubscript{n}) versus transpulmonary pressure (P\textsubscript{t}). Pt was used as an index of the activity arising from the slowly adapting pulmonary stretch receptors (PSRs). Changes in inspired CO\textsubscript{2} levels that ranged from 20 to 80 mmHg. The data obtained from 41 E neurons were used to derive an empirical model that quantifies the average relationship for F\textsubscript{n} versus both P\textsubscript{t} and PaCO\textsubscript{2}. This model can be used to predict the time course and magnitude of E neuronal responses to these inputs. These data suggest that the interaction between PaCO\textsubscript{2} and PSR-mediated excitation and inhibition of F\textsubscript{n} is mainly additive, but synergism between PaCO\textsubscript{2} and excitatory inputs is also present. The implications of these findings are discussed.

control of breathing; central integration; central chemodrive; pulmonary stretch receptors

EXPIRATORY (E) bulbospinal neurons make up a great majority of the E neurons in the caudal portion of the ventral respiratory group (VRG) in the region of the nucleus retroambigualis (4, 21). E bulbospinal neurons are known to have both monosynaptic and polysynaptic connections with contralateral spinal cord thoracic E motoneurons (9, 16, 17). It also appears that lumbar E motoneurons receive mono- and polysynaptic inputs from the contralateral E bulbospinal neurons (22). E bulbospinal neurons are the major source of drive for thoracic and abdominal E motoneurons (3) and, therefore, are responsible for active expiration. In addition, E bulbospinal neurons provide inhibition (presumably via interneurons) to thoracic inspiratory (I) motoneurons during expiration (29).

The excitability of E bulbospinal neurons is highly dependent on arterial CO\textsubscript{2} tension (PaCO\textsubscript{2}) activation of central chemosensory sources with the greatest sensitivity being found over the hypo- to normocapnic range (2). In addition, these neurons receive excitatory inputs from carotid chemoreceptors (18, 30) and both excitatory and inhibitory inputs from pulmonary mechanoreceptors with vagal afferent fibers (4, 9). On the basis of spontaneous discharge patterns and responses to lung inflation, the E bulbospinal neurons in dogs can be divided into two types: type A, augmenting (20–30%), and type D, decrementing (70–80%) (4). Graded inhibition of type A neurons is produced by lung inflation when transpulmonary pressure (P\textsubscript{t}) exceeds 3–4 mmHg. For type D neurons, studies using step and slow positive or negative ramp inflations (1–2 mmHg/s) demonstrate that the relationship between neuronal discharge frequency (F\textsubscript{n}) and P\textsubscript{t} is made up of two major, linear components (4). For 1.5 ≤ P\textsubscript{t} ≤ 4.5 mmHg, the relation is positive (excitatory), whereas for 4.5 ≤ P\textsubscript{t} ≤ 20 mmHg, the relation is negative (inhibitory). The inhibition is strong enough to override the excitation. Because the excitatory and inhibitory responses to step inflations are slowly adapting, it is highly likely that the slowly adapting pulmonary stretch receptors (PSRs) are involved. Two types of canine PSRs have been identified based on the relationship of their discharge frequency to P\textsubscript{t} and on their anatomic location in the airways (23, 24). Because the characteristics of these two PSR types coincide with the response characteristics of the type D (decrementing) E bulbospinal neurons, it is possible that these two different types of PSRs mediate the excitatory and inhibitory components.

The activity of E bulbospinal neurons is sensitive to vagal feedback from pulmonary mechanoreceptors and to central and peripheral chemosensory stimulation and thus can influence most of the mechanical properties of ventilation, such as tidal volume, breathing...
frequency, airflow rate, and end-expiratory lung volume. In response to metabolic demands, they appear to play a significant role in adjusting ventilatory mechanisms to provide efficient performance. These studies were undertaken to characterize the interaction between PSR and central chemosensory inputs on the discharge patterns of the type D E neurons of dogs.

METHODS

Experimental preparation. Experiments were performed on 18 mongrel dogs (10–20 kg) anesthetized with thiopental sodium (induction dose: 15 mg/kg iv; additional doses given as needed during preparation; maintenance dose during data collection, 4–8 mg·kg⁻¹·h⁻¹ iv continuous infusion). Positive-pressure constant flow ventilation was produced by an alternating two-valve solenoid ventilator through a cuffed endotracheal tube using 100% O₂. Airway CO₂ was measured with an infrared analyzer (Instrumentation Laboratory IL-200), and tracheal pressure was measured from an airway sideport with an air-filled catheter connected to a Gould-Statham P23ID transducer. Arterial pressure was measured from a femoral artery fluid-filled catheter using a Gould-Statham P23ID transducer. Arterial blood samples were obtained hourly for the measurement of pH, PaCO₂, and PaO₂ using a Radiometer ABL 1 analyzer. When required, metabolic acidosis (base deficit >5 mM) was corrected with an appropriate amount of sodium bicarbonate in saline. The dogs were monitored for signs of inadequate anesthesia, including movement, salivation, lacrimation, and/or increases in blood pressure and heart rate. The anesthetic depth was increased immediately if such signs were present. Esophageal temperature was maintained at 38 ± 1°C using a servocontrolled heating pad.

The dogs were positioned in a Kopf (model 1530) stereotaxic apparatus with the head ventrally flexed by 30°. The right C₅ phrenic nerve rootlet and both vagi were exposed by dorsolateral neck dissection. The medulla oblongata was exposed by occipital craniotomy, cutting the dura mater along the midline, and retracting the dura with silk sutures. This procedure exposed the dorsal surface of the medulla from 2 mm rostral to 10 mm caudal to the obex and 5 mm bilaterally from the midline. To further stabilize the brain stem before recording unit activity and to minimize feedback from nonvagal, chest wall afferents, the animals were paralyzed with pancuronium bromide (Pavulon), initial dose of 0.1 mg/kg iv, followed by supplemental doses of 0.05 mg/kg as required, and a bilateral pneumothorax was created. Thus in these studies, tracheal and transpulmonary pressure, Pₜ, are equivalent.

Data recording. Efferent phrenic activity, spike potentials from the brain stem neurons, airway CO₂ concentration, tracheal pressure, and blood pressure were recorded on an FM tape recorder (Vetter, model D). The above-mentioned parameters and time-averaged phrenic activity (PNG), neural spikes/100 ms, I duration (Tᵢ), and E duration (Tₑ) were recorded on a Grass model 7 polygraph. Phrenic recordings were obtained with bipolar electrodes from the desheathed central end of the C₅ rootlet, which was immersed in a mineral oil pool formed from a neck pouch. The phrenic nerve signal was amplified with a band pass of 0.1–3 kHz. The online moving time average of the phrenic activity was obtained by full-wave rectification and low-pass filtering (averaging window = 50 ms). The positive PNG slope at the onset of phrenic activity and the negative PNG slope at the onset of the abrupt decline in phrenic activity were used to generate I and E timing pulses, respectively. These timing pulses were used to compute online values of Tᵢ and Tₑ.

Extracellular single-unit recordings from caudal VRG E neurons were obtained using tungsten metal microelectrodes (10–15 MΩ at 1,000 Hz). Locations of recorded neurons relative to obex were in a region 2–4 mm caudal, 2.5–4.5 mm lateral to the midline, and 2–4 mm below the dorsal medullary surface. A time-amplitude window discrimination was used to generate a standard pulse for each spike. Online neuronal spike frequency was determined as spikes per 100 milliseconds, whereas offline data analysis used spikes per 10 milliseconds.

Protocol. During control cycles, the ventilator frequency was adjusted to be near that of the central respiratory rhythm (as indicated by PNG) and entrained the central pattern via PSR feedback. Once a type D expiratory neuron was located in the caudal VRG at normocapnia, ventilatory tidal volume was increased to produce a hypocapnic PaCO₂ level of 12–20 mmHg. After airway end-tidal CO₂, Fₑ, and peak PNG (if central inspiratory rhythm was still present) had reached a steady state (usually after 3–5 min), four slow positive (+) and four negative (−) test ramp inflations separated by six to ten control ventilator cycles were applied. These test inflations had duration of 6–10 s and produced peak Pₜ values of 12–20 mmHg. Arterial blood samples were drawn for measurement of PaCO₂, pH, and PaO₂. To obtain several steady-state PaCO₂ levels over the range of 20–80 mmHg, CO₂ was added to the inspired O₂ via a gas blender. Neuronal responses to the ramp inflations were obtained for one to eight steady PaCO₂ levels, averaging four PaCO₂ levels per neuron.

Data reduction. Offline data analyses were carried out on a Hewlett-Packard model 360 computer with a data converter interfaced through an IEEE 488 data port. A conversion rate of 100 Hz was used to enter 5- or 10-min epochs of the number of neuronal spikes per 10 milliseconds, phrenic activity, time averaged for 10 ms, tracheal pressure, and a ventilator I phase indicator into the computer memory and subsequently onto a disk file. Software routines assigned a number to each consecutive ventilation cycle and displayed the signals on the monitor. Ventilator cycles were numbered consecutively, and the numbers corresponding to a given test inflation pattern, (+) or (−) ramps, at a given PaCO₂ level were identified and used to generate cycle-triggered histograms (CTHs) of unit activity and ensemble averages of both phrenic activity and tracheal pressure patterns. The temporal alignment of the CTHs and ensemble averages was accurate to within 10 ms of the phase onset indicator signal. CTHs and ensemble averages were saved on disk files for further analyses, which included the generation of Fₑ versus Pₑ plots for test inflation cycles.

Least-squared-error linear and nonlinear regressions were used to quantify the Fₑ-dependent effects on Fₑ, and the PaCO₂-dependent effects on the Fₑ-Pₑ relationship parameters. Data for these analyses were obtained from Fₑ-Pₑ plots using the CTHs for Fₑ and corresponding ensemble averages of Pₑ. Data are presented as mean values with SEs, unless otherwise stated. Probability levels of P < 0.05 were used to indicate significant differences.

RESULTS

Expiratory neuronal responses to step lung inflations delivered during the expiratory phase. The E neuronal responses to step inflations during the E phase are...
slowly adapting and related to the magnitude of the step $P_t$, in a biphasic manner. In the example of Fig. 1, the 4-s-long step test inflations ($P_t$) were delayed 300 ms from the beginning of the E phase and were separated by 8–10 control cycles to minimize changes in $P_{aCO_2}$ during subsequent test cycles. During control and test cycles, 1-s duration inflations were delivered during the I phase to provide steady-state ventilation. For $P_t$ values >5 mmHg, step inflations produced reflex increases in $T_E$ and increases in $F_n$ (Fig. 1A). A step inflation of 1.3 mmHg prolonged the E phase and the neuronal discharge (Fig. 1A, S1) of an E neuron with a decrementing control pattern, whereas larger step inflations of 2.6 and 4.8 mmHg increased $F_n$ of the step responses (Fig. 1, A and B, S2 and S3). For $P_t$ >5 mmHg, step inflations reduced $F_n$ below maximum response values and the responses remained relatively independent of time during the step inflation (e.g., Fig. 1B, S4 = 10.6 mmHg). The neuronal responses are mainly dependent...
on inflation pressure and only to a small degree on time. This allowed us to quantify the biphasic neuronal response to inflation using plots of $F_n$ versus $P_t$, where $P_t$ is used as an index of slowly adapting PSR activity (Fig. 1B).

E neuronal responses to slow ramp lung inflations delivered at different times during the E phase. The relationship between $F_n$ and $P_t$ can also be obtained when slow ramp inflations are used to scan the entire $P_t$ range from 0 to 15–20 mmHg in a single test cycle. This is possible because time-dependent effects on these responses are minimal. This is illustrated for an E neuron where slow ramp test inflations were delivered in the E phase with different delays with respect to the onset of the PNG (Fig. 2A). The biphasic nature of the response can be seen for the ramp inflation with the larger delay (Fig. 2A). This delay was the largest one that could be used, because the control $T_E$ was $<2$ s ($T_{EO}$, Fig. 2A) and the next I phase would start before the inflation reflexly prolonged the E phase. Near the beginning of the E phase, the early portion of the control decrementing pattern can be seen before the ramp inflation starts (Fig. 2A, $F_n$). As the ramp $P_t$ increased, $F_n$ increased and reached a maximum at $P_t \approx 5$ mmHg, then decreased with increasing $P_t$. Note that the amount of inhibition is more than able to suppress the $P_t$-induced excitation. When the ramp inflation was terminated, $F_n$ rapidly increased and then decreased as the subsequent I phase began. For those cycles in which the slow ramp inflations began 2 s earlier, the early portion of the response was not seen because I-phase inhibition produced neuronal silence (Fig. 2A). The lack of time dependence in these neuronal inflation responses, separated by 2 s, is demonstrated by the high degree of overlap in the $F_n$ traces when the two $P_t$ traces are superimposed (Fig. 2B). The plot of $F_n$ versus $P_t$ for the more delayed ramp inflation quantifies this biphasic relationship (Fig. 2B). This relationship is similar to the one for step inflations (Fig. 1B).

Fig. 3. Effect of arterial $\text{PaCO}_2$ ($\text{PaCO}_2$) on E neuronal responses to slow positive ramp inflations. $F_n$: ratemeter output (0.1-s intervals). Vertical dashed lines: onset of the E phase of the test cycle. Slow ramps delayed 1.8 s from onset of E phase. $F_n$ of both control and biphasic inflation response patterns increased with increased $\text{PaCO}_2$.

Fig. 4. Effect of $\text{PaCO}_2$ on E neuronal responses to slow negative ramps for the same neuron shown in Fig. 3. Vertical dashed lines: onset of the E phase of the test cycle. E airflow resistance added during test cycle to produce slow negative ramps with no delay. As $P_t$ decreased, the decline in inhibition resulted in increased $F_n$. With further decreases in $P_t$, excitation was also removed. Peak $F_n$ of both control and test cycles increased with increased $\text{PaCO}_2$.
The effects of PaCO2 on E neuronal responses to positive and negative slow ramp inflations. We used both positive and negative test ramp inflations for the same neuron to better isolate Pt-dependent effects from time-dependent effects. By using “contrasting” pressure-time profiles, the amount of time dependence affecting the neuronal response to inflation will be reflected in the difference between $F_n$-Pt plots for each type of inflation pattern. In the absence of time dependence, $F_n$-Pt plots obtained from positive and negative ramps should coincide.

Ventilation patterns synchronized with central rhythm. Examples of the responses to positive and negative ramps for the same neuron at different levels of PaCO2 are shown in Figs. 3 and 4. Both inflation patterns were alternately presented at each PaCO2 level. Increases in PaCO2 increased the peak $F_n$ of control cycles and enhanced the inflation-induced responses. For the positive ramps (Fig. 3), the biphasic nature of the response is evident and similar to those of Fig. 2. One-second duration control inflations were delivered during the I phase. However, to produce a slower central rhythm, a 1-s delay from the onset of the PNG was used to shift the inflation later into the I phase and subsequent deflation later into the E phase. This prevented too much reflex shortening of $T_i$ and produced longer $T_e$ values. On test cycles, no delay was used for the I-phase inflation to allow sufficient time for deflation and $P_t$ to return to baseline before the slow test ramp was initiated (1.8-s delay from onset of E phase, Fig. 3) that had to occur early enough to reflexly prolong $T_e$. The negative ramps were produced by an increase in E flow resistance during the test cycle ($P_t$, Fig. 4). Because $P_t$ is highest at the onset of the E phase, $F_n$ is initially reduced and gradually increases as the $P_t$-induced inhibition decreases, unmasking the $P_t$-induced excitation. The neuronal response peaks as $P_t$ passes through the inhibitory threshold and then declines as the $P_t$-induced excitation decreases. The peak $F_n$ is again highly dependent on PaCO2 (Fig. 4).

Central rhythm entrained by ventilation pattern. To investigate the effects of PaCO2 both above and below
the apneic threshold, the phenomenon of entrainment was used to time lock the central respiratory pattern to the ventilation pattern. For this purpose, the ventilator rate for control cycles is set close to the central respiratory rhythm rate, which can be determined from a few PNG cycles without ventilation. In the example of Fig. 5, the control inflation ($P_t$) can be seen to consistently occur during the early part of the E phase. For the test cycles, slow ramps replaced the control inflation pattern. Slow negative ramps were produced by adding an E flow resistance during test inflations. In addition, the I flow rate was increased to produce greater peak $P_t$ levels during the negative ramp test cycles (15–20 mmHg).

At all $P_{aCO_2}$ levels, a $P_t$-induced reduction in spike discharge frequency was seen during both the control and test cycles (Fig. 5). For the three levels of $P_{aCO_2}$ indicated, the traces are time aligned with respect to $P_t$. As $P_{aCO_2}$ decreased, the peak PNG also decreased and disappeared at the 35 mmHg $P_{aCO_2}$ level (Fig. 5, bottom trace). The E neuronal discharge rate also decreased, but the underlying pattern was preserved. Longer periods of neuronal silence occurred during the test inflation at the lower $P_{aCO_2}$ levels, indicating a baseline shift in discharge activity.

Typical plots of $F_n$ versus $P_t$ for positive (+) and negative (−) $P_t$ ramps at several $P_{aCO_2}$ levels are shown in Fig. 6, A and B, for two E neurons. Data values for these plots were obtained from CTHs of neuronal activity and ensemble averages of $P_t$ at corresponding times for all 50-ms bins during the test inflation. This analysis demonstrates that the typical biphasic nature of the $F_n$ versus $P_t$ relationship (e.g., Figs. 1B and 2B) is preserved regardless of whether positive or negative test inflations were used. A noticeable difference between positive and negative test inflation plots is the missing data points at low transpulmonary pressures (0–3 mmHg range) for negative test inflations. This is due to the reappearance of inspiratory activity when $P_t$ levels approached 0 mmHg and thus no longer reflexly prolonged the E phase. This effect manifests itself as a sharp fall in $F_n$ as $P_t$ decreases toward zero. Another difference is that the $F_n$-$P_t$ plots for the positive ramp inflations appear to be more skewed to the right, especially at the higher $P_{aCO_2}$ levels. This may be due to a small amount of time dependence in the response. In all $F_n$-$P_t$ plots, $F_n$ and the sensitivity of the response to $P_t$ increased with increasing $P_{aCO_2}$ levels. In addition, the $P_t$ value at which $F_n$ is maximal shifted to higher values.

Pressure-dependent and time-dependent response components. Although it is clear that the canine E neuronal inflation response is highly dependent on $P_t$ via the PSRs, the response also appears to depend to a minor degree on time ($t$), i.e., $F_n = F(P_t, t)$. To separate these two effects, we assumed, as a preliminary hypothesis, that the time component was linear or $F(t) = at$, where $a$ is the slope, which can be positive or negative and has the units of Hertz per second. This assumption appears reasonable based on the neuronal responses to relatively constant inputs such as those of Fig. 1 and those in which step frequency, electrically induced PSR inputs were used (32). From a practical viewpoint, it would be very difficult to separate the pressure and time-dependent components, if a more complex form of time function is used. Thus $F_n = F(P_t) + at$, and the pressure-dependent component is $F(P_t) = F_n - at$. Data for the responses to both the positive and negative ramps were used to calculate an average value of $a$ over the time span of the test inflations (see APPENDIX I for details). Figure 7 shows an example of $F_n$-$P_t$ plots before (Fig. 7, top) and after (Fig. 7, bottom) the removal of the time-dependent
component, α. The latter provides a better estimate of the $P_t$-dependent relationship. Thus, if the neuronal response is dependent only on $P_t$, then the same $F_n$-$P_t$ plot should be obtained whether response data from (+) or (−) test ramps are used. To estimate the ability of this method to reduce the differences between the $F_n$-$P_t$ plots for the (+) and (−) ramp inflations, an average error index was calculated before and after the time compensation procedure. The error index was the average of the absolute difference between the two plots for the range of overlap. The average error between plots was reduced by 25.3% from $7.94 \pm 0.42$ to $5.93 \pm 0.34$ Hz ($P < 0.0001$) for the 41 neurons with multiple $P_{aCO_2}$ levels.

Time-dependent component. The α values at each $P_{aCO_2}$ level for each neuron were also analyzed for their dependence on $P_{aCO_2}$. The slope and intercept values for plots of α vs. $P_{aCO_2}$ were obtained by linear regression for each of the 41 neurons. The mean values of the slopes and intercepts, weighted according to the number of $P_{aCO_2}$ levels per neuron (average no. of levels = 2.8) yielded the following average relationship: $\alpha = 0.62 \pm 0.027 \cdot (P_{aCO_2} - 40)$ Hz/s, where the slope (i.e., 0.027 ± 0.008) and the intercept at $P_{aCO_2} = 40$ mmHg (0.62 ± 0.19) were significantly different from zero. This relationship indicates that the time-dependent effect is relatively small. For example, at a $P_{aCO_2}$ of 40 mmHg, $\alpha = 0.62$ Hz/s and for a 10-s duration inflation, this component would contribute 6.2 Hz at the end of the response. At a $P_{aCO_2}$ of 60, the contribution would be 11.6 Hz.

$P_{aCO_2}$ effect on $F_n$-$P_t$ relationship. To analyze the $P_{aCO_2}$ effect on the $F_n$-$P_t$ relationship, the salient features of the plots were quantified using a piecewise linear approximation of the relationship after the time-dependent component was removed. To facilitate the analysis, the $F_n$-$P_t$ data were sorted according to $P_t$ value and placed in a histogram format and cursors were used to define the analysis ranges. The $F_n$-$P_t$ relationship was then divided into three linear segments: one with a positive slope (S$\lambda_p$), and two with negative slopes (S$\lambda_1$ and S$\lambda_2$; Fig. 8). Standard linear

Fig. 7. Example of $F_n$ vs. $P_t$ plots for both positive (P) and negative (N) ramp inflation patterns. Top: before time correction, average error between plots: 11.1 Hz. Bottom: after time correction procedure, average error between plots: 4.0 Hz. $P_{aCO_2}$: 79 mmHg; $\alpha$: 5.5 Hz/s.

Fig. 8. Example illustrating method used to quantify the inflation response. A piecewise linear approximation of the relationship was used after time correction. See $P_{aCO_2}$ effect on $F_n$-$P_t$ relationship for further details. $F_{max}$, peak $F_n$. 

Fig.
regression techniques were used to obtain the best-fit lines. The intersection of the positive and negative slope lines was used to determine the $P_{th}$ value or threshold ($P_{th1}$) where the FSR-mediated inhibition began to reduce $F_n$. The actual $F_n$ value, which corresponds to $P_{th1}$, was defined as $F_{max}$. A second $P_t$ value ($P_{th2}$) was defined by the intersection of the two negative slope line segments. In addition, a third $P_t$ value ($P_{th3}$) was defined as the $P_t$ at which lung inflation began to produce excitation of the E type-D neurons. This value was easiest to obtain at the lower $P_{CO2}$ levels when central inspiratory rhythm was slower or absent. However, it was also obtained at higher $P_{CO2}$ levels in cases where I-phase inhibition did not coincide in time with the low-$P_t$ portion of the test ramp inflations. Typical plots of these parameters versus $P_{CO2}$ for an E neuron are shown in Fig. 9. Linear regression was used to quantify the relationship between each parameter and $P_{CO2}$ (lines through data points). The plots of $F_{max}$ versus $P_{CO2}$ were best fit by a sigmoidal function of the form: $F_{max} = F_0/[1 + (x/k)]$, where $x = (P_{CO2}/P_{CO2}^*)^k$, $k$ is a noninteger exponent related to steepness, $P_{CO2}^*$ is the value of $P_{CO2}$ at which $F_{max} = F_0/2$, and $F_0$ is the asymptotic maximum of the sigmoid function.

Average $P_{CO2}$-dependent parameters of the $F_n-P_t$ relationship. After correction for time dependence, the corresponding parameters obtained from the best fits of the $F_n-P_t$ plots (e.g., Fig. 8) for the (+) and (-) ramp responses were averaged at each $P_{CO2}$ level for each neuron. Table 1 summarizes the pooled data of 41 neurons. The intercepts have been translated to a $P_{CO2}$ value of 40 mmHg. Thirty-nine of forty-one neurons exhibited an $S_{lp2}$ segment. Data for the sigmoidal type relationship indicates that the average maximum $F_n$ at high $P_{CO2}$ was 91.9 Hz and that 50% of this value was achieved at a $P_{CO2} = 37.1$ mmHg. Significant linear relationships were found for $S_{lp0}$, $S_{lp2}$, $P_{th0}$, $P_{th1}$, and $P_{th2}$, but not for $S_{lp1}$ (Table 1).

Average $F_n$-$P_t$ relationship as a function of $P_{CO2}$. With the use of the mean values of Table 1, it is possible to produce a family of $F_n$-$P_t$ relationships in which $P_{CO2}$ is the family parameter. Similar to the analysis, the empirical model for $F_n$-$P_t$ relationship is comprised of line segments that are functions of $P_t$ ($F_a$, $F_b$, $F_c$, and $F_d$, Fig. 10, top). These explicit $P_t$-dependent functions, which were used to approximate the $F_n$-$P_t$ relationship, are given in Fig. 10, middle. The point $P_{th1}$,$F_{max}$ is the key starting point from which the $F_n$-$P_t$ relationship is constructed. Because the line segments are also functions of $P_{CO2}$, the slope, intercept, and intersection point parameters of these line segments are functions of $P_{CO2}$, as defined in Fig. 10, bottom. These analytic relations were used to generate a three-dimensional surface plot of $F_n$ as a function of both $P_t$ and $P_{CO2}$ (Fig. 11). The strong dependence of $F_n$ on both of these variables can be appreciated. The inflation-mediated excitation is much more sensitive to $P_{CO2}$ than the inflation-mediated inhibition (compare $S_{lp0}$ with $S_{lp1}$ and $S_{lp2}$, Fig. 10, bottom, and Fig. 11). The $F_n$-$P_t$ relationship is maintained in the absence of central respiratory rhythm at $P_{CO2}$ levels typically <30 mmHg. The increases in threshold levels for $P_{th0}$, $P_{th1}$, and $P_{th2}$ with increases of $P_{CO2}$ can also be seen.

Table 1. $F_n$-$P_t$ parameters as function of $P_{CO2}$ after time correction and averaged for ± ramp inflations

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<th>$F_{max}$</th>
<th>$S_{lp0}$</th>
<th>$S_{lp1}$</th>
<th>$S_{lp2}$</th>
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<td>$Intc$</td>
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<tr>
<td>Mean</td>
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<td>0.236</td>
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</table>

Values are the pooled data of 41 neurons. $S_{lp0}$ (positive slope) is the regression slope and $S_{lp1}$, intercept (Intc) is regression intercept at arterial $CO2$ tension ($P_{CO2}$ = 40 mmHg; $F_n$, neuronal discharge frequency; $P_t$, transpulmonary pressure; $F_{max}$, peak neuronal frequency; sigmoidal parameters: $F_{max}$, asymptotic maximum value of $F_{max}$; $X_{1/2}$, $P_{CO2}$ value when $F_{max} = F_0/2$; $k$, coefficient of steepness. $S_{lp}$ and $S_{lp2}$, negative slopes; $P_{th0}$, $P_{th1}$, $P_{th2}$, transpulmonary pressure threshold for positive and negative slopes. *P < 0.001 of regression slope ($S_{lp}$) be different from zero; †P < 0.01; ‡P < 0.05. NS, nonsignificant.
The calculated relationship between $F_n$ and $\text{PaCO}_2$, at fixed $P_t$ levels is sigmoidal in shape (Fig. 12). Generally, $F_n$ increases with $\text{PaCO}_2$, at all $P_t$ levels, suggesting that part of the inflation-mediated inhibition of the E neurons can be offset by increases in $\text{PaCO}_2$.

DISCUSSION

This study characterizes the integration of mechanosensory and chemosensory inputs by canine E neurons of the caudal VRG. Most of these neurons are presumed to be bulbospinal neurons on the basis of previous studies of E neurons in the same location, where $>93\%$ of those neurons were antidromically activated from the cervical spinal cord (5). An empirical model has been developed that can predict the instantaneous discharge frequency, $F_n$, of an average E bulbospinal neuron for given $\text{PaCO}_2$ and transpulmonary pressure, $P_t$, values. In addition, the profile of the discharge pattern is also predictable for a given trajectory of $P_t$.

Previous studies have investigated the response of these neurons to each input in isolation (2, 4, 9, 31), but not to the combination of both inputs. Open-loop conditions were used to minimize confounding inputs from other sources or secondary effects. In this regard, neuromuscular blockade and bilateral pneumothorax were used to eliminate phasic afferent inputs from areas other than the lung, and test inflation patterns, separated by several control respiratory cycles, were used during hyperoxia ($\text{PaO}_2$ > 300 mmHg) to minimize the effects of transient changes in $\text{PaCO}_2$ during test cycles. The delayed responses of neuronal and phrenic activities to step changes in inspired $\text{CO}_2$ concentration, which required 3–5 min to reach steady state, suggest that the hyperoxic conditions minimized inputs from the peripheral chemoreceptors that have a fast response time. Thus, in this study, the major source of chemosensory input to the E bulbospinal neurons is of...
Central origin. The PSR-mediated reflex is also controlled by a GABAergic gain-modulating mechanism (20) that may be affected by barbiturates. However, a constant infusion of the thiopental anesthetic was used to maintain a stable blood concentration and hence a relatively constant level of anesthesia. Thus modulation of the GABAergic input by the anesthetic, if present, would be at a constant level and the nature of the interaction between PaCO₂ and the PSR input should be unaltered.

On the basis of the sustained E neuronal responses to step inflation patterns, our previous (4) and current study (e.g., Fig. 1) suggest that both the excitatory and inhibitory components of the inflation response are mediated by the slowly adapting PSRs. P₁ was used as an index of PSR activity, and vagotomy eliminates the inflation response (4). It is possible that the excitatory and inhibitory components are mediated by the two types of PSRs with appropriate characteristics that have been described in dogs (23–25) or by a single set of PSRs in conjunction with a central mechanism (4).

Pressure and time dependence of the E neuronal response. Although our analysis shows that a time-dependent factor contributes to the discharge pattern of these E neurons, its effect is relatively small and appears to be overridden by inputs from the PSRs, such that the time course of the discharge pattern is highly dependent on the time course of transpulmonary pressure (4). The small contribution of the time-related changes in Fₙ is best illustrated by the E neuronal responses to step inflations, delayed ramp inflations, and by overlap of the Fₙ versus P₁ plots for data from positive and negative ramp inflations, as demonstrated in Figs. 1, 2, and 7, respectively. When the Fₙ-P₁ plots were corrected for time-dependent effects, the average error between plots for the positive and negative ramp inflations for the 41 neurons at the various PaCO₂ levels was reduced from 7.9 to 5.9 Hz.

The average coefficient, α, for the assumed time-dependent linear component, α₁, at a PaCO₂ of 40 mmHg was 0.62 ± 0.19 Hz/s, with 68% of the neurons having values within the range of −0.57 (mean − SD) and 1.81 Hz/s (mean + SD). Thus, at the end of a 10-s test inflation, the time-dependent component could reduce Fₙ by 5.7 Hz for neurons at the low end of the range or increase Fₙ by 18.1 Hz for neurons at the high end of the range. These contributions would be proportionately less during eupnea, where E durations are 2–4 s. The α coefficient was also dependent to a small degree on PaCO₂ with a mean ± SD value of 0.027 ± 0.052 Hz·s⁻¹·mmHg PaCO₂⁻¹. A 10-mmHg increase in PaCO₂ would increase α on average by 0.27 Hz/s.

In contrast to dogs, E bulbospinal neurons in cats exhibit a marked time-dependent component, which manifests itself as an augmenting ramp discharge pattern. Neuronal excitation was observed for the low-P₁ range and inhibition for the higher P₁ range in cats (see Fig. 10, top, of Ref. 9); however, the pattern maintained its augmenting profile.

Central chemodrive dependence of the E neuronal response. The activities of both the control and test inflation cycles increased with increases in PaCO₂ (e.g., Figs. 3 and 4). Plots of the peak Fₙ (Fₙ max, Fig. 8), as well as Fₙ at various P₁ levels (Fig. 12), versus PaCO₂ were sigmoidal in shape (Fig. 9, middle). The steepest part of the Fₙ max curve, which occurred at 50% of maximum, was located at an average ± SE PaCO₂ of 37.1 ± 1.5 mmHg, and the PaCO₂ range for the 20–80% response was 26–62 mmHg. In chloralose-urethane-anesthetized cats, Bainton and Kirkwood (2) also noted that plots of Fₙ versus alveolar PCO₂ (PACO₂) for E bulbospinal neurons were steep and sigmoidal; however, the greatest sensitivity was found to occur at PaCO₂ values from 20 to 30 mmHg. These results suggest that the greatest sensitivity of the caudal VRG E neurons to PaCO₂ occurs over a PaCO₂ range somewhat lower than that for inspiratory (phrenic) activity (13).

Nature of interaction between chemosensory and mechanosensory inputs. On the basis of the plot of Fₙ vs. P₁ and PaCO₂ of the average E neuron (Fig. 11), the general impression is that these two inputs are mainly additive. That is, an increase in PaCO₂ shifts the Fₙ-P₁ relationship upward. However, the quantitative relationships used to generate Fig. 11 indicate that there is a synergistic interaction between PaCO₂ and the excitatory component of the P₁ response. The positive slope, Slpₒ, increases by 38% for a change in PaCO₂ from 30 to 50 mmHg and results in higher Fₙ max values. The negative slope, Slp₁, is not altered by PaCO₂ and can be seen as a parallel shift if the Fₙ-P₁ relationship (Fig. 11). The negative slope, Slp₂, becomes less negative with increases in PaCO₂ and the inhibition of the higher P₁ range is less effective at higher PaCO₂ levels.

The bidirectional Fₙ-P₁ relationship was preserved regardless if ventilation was synchronized with central I activity (e.g., Fig. 3) or if central I activity was entrained by ventilation pattern (e.g., Fig. 5). In addition, for >80% of the cases, central neural apnea (peak PNG decreased to zero) occurred in these barbiturate-anesthetized dogs at PaCO₂ < 45 mmHg. In some cases, such as Fig. 5, central I inhibition of E neuronal activity was observed. However, with lower PaCO₂ levels, the central I inhibition disappears and tonic E activity can be observed when PSR input is prevented by temporarily halting ventilation (data not shown). The transition from rhythm to central neural apnea appears to have no affect on the Fₙ-P₁ relationship, suggesting that the functioning of this reflex is not conditional on the presence of rhythm or phasic activities. This is consistent with the finding that the same E bulbospinal neurons are capable of relaying both phasic and tonic excitation to spinal respiratory motoneurons and that rhythmic excitation of E muscles results from a periodic I phase inhibition of the E bulbospinal neurons that are subjected to a graded, tonic, CO₂-dependent excitation (2, 3).

Furthermore, the preservation of the Fₙ-P₁ relationship below the apneic threshold suggests that more direct neural pathways, which bypass the rhythm gen-
eration structures, may mediate integration of the mechano- and chemosensory inputs by the E bulbospinal neurons. PSRs synapse within the nucleus of the solitary tract and second- and possibly higher-order neurons may relay the Pt-related information to the E bulbospinal neurons (8, 32).

**Physiological significance of the model parameters.** Although the empirical model for the $F_n$-Pt relationship (Fig. 10) is useful in quantifying the neuronal responses to arbitrary inflation patterns, the model parameters themselves have physiological correlates. $P_{thr0}$ indicates the threshold pressure at which the PSR activation begins to excite the E neurons, and $Slp_0$ represents the sensitivity of that reflex component. $P_{thr1}$ appears to represent the point where the Pt-related progressive recruitment of the PSRs and/or PSR activity, which mediate the inhibitory component of the E neuronal response to lung inflation, opposes further neuronal excitation, and actually reverses it. It is not known if there exists a separate group or type of PSRs that mediates the inhibitory response. This inflation-mediated excitatory/inhibitory interaction determines $F_{max}$ and the sensitivity of the inhibitory component, $Slp_1$, as $Pt$ increases. Because $P_{thr2}$ is the $Pt$ value at which the two piecewise linear approximations of the curvilinear inhibitory response component intersect, it represents a point of maximum slope inflection. $Slp_2$ represents the sensitivity of the second subcomponent of the inhibitory portion of the $F_n$-$Pt$ relationship.

Increases in $PaCO_2$ produced small, but statistically significant, increases in the three $Pt$ threshold values, $P_{thr0}$, $P_{thr1}$, and $P_{thr2}$ (Table 1). The upward shift in these parameters with increases in $PaCO_2$ may be due the effects of $PaCO_2$ on the PSRs, per se, because increases in $PaCO_2$ have been shown to reduce PSR activity and increase their activation thresholds (10, 14, 27, 28).

**Correlation of E bulbospinal neuronal with E muscular responses.** The various thoracic and abdominal E muscles respond differentially to changes in $PaCO_2$ and PSR inputs (1). It is possible that these differences may be accounted for by the variability in the sensitivities of the model parameters, although the behavior of the 41 type D neurons of this study was qualitatively similar. A measure of the degree of parameter variation is given by the 10th and 90th percentile values of Table 1. For example, in 80% of these neurons, the slope of the excitatory portion of the $F_n$-Pt relationship ranged from 5 to 30 Hz/mmHg and that of the inhibitory portion ranged from $-5.2$ to $-20.4$ Hz/mmHg (Table 1, intercept of $Slp_0$ and $Slp_1$ at a $PaCO_2$ of 40 mmHg). In addition to parameter variability, it is possible that some E muscles do not depend on PSR feedback. For example, during postural changes and use of the rib cage, triangularis sterni E muscles are largely independent of vagal inputs, in contrast to E abdominal muscles, which rely on vagal feedback for this purpose (11).

**Perspectives**

During spontaneous breathing, E airflow normally is retarded by the combined effects of increased laryngeal airflow resistance, post I activity of the diaphragm, and inhibition of E muscle activity (26, 33). The afferent limb is composed of extrathoracic and intrathoracic pulmonary and tracheal-bronchial stretch receptors with vagal fibers. During expiration, this reflex continuously compensates for changes in upper airway resistance and tends to maintain a normal E flow rate possibly to improve gas exchange and prevent alveolar collapse.

PSR-mediated inhibition of E bulbospinal neurons appears to play an important role in E airflow control.
At end-inspiration, elastic recoil pressure and $P_t$ are greatest, and maximum inhibition of E bulbospinal neuronal activity occurs. As deflation proceeds, disinhibition results in an augmenting E neuronal discharge pattern, which would act to maintain E airflow when elastic recoil pressure is decreasing. Hypercapnia increases E neuronal $F_n$; however, tidal volume and peak $P_t$ are also increased and PSR-mediated inhibition of $F_n$ would minimize the contribution of E muscles in early expiration. Due to the higher recoil pressure, the lung would empty faster and disinhibition of the $P_{ACO_2}$-elevated E neuronal activity would aid in emptying the lung during the later part of the E phase. In addition, as lung volume and $P_t$ decrease below the $P_{th}$, $F_n$ decreases due to the reduction in the PSR-mediated excitation, effectively braking active expiration and limiting deflation below function residual capacity. Expiratory phase duration is also highly dependent on the E volume trajectory and history via the Hering-Breuer E facilitatory reflex (34), and the discharge pattern of the E bulbospinal neurons would therefore contribute to the control of $T_E$ by altering PSR feedback.

E bulbospinal neurons appear to provide a good neural model in which the central integration of different types of information may be studied, such as those arising from mechanosensory (5, 7), chemosensory (12), and central pattern-generating (6, 15, 19) inputs. The model also allows the study of the neurotransmitters and synaptic mechanisms involved.

In summary, this study characterizes the control of the E neurons in the caudal VRG of dogs by the combination of inputs arising from PSRs and central chemosensory sources. The interaction between these two types of input appears to be mainly additive with regard to the PSR-mediated inhibition and synergistic with regard to the PSR-mediated excitation.

**APPENDIX I**

Method used to estimate and separate the time-dependent component from the pressure-dependent component. The response to lung inflation was assumed to be a function of transpulmonary pressure, $P$, and time: $F_n(t) = f[P(t), t]$. As a first approximation, we assumed that the time-dependent component was a linear function of time into the E phase, that is: $F(t) = \alpha t$, where $\alpha$ is the slope, which can be either positive or negative, and has the units of Hertz per second. The neuronal discharge patterns to a positive ramp inflation ($P$, Fig. A1, left center) and the corresponding negative ramp inflation ($P$, Fig. A1, right center) are

$$ F_1(t) = f[P(t)] + \alpha t 
(1) $$

$$ F_2(t) = f[P(t)] + \alpha t 
(2) $$

For a given $P$ level ($P$), $t_1$ and $t_2$ were found such that $P_1(t_1) = P_2(t_2) = P$ [circles on (+) and (-) ramps, Fig. A1, right and left center]. Under this condition, Eqs. 1 and 2 become

$$ F_1(t_1) = f[P(t_1)] + \alpha t_1 
(3) $$

$$ F_2(t_2) = f[P(t_2)] + \alpha t_2 
(4) $$

Taking the difference between Eqs. 3 and 4 removes the $P_t$-dependent component that is common to both equations ($f[P]$) and yields

$$ F_1(t_1) - F_2(t_2) = \alpha (t_1 - t_2) 
(5) $$

Thus

$$ \alpha = \frac{F_1(t_1) - F_2(t_2)}{t_1 - t_2} 
(6) $$

For the overlapping $P_t$ range of both ramps (Fig. A1, left bottom), $\alpha$ values are calculated for each $P$ value in 0.25-mmHg increments and then averaged to reduce variation and obtain a better estimate of $\alpha$.

The $P_t$-dependent components were then obtained by rearrangement of Eqs. 1 and 2

$$ \tilde{F}[P(t)] = F_1(t) - \alpha t 
(6) $$

$$ \tilde{F}[P(t)] = F_2(t) - \alpha t 
(7) $$

Plots of $\tilde{F}_1$ versus $P_1$ and $F_2$ versus $P_2$ then coincide (Fig. A1, right bottom), demonstrating independence from time and showing only the pressure-dependent component of the E neuronal response to lung inflation (also see Fig. 7).

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