Role of Carbon Dioxide Oscillation in the Control of Respiration in the Anesthetized Dog

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Abstract In order to examine the role of respiratory oscillation of Pa_{CO_2} (CO_2 oscillation) in the control of respiration, we performed veno-venous bypass using a membrane lung in 10 anesthetized paralyzed dogs, where the dog was put on fixed mechanical ventilation so that we could keep average Pa_{CO_2} and Pa_{O_2} constant by adjusting Fi_{CO_2} and Fi_{O_2} during CO_2 loading/unloading. By venous CO_2 loading/unloading we could widely change the CO_2 output from the lung (11–440% of the control) resulting in large changes in the arterial CO_2 oscillations (50–280% of the control for the maximum rate of rise of Pa_{CO_2} and 40–350% of the control for the maximum rate of fall of Pa_{CO_2}), which was measured by a rapidly responding intra-arterial pH electrode. Despite such wide variations in CO_2 oscillations we did not find any consistent change in the respiratory center output (minute phrenic activity). This held true after compensating the changes in the minute phrenic activity for the CO_2 sensitivity of each individual dog. Thus, the present results may suggest that the CO_2 oscillation plays little role in the control of respiration in mild increase in $\dot{V}_{CO_2}$.

Key words: control of respiration, CO_2 oscillation, veno-venous bypass.

Since the first experiment conducted by Yamamoto and Edwards in 1960 (YAMAMOTO and EDWARDS, 1960), many investigators have addressed the issue of respiratory CO_2 oscillations as a mediator of the information of metabolism to ventilation. This is mainly based on the following experimental findings: 1) the rate of rise of the respiratory CO_2 oscillation reflects $\dot{V}_{CO_2}$ (SAUNDERS, 1980; COCHRANE *et al.*, 1982; ALLEN and JONES, 1984), 2) there is a linear relationship between $\dot{V}_{CO_2}$ and ventilation (WASSERMAN *et al.*, 1967; PHILLIPSON *et al.*, 1981a); and 3) the peripheral chemoreceptor appears to provide a rate sensitivity to Pa_{CO_2} (BLACK *et al.*, 1971; PONTE and PURVES, 1974). In addition, there have been many experiments which indirectly support this hypothesis (e.g., YAMAMOTO and EDWARDS, 1960; PHILLIPSON *et al.*, 1981b; CROSS *et al.*, 1982).

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The most straightforward experiment to test the role of CO_2 oscillation in the control of ventilation may be to examine the respiratory center output while CO_2 oscillations are widely changed without affecting the average Pa_{CO_2} or Pa_{O_2} . The experiment should be conducted by an open-loop method to prevent the output of the system (respiratory center output) from influencing the input of the system (Pa_{CO_2}). There has been only one such an experiment reported in a preliminary form (MUELLER *et al.*, 1976) where they produced large CO_2 oscillations (up to 28 mmHg peak-to-peak) in cats while keeping mean Pa_{CO_2} and pHa constant using a respirator. They found only minor effects on ventilation.

We undertook the present experiment in anesthetized dogs using an open-loop technique. We conducted veno-venous bypass to obtain venous CO_2 loading/unloading so as to control the CO_2 output from the lung and hence the CO_2 oscillations. The dog was paralyzed and put on mechanical ventilation to open the respiratory control loop. We adjusted $\dot{V}_{\text{I}\text{CO}_2}$ to keep mean Pa_{CO_2} constant. Our result showed no appreciable response in the respiratory center output to CO_2 loading/unloadings.

MATERIALS AND METHODS

Dog preparations. We studied 10 dogs weighing 18–29 kg. The dogs were anesthetized with intramuscular fentanyl and droperidol (0.04 and 2 mg/kg, respectively; Innovar-vet) followed by a slow intravenous injection of a mixture of urethane and chloralose (400 and 40 mg/kg, respectively; supplemental dose of 40 and 4 mg/kg every 1 h, respectively). A tracheostomy was then created through which a cuffed tracheostomy tube (10 mm ID, Portex) was inserted. A polyethylene catheter was inserted from the left femoral artery to the level of the thoracic aorta for blood pressure measurement using a pressure transducer (Gould, P231D) and for the sampling of arterial blood. The right femoral artery was exposed for the insertion of the intravascular pH electrode. Right jugular vein, left and right femoral veins were exposed for the insertion of cannulas for the extracorporeal circulation, while the left jugular vein was left intact. The C_5 root of the left phrenic nerve was carefully separated from the surrounding tissue at the level of the neck, cut, and the central end of the nerve placed on a bipolar stainless steel electrode for measurement of the phrenic neurogram. The nerve and electrode were immersed in mineral oil to prevent drying. We performed a bilateral cervical vagotomy in 4 dogs.

Extracorporeal circulation. In order to conduct venous CO_2 loading/unloading we used veno-venous bypass using a membrane lung (surface area 0.85 m^2 ; Cobe, 50-103 Lab unit). The circuit was flushed with saline and then primed with heparinized fresh canine blood collected from a donor dog anesthetized with the same anesthetics. Following total heparinization of the dog (300 units/kg), polyethylene cannulas (4 mm ID) were inserted into the right and left femoral veins for blood drainage which were connected to a blood reservoir attached to the membrane lung. A non-occlusive roller pump (Travenol) pumped the blood out to

the superior vena cava through the jugular venous cannula (4 mm ID). We started bypass with a very low flow rate and gradually increased the flow to 0.6–1 l/min with no gas flow being applied to the membrane lung. This condition was maintained at least 30 min to stabilize the dog. We added saline-dextran (Rheomacrodex) in case of hypovolemia as assessed by the blood level of the reservoir. The temperature of the dog was monitored with a thermistor included in the intravascular pH electrode. We adjusted the temperature of the heat exchanger of the membrane lung system so that the changes in blood temperature of the dog became less than 0.5°C. Supplemental heparin (100 units/kg) was added every 1 h to the circuit. Sodium bicarbonate was injected to the circuit to correct base deficit if necessary.

In vivo arterial pH measurement. Since respiratory oscillations of arterial pH are thought to reflect the respiratory CO₂ oscillations, we measured *in vivo* arterial pH continuously by a rapidly responding catheter tip ISFET (ion-sensitive field effect transistor) pH electrode (63% response 120 ms; Kuraray, PH-1035) in which a small H⁺ sensitive ISFET (5.5 × 0.4 × 0.15 mm) is encapsulated in a flexible catheter (35 cm length, 0.65 mm OD; SHIMADA *et al.*, 1980). A thermistor is also fabricated on the same semiconductor chip, providing direct measurement of temperature at the site of pH measurement. With an appropriate amplifier (Kuraray, KR-500) the pH monitoring system provides a sensitivity of 0.1 unit/V. The electrode was calibrated with two phosphate buffers (pH = 7.2 and 7.6) at 37 ± 0.1°C. The pH of the buffer solutions were carefully cross checked (with an internal accuracy of 0.001 unit) in the glass electrode system which was used for the *in vitro* arterial blood gas measurements (Corning, 168). We inserted the pH electrode from the right femoral artery and advanced it so that the sensor was placed in the thoracic aorta. A silver/silver chloride reference electrode was fixed under the skin. We calculated CO₂ oscillations from measured pH oscillations assuming that pH and log P_{CO_2} obey a linear relationship (Henderson-Hasselbalch equation),

$$\text{pH} = a + b \cdot \log P_{\text{CO}_2}.$$

Therefore, we calculated the amplitude and slope of CO₂ oscillation from the above equation as follows:

$$\Delta P_{\text{CO}_2} = P_{\text{CO}_2} \cdot (10^{(\Delta \text{pH}/b)} - 1),$$

and

$$dP_{\text{CO}_2}/dt = P_{\text{CO}_2}/b \cdot 2.303 \cdot d\text{pH}/dt,$$

where b denotes the slope of the pH-log P_{CO_2} relationship, which was calculated by a least square error method for each individual dog.

Ventilatory measurements. We measured the respiratory flow by a heated pneumotachograph (Fleish, No. 2) which was connected to the tracheostomy tube. The flow signal was then electrically integrated (Gould, 13-4615-70) to provide tidal volume. Airway CO₂ fraction was measured by an infrared CO₂ analyzer

(Beckman, LB-2) where a heated sampling tube was connected just distal to the pneumotachograph. Oxygen saturation was measured by a pulse oximeter (Ohmeda, Biox 3700) with the probe placed on the tongue. We computed the rate of change (slope) of the respiratory pH oscillations by electrically differentiating the intra-arterial pH signal using a differentiator (cut-off frequency 1 Hz; Gould, 13-4615-71). We calculated CO_2 output from the lung ($\dot{V}_{\text{CO}_2}$) by collecting expired gas to a spirometer (Med-Science Electronics, 270) using the following equation:

$$\dot{V}_{\text{CO}_2} = (F_{\text{E}\text{CO}_2} - F_{\text{I}\text{CO}_2}) \cdot V,$$

where $F_{\text{E}\text{CO}_2}$, $F_{\text{I}\text{CO}_2}$, and V are fractional concentrations of CO_2 of mixed expired gas and inspired gas, and expiratory gas volume per minute, respectively. We used minute phrenic activity (i.e., product of the height of the moving time average of the phrenic neurogram and the frequency of the burst/min) as an index of the central respiratory drive (ELDRIDGE, 1976). In order to obtain the moving time average of the phrenic neurogram, the phrenic electrodes were connected to a high impedance probe (Grass, HIP511GA) and then the signal was amplified (frequency band, 30 Hz–10 kHz; Grass, P511K). The moving time average of the neurogram was provided by a moving time averager (time constant, 100 ms, Coulbourn). All the signals were amplified and recorded on paper (Gould, 2800S). We also monitored the raw neurogram using an audio amplifier (Grass, AM-8).

Procedures. Our purpose was to examine the effects of changing arterial CO_2 (pH) oscillations on the respiratory center output while maintaining the mean arterial blood gases constant. In order to control mean $P_{\text{a}\text{CO}_2}$ the dog was put on mechanical ventilation (Harvard) following paralysis by intravenous injection of pancuronium bromide (Pavulon). We did not change the minute ventilation of the mechanical ventilator to keep mean $P_{\text{a}\text{CO}_2}$ constant during various CO_2 loading/unloadings since changes in positive pressure ventilation are known to produce significant effects on the venous return and therefore blood pressure. Instead we regulated $F_{\text{I}\text{CO}_2}$ so that mean $P_{\text{a}\text{CO}_2}$ was kept constant during CO_2 loading/unloadings. Namely, the dog was hyperventilated (tidal volume, 0.5 or 0.7 l, depending on the size of the dog; rate, 15 breaths/min) and $F_{\text{I}\text{CO}_2}$ was simultaneously increased to keep mean $P_{\text{a}\text{CO}_2}$ normocapnic level (usually 40 mmHg). No gas flow to the membrane lung was applied here. After all the respiratory and circulatory variables became steady, we conducted control measurements of arterial pressure, minute ventilation, arterial oxygen saturation, airway CO_2 , *in vivo* arterial pH, phrenic neurogram, arterial blood gases, and $\dot{V}_{\text{CO}_2}$. The respiratory and pH oscillation data were represented as means of 10 (vagi intact dogs) or 20 (vagotomized dogs) consecutive breaths. Then we infused and/or extracted CO_2 in the venous blood using the membrane lung. During CO_2 loading to the venous system, $F_{\text{I}\text{CO}_2}$ was decreased to zero resulting in constant mean $P_{\text{a}\text{CO}_2}$ with increased $\dot{V}_{\text{CO}_2}$. On the other hand, during CO_2 unloading $F_{\text{I}\text{CO}_2}$ was increased while unloading CO_2 from the venous system resulting in constant $P_{\text{a}\text{CO}_2}$ with decreased $\dot{V}_{\text{CO}_2}$. At least 10 min after starting CO_2 loading/unloading we made measurements

of the respiratory variables and pH oscillations. After the data acquisition, we shut off the gas flow to the membrane lung, waited at least 10 min for steady state, and repeated the control measurement. We tried to adjust $\dot{V}_{\text{CO}_2}$ so that the difference in mean P_{aCO_2} among controls and CO₂ loading/unloading became less than 1 mmHg. These bracketed measurements were repeated under various conditions such as hypoxia, hypercapnia, and hypoxic hypercapnia. In order to assess the responsiveness of each dog to steady state changes in P_{aO_2} or P_{aCO_2} , we conducted steady state inhalation of hypoxic or hypercapnic gas, where the target S_{aO_2} and P_{aCO_2} were 80% and 60 mmHg, respectively.

Data analysis. Since each experimental run consisted of the first control, CO₂ loading/unloading, and the second control, we first examined the stability of the baseline of minute phrenic activity by comparing those of the first and second control. We discarded the run if changes in the minute phrenic activity between the two controls were more than 20% of the first control. Then, we interpolated the base line during CO₂ loading/unloading from the two controls and took the difference of the minute phrenic activity from that base line. Since the minute phrenic activity was measured in arbitrary units, it was difficult to compare the response among dogs. Therefore, we at first treated the changes in the minute phrenic activity as nominal data: the direction of the change, i.e. sign of change in the minute phrenic activity divided by the change in $\dot{V}_{\text{CO}_2}$, which had been expected to be positive, was examined by the χ^2 test with the Yates correction for continuity. Then we normalized the changes in the minute phrenic activity and compared the data from all the dogs on the same axis. Normalization was performed by dividing the changes in minute phrenic activity during CO₂ loading/unloading by the slope of the CO₂ response curve measured separately. The idea of the normalization is to convert the change in the minute phrenic activity obtained by venous CO₂ loading/unloading (measured in arbitrary unit) to change in P_{aCO_2} (in mmHg) which would result in the same amount of change in the minute phrenic activity by airway CO₂ loading. We plotted these normalized changes in the minute phrenic activity against changes in $\dot{V}_{\text{CO}_2}$. Linear regression analysis was performed by a least square error method between these two variables. The statistical significance of the regression was examined by the *F*-test and the *t*-test. Means were represented with $\pm$ S.D. Statistical significance of the mean was tested by the *t*-test where the level of significance was $p < 0.05$.

RESULTS

We performed 42 CO₂ loading/unloadings in 10 dogs in which 29 and 13 runs were conducted in vagi intact and vagotomized dogs, respectively. Of these, 16 had to be discarded because of base line shift from the first to the second control. The remaining analysis was carried out on 26 CO₂ loading/unloadings in 9 dogs in which 17 studies were collected from dogs with intact vagi and 9 in vagotomized dogs. We performed 18 and 14 CO₂ loadings/unloadings of 26 total runs in hypercapnia

(average $Pa_{CO_2} = 61$ mmHg) and hypoxia (average $Sa_{O_2} = 82\%$), respectively.

The dogs had significant responses to both steady state inhalation of hypercapnic or hypoxic gas, i.e. $\Delta(\text{minute phrenic activity})/\Delta Pa_{CO_2} = 6.82 \pm 3.50$ arbitrary unit/mmHg ($n=9$) and $\Delta(\text{minute phrenic activity})/\Delta Sa_{O_2} = -3.32 \pm 3.80$ arbitrary unit/ $\%$ ($n=8$), respectively. Figure 1 shows the changes during the transition from the control run to a CO_2 loading experiment. By carefully adjusting $F_{I_{CO_2}}$, it was possible to increase the pH oscillations (amplitude and dPa_{CO_2}/dt) and hence the CO_2 oscillations by venous CO_2 infusion with little changes in the average pHa (Pa_{CO_2}) and Sa_{O_2} (occasionally $F_{I_{O_2}}$ had to be adjusted). By intravenous CO_2 loading/unloadings we could change the CO_2 output from the lung from the control of 0.147 ± 0.050 l/min ($n=34$) up to 0.646 l/min (440% of the control) or down to 0.016 l/min (11% of the control). As a result, pH oscillations and therefore CO_2 oscillations changed considerably. The amplitude (ΔPa_{CO_2} , 1.91 ± 0.77 mmHg in control, $n=35$), the maximum rate of rise (Max dPa_{CO_2}/dt , 1.58 ± 0.57 mmHg/s in control, $n=35$), and the maximum rate of fall (Max $-dPa_{CO_2}/dt$, 2.03 ± 0.71 mmHg/s, $n=35$) of CO_2 oscillation had linear relationship with the CO_2 output from the lung as follows (Figs. 2–4):

$$\Delta Pa_{CO_2} = 9.10 \cdot \dot{V}_{CO_2} + 0.656 \quad (r=0.817, \text{ Fig. 2}),$$

$$\text{Max } dPa_{CO_2}/dt = 5.52 \cdot \dot{V}_{CO_2} + 0.788 \quad (r=0.786, \text{ Fig. 3}),$$

$$\text{Max } -dPa_{CO_2}/dt = 9.89 \cdot \dot{V}_{CO_2} + 0.613 \quad (r=0.844, \text{ Fig. 4}).$$

The slope of the regression line for the maximum rate of fall of Pa_{CO_2} versus $\dot{V}_{CO_2}$ was significantly higher than that for the maximum rate of rise of Pa_{CO_2} versus $\dot{V}_{CO_2}$. During CO_2 loading/unloadings we could keep changes in the mean Pa_{CO_2} and Sa_{O_2} minimal. In fact, changes in the average Pa_{CO_2} and average Sa_{O_2} were 0.04 ± 1.34 mmHg (95% confidence limit: -0.50 to 0.58 mmHg, $n=26$) and $-0.29 \pm 1.52\%$ (95% confidence limit: -0.90 to 0.32% , $n=26$).

Despite the considerable changes in the CO_2 output from the lung associated with little changes in the average blood gases, the minute phrenic activity was unchanged among the control and CO_2 loading/unloading experiments (Table 1, Fig. 5). Regarding the changes in the minute phrenic activity as nominal data, we found the expected change (increase in the minute phrenic activity during CO_2 loading and decrease during CO_2 unloading) in only 65% of the total runs (Table 1). On the other hand, if we examine only the data from the vagotomized dogs, 89% of the runs resulted in the expected change, which was statistically significant (Table 1). Since all the dogs provided significant ventilatory response to steady state CO_2 inhalation ($\Delta(\text{minute phrenic activity})/\Delta Pa_{CO_2} = 3.41 \pm 0.49$ arbitrary unit/mmHg in vagotomized dog and 9.62 ± 1.50 arbitrary unit/mmHg in vagi intact dog), we normalized the changes in the minute phrenic activity during CO_2 loading/unloading by the slope of the CO_2 response curve (Fig. 6). We could not find any significant relationship between the normalized minute phrenic activity and the CO_2 output from the lung even in the vagotomized dogs ($p > 0.1$).

Fig. 1. Recorder tracings in one dog showing the transition from a control to a venous CO₂ loading. Arterial blood was sampled at points A and B. The arterial line was flushed with saline after point B, which was reflected in pHa tracings (point C). Note that the sensitivity for $dpHa/dt$ was halved in the middle of the tracing.

Fig. 2. Relationship between the CO_2 output from the lung ($\dot{V}_{\text{CO}_2}$) and the amplitude of CO_2 oscillation (ΔPa_{CO_2}). Regression equation is: $\Delta Pa_{\text{CO}_2} = 9.10 \cdot \dot{V}_{\text{CO}_2} + 0.656$ ($r = 0.817$).

Fig. 3. Relationship between the CO_2 output from the lung ($\dot{V}_{\text{CO}_2}$) and the maximum rate of rise of CO_2 oscillation ($\text{Max } dPa_{\text{CO}_2}/dt$). Regression equation is: $\text{Max } dPa_{\text{CO}_2}/dt = 5.52 \cdot \dot{V}_{\text{CO}_2} + 0.788$ ($r = 0.786$).

DISCUSSION

The objective of the present study was to examine the effect of CO_2 oscillations on the central neural respiratory output without affecting the stationary components of arterial blood gases. In order to change arterial CO_2 oscillations, we conducted veno-venous bypass using a membrane lung by which we controlled CO_2 output from the lung by adding or extracting CO_2 in the venous blood. At the same time we controlled $\dot{V}_{\text{CO}_2}$ so that the mean Pa_{CO_2} was kept constant without changing the minute volume of mechanical ventilation. We did not find consistent relationship in the direction of the change in minute phrenic activity to CO_2 loading/

Fig. 4. Relationship between the CO₂ output from the lung ($\dot{V}_{\text{CO}_2}$) and the maximum rate of fall of CO₂ oscillation (Max $-\text{d}P_{\text{aCO}_2}/\text{d}t$). Regression equation is: Max $-\text{d}P_{\text{aCO}_2}/\text{d}t = 9.89 \cdot \dot{V}_{\text{CO}_2} + 0.613$ ($r = 0.844$).

Table 1. Directions of the response in the minute phrenic activity to changes in $\dot{V}_{\text{CO}_2}$.

	Direction of response ($\Delta(\text{minute phrenic activity})/\Delta\dot{V}_{\text{CO}_2}$)		χ^2 test
	+	-	
Vagi intact	9	8	N.S.
Vagotomized	8	1	$0.025 < p < 0.05$
Total	17	9	N.S.

unloadings. Even when the changes in minute phrenic activity was compensated for by the CO₂ sensitivity of each individual dog, we did not find a significant relationship between the respiratory center output and $\dot{V}_{\text{CO}_2}$.

Before we discuss the response of the medullary respiratory center to CO₂ oscillation, we have to examine if we have provided a large enough input signal to the system to yield a satisfactory signal-to-noise ratio of the measurements. We could change the CO₂ output from the lung from 11 to 440% of the control $\dot{V}_{\text{CO}_2}$ by the veno-venous bypass with a membrane lung. We expected that this change in the CO₂ output from the lung would be large enough to produce wide variations of respiratory center output if we assume a linear relationship between $\dot{V}_{\text{CO}_2}$ and ventilation as demonstrated by many investigators (PHILLIPSON *et al.*, 1981a; WASSERMAN *et al.*, 1967). By controlling the CO₂ output from the lung, we have also obtained appreciable changes in the CO₂ oscillation. In the present study, we found linear relationships between the amplitude, the maximum rate of rise of P_{aCO_2} , and

Fig. 5. Changes in minute phrenic activity during CO₂ loading (upper panel) and CO₂ unloading (lower panel). $\Delta \dot{V}_{CO_2}$ denotes the difference in $\dot{V}_{CO_2}$ between control and CO₂ loading/unloading. Note that the control minute phrenic activity was obtained by interpolation using the controls before and after CO₂ loading/unloading.

the maximum rate of fall of P_{aCO_2} and $\dot{V}_{CO_2}$ (Figs. 2–4). The slope of $\dot{V}_{CO_2}$ -the maximum rate of rise of P_{aCO_2} line was 5.52 mmHg/s/(l/min), which is in accordance with the theoretically induced value of 4.5 mmHg/s/(l/min) by

Fig. 6. Relationship between the changes in $\dot{V}_{CO_2}$ from control to CO₂ loading/unloading and the minute phrenic activity. The minute phrenic activity was normalized using the slope of CO₂ response line measured separately. The regression equation for all the data (solid line) is: normalized minute phrenic activity = $12.6 \cdot \Delta \dot{V}_{CO_2} - 2.45$ ($r=0.325$), and for the vagotomized dogs (broken line) is: normalized minute phrenic activity = $17.9 \cdot \Delta \dot{V}_{CO_2} - 5.97$ ($r=0.555$).

SAUNDERS (1980). THUS, BY CONTROLLING $\dot{V}_{CO_2}$, we could obtain large enough changes in both the rate of rise and the rate of fall of Pa_{CO_2} ranging about 50 to 280% and 40 to 350% of the control, respectively.

Besides providing large enough input to the respiratory control system, it is important to examine if the respiratory control system was sensitive to the input signal. Therefore, we confirmed the CO₂ responsiveness of the dog by steady state CO₂ inhalation and the function of carotid bodies by steady state inhalation of hypoxic gas.

Although we provided large enough input signal to the respiratory control system which could respond to steady state CO₂, we could not find any appreciable response in the minute phrenic activity to CO₂ oscillations. Possible problems in the measurement will be discussed hereinafter.

We did not change minute ventilation during CO₂ loading/unloading but altered $F_{I_{CO_2}}$ to maintain Pa_{CO_2} isocapnic. Since $\dot{V}_{CO_2}$ under a fixed mechanical ventilation is proportional to $(F_{A_{CO_2}} - F_{I_{CO_2}})$, we sometimes made the dog hypercapnic to obtain larger maximum CO₂ output from the lung. We also conducted runs during mild hypoxia hoping this would increase the sensitivity of the carotid bodies. There may have been, however, several unfavorable effects due to hypercapnia or hypoxia. FITZGERALD and PARKS (1971) found in cats that the neural

output of the carotid bodies does not respond to changes in P_{aCO_2} when P_{aCO_2} is above 60 mmHg during hypoxia. Although we found that all the dogs in the present experiments provided significant CO_2 sensitivities, it may be possible that the actual operating point of P_{aCO_2} on which the CO_2 loading/unloadings were conducted lay near such a plateau. To exclude such a possibility we collected the data of which CO_2 loading/unloadings were conducted only during normoxia, since it has been shown that the relationship between the neural output of the carotid body and P_{aCO_2} is straight up to $P_{aCO_2} = 80$ mmHg if the blood is normoxic (FITZGERALD and PARKS, 1971; LAHIRI and DELANEY, 1975; FITZGERALD and DEGHANI, 1982). Even in that case the direction of the changes in minute phrenic activity to CO_2 loading/unloading appeared to be random: only 6 out of 13 runs showed the expected result in terms of the direction of change, i.e., increase in minute phrenic activity to CO_2 loading and decrease to CO_2 unloading.

Dependence of respiratory center output on the timing of carotid body stimulation within a respiratory cycle has been demonstrated (ELDRIDGE, 1972; CROSS *et al.*, 1979; TEPPEMA *et al.*, 1985). In the present experiment, vagotomy changed the phase relationship between mechanical ventilation (hence arterial CO_2 oscillation) and the respiratory center output of the dog. In the vagi intact dogs, the phase relationship was locked almost in-phase due to the entrainment of the spontaneous respiratory rhythm to mechanical ventilation since there still was a feedback loop, a feedback from the lung stretch receptors. On the other hand, in vagotomized dogs, the phase relationship seemed random. We took a relatively long period of observation to calculate the respiratory variables (usually 20 breaths) to exclude the effect of the breath-by-breath changes in the phase in vagotomized dogs. As a result, it was anticipated that the response of the minute phrenic activity to CO_2 loading/unloading was "diluted" because of varying phase differences within the period of observation. Therefore, it may be reasonable to examine the response of the minute phrenic activity obtained only from the vagi intact dogs where the phase relationship may be more physiological. Seventeen CO_2 loading/unloadings were collected in vagi intact dogs. However, only 9 of the experimental runs showed the expected response.

It has been demonstrated that the acidic slope of arterial pH oscillation (therefore the rate of rise of P_{aCO_2} oscillation) correlates with CO_2 output from the lung, which has prompted investigators to speculate that the CO_2 oscillation is a link between metabolism and ventilation. For the hypothesis to be feasible, at least the following two points should be satisfied: 1) the carotid body provides a rate sensitivity to P_{aCO_2} change; and 2) the carotid body responds only to the rate of rise of P_{aCO_2} . The feasibility of these assumptions will be discussed hereinafter (some discussion has already been made by CUNNINGHAM *et al.*, 1986).

Many investigators have demonstrated that the neural discharge of the carotid body chemoreceptor consists of the stationary and oscillatory components and that the latter is specifically due to the respiratory oscillations of P_{aCO_2} (BISCOE and PURVES, 1967; GEHRICH and MOORE, 1973; GOODMAN and NAIL, 1973). It also has

been in general agreement that the ratio of the oscillatory and stationary components in the neural discharge is much larger than that of the amplitude of P_{aCO_2} oscillation and the average level of P_{aCO_2} (GEHRICH and MOORE, 1973; BAND *et al.*, 1978; CROSS *et al.*, 1986), suggesting that the response characteristic of the carotid body is proportional plus rate sensitive to P_{aCO_2} (provided the threshold of the carotid body is not so high). However, the rate sensitivity of the carotid body seems to be significant only when the stimulus is strong enough; i.e. the rate of change of P_{aCO_2} is several times larger than the resting (BLACK *et al.*, 1971; DUTTON and PERMUTT, 1975; PLAAS-LINK and LOESCHCKE, 1983). Therefore, the specific effect of CO₂ oscillation on the carotid body might be too small at rest (BAND *et al.*, 1978) or even in the mild exercise as well as the present experiment in which the rate of rise of P_{aCO_2} was increased to 280% of the control.

Whether the carotid body responds only to the rate of rise of P_{aCO_2} is of great importance since, if the carotid body could sense both the rate of rise and fall of P_{aCO_2} , information regarding the CO₂ output from the lung would hardly be reflected in the neural output of the carotid body since only the rate of rise of P_{aCO_2} may accurately reflect the CO₂ output from the lung. There may be at least two possibilities that the neural output of the carotid body mainly reflects only the rate of rise of P_{aCO_2} . The first one is that the rate sensitivity of the carotid body to decreasing P_{aCO_2} might be considerably smaller than that to rising. In the experiments where the on-and-off response to hypercapnic step was examined, BLACK *et al.* (1971) found the adaptation of the neural output in the off transient was smaller than that of the on transient. Such asymmetry in the response of sinus nerve activity can be also seen in the experiment of PLAAS-LINK and LOESCHCKE (1983). The second possibility is that the undershoot of the neural discharge in response to the rapid fall of P_{aCO_2} might be clipped at the threshold of the carotid body where the neural discharge ceases, and hence the effect of the rate of fall of P_{aCO_2} is attenuated. BLACK *et al.* (1971) demonstrated this could happen. We suppose, however, that the clipping of the neural output is unlikely to occur in the physiological CO₂ oscillation range since the neural threshold of the carotid body is low (10 to 20 mmHg in the cat, LAHIRI *et al.*, 1978). Thus, we suppose that the carotid body provides an asymmetrical rate sensitivity to changes in P_{aCO_2} which may not be effective at rest or during mild increase in $\dot{V}_{CO_2}$.

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