Ventilatory response to high inspired carbon dioxide concentrations in anesthetized dogs

Jack A. Loeppky¹, Ray Risling²

(Work Affiliation) Department of Physiology and Pharmacology, University of Saskatchewan, Saskatoon, Canada.
¹(Present affiliations) 2725 7th Street South, Cranbrook, British Columbia, V1C 4R8, Canada.
²(Present affiliations) 128 Ottawa Avenue South, Saskatoon, Saskatchewan, S7M 3L5, Canada.

Citation: Loeppky JA, Risling R. Ventilatory response to high inspired carbon dioxide concentrations in anesthetized dogs. North Am J Med Sci 2011; 3: 63-69.
Doi: 10.4297/najms.2011.363
Availability: www.najms.org
ISSN: 1947 – 2714

Abstract

Background: The ventilation (V₁) response to inspired CO₂ has been extensively studied, but rarely with concentrations >10%. Aims: These experiments were performed to determine whether V₁ would increase correspondingly to higher concentrations and according to conventional chemoreceptor time delays. Materials and Methods: We exposed anesthetized dogs acutely, with and without vagotomy and electrical stimulation of the right vagus, to 20-100% CO₂-balance O₂, and to 0 and 10% O₂-balance N₂. Results: The V₁ time delays decreased and response magnitude increased with increasing concentrations (p<0.01), but at higher concentrations the time delays were shorter than expected, i.e., 0.5 s to double V₁ at 100% CO₂, with the response to 0% O₂ being ~3 s slower. Right vagotomy significantly reduced baseline breathing frequency (fR), increased tidal volume (VT) and increased the time delay by ~3 s. Bilateral vagotomy further reduced baseline fR and V₁, and reduced the response to CO₂ and increased the time delay by ~12 s. Electro-stimulation of the peripheral right vagus while inspired CO₂ caused a 13 s asystole and further reduced and delayed the V₁ response, especially after bilateral vagotomy, shifting the mode from VT to fR. Conclusions: Results indicate that airway or lung receptors responded to the rapid increase in lung H⁺ and that vagal afferents and unimpaired circulation seem necessary for the initial rapid response to high CO₂ concentrations by receptors upstream from the aortic bodies.

Keywords: Central chemoreceptors, lung chemoreceptors, nociceptors, peripheral chemoreceptors, vagotomy.

Correspondence to: Jack A. Loeppky, PhD., 2725 7th Street South, Cranbrook, British Columbia, V1C 4R8, Canada. Tel.: 250-489 4597, Email address: Loeppkyj@telus.net

Introduction

There are few studies that have investigated the relationship between the acute inspiration of very high CO₂ levels and the time delay and magnitude of the resulting change in ventilation (V₁). At lower, more physiological, levels it is generally presumed that the magnitude of the response is directly related to the concentration; however the time delay is determined by a number of factors that need not be related to the concentration, such as: (a) lung to peripheral and central chemoreceptor circulation time, (b) nerve conduction velocity from receptor to brain to effector organs and (c) baseline alveolar ventilation that determines the initial alveolar Pco₂ (PAco₂) and Po₂ (PAO₂).

Two factors that are potentially related to the CO₂ concentration are (a) the rate of rise of Pco₂ in the lung and receptor sites and (b) the associated stimulation of other receptors (nociceptors) in the larynx, airway, lungs or pulmonary veins by high CO₂. In order to determine whether the latter may be involved, these experiments were undertaken to measure the acute responses to inspired CO₂ levels from 20 to 100% in anesthetized dogs. Measurements were made before and after right and left vagotomy and superimposed stimulation of the right vagus nerve.

Materials and Methods

All experiments were performed on two female mongrel
dogs, weighing 8.4 kg each, on separate days, after which the animals were sacrificed. All procedures were in accordance with the Canadian Council of Animal Care guidelines. Dogs were anesthetized with 30 mg/kg Nembutal. The trachea was cannulated and the right and left vagus nerves isolated at the neck. A Y-tube was fitted to the tracheal cannula. Gas mixtures were prepared in a Douglas bag and valve, attached to one arm of the Y-connector placed in the tracheal cannula. Following a period of ~30 s, when baseline $V_1$ was recorded, the valve from the bag was opened just prior to an inspiration, a time event marker indicated time zero for establishing the time of subsequent inspirations.Expiration took place through the other arm of the Y-connector that had a one-way valve that closed during inspiration. Recordings were continued until the tidal volume (VT) response to the test gas appeared maximal on the tracing.

Breath-by-breath chest expansion was obtained by an impedance pneumograph, and ventilation frequency (fR) and timing were obtained from subsequent measurements from polygraph recordings (1.0 cm/s) and time event markers. Heart rate (fH) was obtained by chest lead ECG. The polygraph recordings were uncalibrated, the height was expressed in units (U) to represent VT because the position of the impedance band may have moved and the contribution of the diaphragm to true VT may vary between trials. Breath-by-breath $V_1$ was calculated as U/min for each breath from the product of VT and fR calculated from the time between a given inspiration at the measured time and the previous one. $V_1$ was then re-plotted on a time base relative to baseline $V_1$ and averaged at specific times for the same conditions (up to six) at 0.5, 1.0 and 2.0 s intervals. Then average time delays from repeated trials with the various test gases were compared from the time of the first inspiration to where $V_1$ was doubled (2 $V_1$/B) and quadrupled (4 $V_1$/B) from baseline $V_1$. Time delays from time zero to where interpolated $V_1$ exceeded 3 SD of baseline $V_1$ were also noted as “response times.”

All CO2 mixtures were given in alternating order from low to high CO2 (20, 40, 70 and 100%), with balance O2 (80, 60, 30 and 0%, respectively). Hypoxic mixtures, 10% O2-90% N2 and 0% O2-100% N2, and air controls were interspersed randomly with the CO2 trials. After these trials with both vagi intact, the 100% CO2 and 0% O2-100% N2 trials were repeated with the right vagus and then both vagi cut. In addition the latter were repeated with the peripheral (efferent) end of the right vagus stimulated (60 Hz, 10 V, 5 ms) with a square wave generator at the same time that the gas was inspired to induce asystole to curtail the circulation. The baseline fH averaged 125/min after one or both vagi were cut before stimulation. Stimulation of the cut right vagus, with and without the left vagus cut, resulted in a 13 s asystole (range: 5 to 17 s), with vagal escape occurring over the next 10 s and fH then stabilizing at ~44/min during stimulation.

The time course of $\text{PacO}_2$ and $\text{PAO}_2$ following inspiration of the test gas was estimated breath-by-breath from a) the mixing of measured VT and functional residual capacity (FRC) with the bag O2 and CO2 concentrations, assuming baseline $\text{PacO}_2 = 35$ mmHg and $\text{PAO}_2= 99$ mmHg and a baseline VT of 120 ml and FRC of 600 ml, as measured in dogs of similar weight by Muggenburg et al. [1] and b) an O2 consumption of 5 ml/min/kg and baseline respiratory exchange rate of 0.8. The change in pH value ($\Delta$H) corresponding to changes in $\text{PacO}_2$ was calculated from the Henderson-Hasselbalch equation, assuming instantaneous equilibration between arterial $\text{Paco}_2$ and $\text{PAO}_2$, a pK of 6.1, a fixed bicarbonate ($\text{HCO}_3^{-}$) concentration of 24 mmol/L and no CO2 storage in lung tissue; the latter would tend to buffer changes in $\text{PacO}_2$ [2].

**Results**

The average $V_1$/B responses to the four CO2 concentrations, two hypoxic mixtures and air controls are depicted in Figure 1, with values in Table 1. The response time and time delay for the four CO2 levels was inversely related to concentration and the magnitude was directly related; the inverse relationship between the highest three concentrations and time delays to $2V_1$/B and $4V_1$/B was linear. The responses to the two levels of hypoxia were similarly related to reduced O2, but attenuated and slower than those for CO2. For 100% CO2 the response time was 0.3 s, with time delays of 0.5 s and 2.0 s at $2V_1$/B and $4V_1$/B, respectively; the rise above baseline occurred on the first inspiration, with a greater VT (124%) and fR (8%) than baseline.

Figure 2 shows the $V_1$/B responses to the four CO2 levels, along with the estimated $\text{Paco}_2$ at each inspiration. The 100% CO2 trial (Fig. 2D) also shows the rapid PAO2 decline with zero inspired O2. The values for the instantaneous change/time of $\text{Paco}_2$ and $V_1$/B are shown at $2V_1$/B and $4V_1$/B, assuming no time delay between $\text{Paco}_2$ and $V_1$/B. The clear pattern is that $\text{Paco}_2$/s markedly increased with inspired CO2 level as the time delay decreased, whereas $\Delta V_1$/B/s was not markedly affected. $V_1$/B was greater at equal times as CO2 concentration increased mainly because of the shorter time delays. The average of the individual time delays to $2V_1$/B and $4V_1$/B were significantly shorter for the 70 and 100% trials than for the 20 and 40% trials (1.8 vs. 23.5 and 3.4 vs. 32.8 s, respectively), with response times of 0.3 and 13.4 s ($p<0.01$ for all).

The percentage changes in fR and VT and estimated $\text{Paco}_2$, $\text{PAO}_2$ and $\Delta$H values at $2V_1$/B and $4V_1$/B for all trials are included in Table 1. With vagi intact most of the $V_1$ increase to $2V_1$/B for all CO2 levels and 0% and 10% O2 were due to increased VT, with an increasing, but still negligible contribution by fR at $4V_1$/B. $\text{PAO}_2$ was ~70 mmHg when $2V_1$/B was reached for the 10% and 0% O2 trials, but the time delay at 0% O2 was 10 s less than at 10%. A comparison of baseline ventilatory
components before and after vagotomy shown in Table 1 shows a significant reduction in baseline fR, an increase in VT and no change in V̇₁ after cutting the right vagus. Bilateral vagotomy further reduced fR, resulting in a significant reduction in V̇₁ compared to right vagotomy alone.

The effects of right and bilateral vagotomy and superimposed right vagus stimulation on the response to 100% CO₂ and 0% O₂ are summarized in Figure 3. Stimulation of the cut right vagus by itself caused a small increase in V̇₁/B (Figs. 3B and 3C), mainly resulting from a greater fR, with the left vagus intact or cut (Table 1). Inspiring 0% O₂ after bilateral vagotomy increased the time delays to 2V̇₁/B and 4V̇₁/B by some 36 s (Fig. 3A), with the increased V̇₁/B resulting predominantly from increasing fR, whereas VT was the main contributor in the intact trials. Inspiration of 100% CO₂ with the right vagus cut (Fig. 3B) resulted in a vigorous response that was delayed about 4 s compared to that with the vagus intact, with VT still the main contributor. When the peripheral end of the right vagus was stimulated as CO₂ was inspired, the vigorous response was delayed an additional 5 s, with fR now the main contributor to the V̇₁/B increase. The effect of bilateral vagotomy on the response to CO₂ was qualitatively similar, but magnified (Fig. 3C). The time delay to 2V̇₁/B was extended an additional 2 s after both vagi were cut, with V̇₁/B reaching a plateau at ~12 s. The relatively greater contribution of VT to the increase to 2V̇₁/B remained about the same as with intact vagi, similar to hypoxia (Fig. 3A). When CO₂ was given during right vagal stimulation with both vagi cut the response was greatly attenuated and the time delay to 2V̇₁/B and 4V̇₁/B increased by an average of 15 s, with the fR contribution increasing compared with no stimulation.

Fig. 1 Average V̇₁/B responses to seven conditions. Numbers in parentheses indicate number of trials for each condition. Time delays at 2V̇₁/B and 4V̇₁/B are indicated as in Table 1.

Fig. 2 Average V̇₁/B responses for four inspired CO₂ concentrations. Open circles indicate estimated Paco₂ for each breath. Pao₂ at 100% CO₂ is indicated by solid circles in panel D. Instantaneous values/time for Paco₂ and V̇₁/B are indicated at 2V̇₁/B and 4V̇₁/B.

Fig. 3 Mean V̇₁/B responses to 100% CO₂ and 0%-100% N₂ before and after bilateral vagotomy (panel A). Panel B shows mean responses to 100% CO₂ before vagotomy, after Rt vagotomy while electrically stimulating the right (Rt) vagus and stimulation alone. Panel C indicates mean responses to 100% CO₂ before vagotomy, after bilateral vagotomy while stimulating the right vagus and stimulation alone. Numbers in italics indicate percentage change in fR and VT above baseline at 4V̇₁/B.
Table 1 Experimental conditions and averaged measurements for 14 trials in anesthetized dogs.

| Experimental condition | n   | $\dot{V}_I$ U x min$^{-1}$ | fR min$^{-1}$ | VT U | Res. time s | $\dot{V}_I/B$ s | Time s | $\Delta fR$ % | $\Delta VT$ % | $\text{PA}_{O_2}$ mmHg | $\text{PA}_{CO_2}$ mmHg | $\Delta pH$ |
|------------------------|-----|-----------------------------|--------------|------|--------------|---------------|--------|--------------|--------------|-------------------------|-------------------------|-----------|
| Control                | 3   | 28.3                        | 15.8         | 1.79 | - (-)        | 1.17          | 2.0    | 1            | 16           | 104                     | 104                     | 0.07      |
| control                | 3   | 4.0                         |              |      |              |               |        |              |              |                         |                         |           |
| Control                | 4   | 18.0                        | 19.4         | 0.93 | 18.3 (7.0)   | 2.0           | 25.1   | -11          | 125          | 441                     | 125                     | -0.55     |
| control                | 4.0 | -                           |              |      |              |               |        |              |              |                         |                         |           |
| 10% O$_2$-90% N$_2$    | 1   | 34.1                        | 26.0         | 1.31 | 1.3 (-)      | 2.0           | 13.7   | 15           | 74           | 66                      | 63                      | 0.43      |
| control                | 4.0 | -                           |              |      |              |               |        |              |              |                         |                         |           |
| 100% O$_2$             | 2   | 11.4                        | 13.1         | 0.87 | 3.3 (2.6)    | 2.0           | 3.4    | 4            | 103          | 73                      | 30                      | 0.07      |
| control                | 4.0 | -                           |              |      |              |               |        |              |              |                         |                         |           |
| 0% O$_2$-100% N$_2$    | 1   | 11.8                        | 6.3          | 1.88 | 24.3 (-)     | 2.0           | 32.8   | 55           | 29           | 18                      | 32                      | 0.04      |
| control                | 4.0 | 50.4                        | 152          | 60   | 0            | 21            | 0      |              |              |                         |                         |           |
| 0% O$_2$-100% N$_2$    | 22  | 20.9 (1.4)                  | 18.7 (1.8)   | 1.12 | 0.0 (1.10)   | 2.0           | 7.1    | 51           | 18           | 104                     | 31                      | 0.05      |
| 20% CO$_2$-80% O$_2$   | 4   | 18.0                        | 19.4         | 0.93 | 18.3 (7.0)   | 2.0           | 25.1   | -11          | 125          | 441                     | 125                     | -0.55     |
| 20% CO$_2$-80% O$_2$   | 4.0 | -                           |              |      |              |               |        |              |              |                         |                         |           |
| 20% CO$_2$-80% O$_2$   | 2   | 16.6                        | 26.0         | 0.64 | 3.7 (0.1)    | 2.0           | 5.1    | -4           | 94           | 213                     | 129                     | -0.57     |
| 20% CO$_2$-80% O$_2$   | 4.0 | -                           |              |      |              |               |        |              |              |                         |                         |           |
| 40% CO$_2$-60% O$_2$   | 2   | 21.0                        | 22.8         | 0.92 | 0.3 (0.2)    | 2.0           | 2.2    | 6            | 90           | 127                     | 163                     | -0.67     |
| 40% CO$_2$-60% O$_2$   | 4.0 | 6.6                         | 11            | 263  | 141          | 233          | 148    |              |              |                         |                         | -0.82     |
| 40% CO$_2$-60% O$_2$   | 2   | 4.3                         | 11            | 263  | 141          | 233          | 148    |              |              |                         |                         | -0.82     |
| 70% CO$_2$-30% O$_2$   | 4   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |
| 70% CO$_2$-30% O$_2$   | 6   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |
| 70% CO$_2$-30% O$_2$   | 6   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |
| 70% CO$_2$-30% O$_2$   | 6   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |
| 70% CO$_2$-30% O$_2$   | 6   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |
| 70% CO$_2$-30% O$_2$   | 6   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |
| 70% CO$_2$-30% O$_2$   | 6   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |
| 70% CO$_2$-30% O$_2$   | 6   | 19.0                        | 15.3         | 1.24 | 0.3 (0.1)    | 2.0           | 0.5    | 5            | 91           | 88                      | 100                     | -0.46     |
| 70% CO$_2$-30% O$_2$   | 4.0 | 2.0                         | 36            | 176  | 69           | 215          | 176    |              |              |                         |                         | -0.79     |

-:

100% CO$_2$ (cut Rt + Lt vag)
\( \varphi: \) inspired ventilation divided by tidal volume; \( \delta R: \) breathing frequency; \( \Delta T: \) tidal volume; \( \delta R: \) response time for interpolated \( \varphi_1 \) to exceed baseline mean +3 SD; \( \varphi_1: \) inspired ventilation divided by baseline ventilation; \( \Delta T: \) time from onset of first inspiration to \( 2\varphi_1/2 \) and -4\( \varphi_1/3 \) from average curve; \( \Delta \varphi: \) percentage change in \( \delta R \) and VT from baseline to +2\( \varphi_1/2 \) and -4\( \varphi_1/3 \); \( \varphi_0: \) estimated at \( 2\varphi_1/2 \) and -4\( \varphi_1/3 \) assuming baseline values of 99 and 33 mmHg, respectively; \( \delta \varphi: \) change estimated from \( \varphi_{200} \) change from baseline (35 mmHg) assuming fixed HCO\(_3\)-; Parentheses: s.e.m.; *: value significantly different (p<0.05) different from that with vagi intact; #: value significantly different (p<0.05) from value with Rt vagotomy

**Discussion**

These experiments strongly suggest that ventilation increases and the time delay decreases as the inspired \( \mathrm{CO}_2 \) level approaches 100%. At levels \( \geq 70\% \) the time delay is shorter than reported for aortic arch and carotid body chemoreceptor response times from previous and subsequent studies. Vagotomy delayed the response to 100% \( \mathrm{CO}_2 \) and restricting the circulation delayed it further.

That our limited experimental set-up was reasonable is partly supported by the following: (a) the changes in \( \delta R \) and VT with vagotomy during baseline (Table 1) agree closely with those reported in anesthetized dogs by Anrep and Samaan [3], who concluded that the slowing of respiration was due to denervation of the lungs and not the peripheral chemoreceptors, (b) the \( \varphi_1/2 \) response to hypoxia (Fig. 1 and Table 1) was not far removed from the 10 s time delay reported in humans and dogs and occurred at estimated \( \varphi_{200} \) values close to those reported for steady state breathing [4], (c) the \( \varphi_1/2 \) response leveled off with 100% \( \mathrm{CO}_2 \) after vagotomy (Fig. 3C), as reported in dogs [5] and (d) the response was greatest and time delay shortest with 100% \( \mathrm{CO}_2 \) when \( \varphi_0 \) fell most rapidly (Fig. 2D), demonstrating the well-known enhanced ventilatory sensitivity to \( \mathrm{CO}_2 \) when combined with hypoxia [6].

Studies of ventilatory responses to \( \mathrm{CO}_2 \) and hypoxia in humans and mammals have typically utilized inspired concentrations of <10% \( \mathrm{CO}_2 \) (inspired \( \mathrm{PCO}_2<71 \text{ mmHg} \) at sea level) and >10% \( \mathrm{O}_2 \). Ventilatory studies using non-physiological concentrations >20% \( \mathrm{CO}_2 \) have rarely been reported; when breathing concentrations >35% for some minutes it is an effective anesthetic in dogs [7]. In humans, repeated applications of 12 inspirations of a 30% \( \mathrm{CO}_2-70\% \) \( \mathrm{O}_2 \) gas mixture were utilized by Meduna [8] some 6 decades ago to treat psychoneuroses and anxiety disorders with some success. The reaction to a mixture of 35% \( \mathrm{CO}_2-65\% \) \( \mathrm{O}_2 \) has also been used as a trait marker for panic disorders [9]. Barcroft and Margaria [10] compared the ventilatory effect of \( \mathrm{CO}_2 \) inhalation and exercise on themselves and stated, “The breathing of 7.5% of \( \mathrm{CO}_2 \) for 20 minutes produces a shock from which the system does not wholly escape for some hours or perhaps even a longer time.” They also measured the change in \( \delta R \) with the inspiration of 64% \( \mathrm{CO}_2 \) in anesthetized cats [11] and noted that the increase was inversely related to baseline \( \delta R \) and that bilateral vagotomy resulted in an erratic response. Their recordings suggest a time delay of 4 to 5 s between first inspiration and ventilation increase. Dejours stated, “The existence of lung air chemoreceptors acting reflexly on the ventilatory regimen is generally not admitted,” because, “These results have been observed only as a result of enormous and quite unphysiological shifts of \( \mathrm{PCO}_2 \),” and, “The hyperventilation resulting from breathing \( \mathrm{CO}_2 \)-rich mixtures does not occur before a lag of many seconds” [12]. On the other hand, Pi-suher, in summarizing extensive chemoreceptor research prior to the early 1940s [13], took exception to the statement by Cordier and Heymans [14] that, “authors have administered by inhalation air with concentrations of \( \mathrm{CO}_2 \) which pass beyond physiological limits and even beyond the pathological”. Pi-suher concluded from numerous experiments, “In addition to the well known action on the respiratory centers, there is exerted a parallel or perhaps previous peripheral influence due to the excitation of end-organs which are sensitive to stimuli of chemical nature by the \( \mathrm{CO}_2 \) contained in the inspired air” [13]. Our results support the latter in the continuing controversy regarding lung chemoreceptors, the same as many early studies based on the ventilation response to higher concentrations.

The aortic arch and carotid body (peripheral) and central medullary chemoreceptors all respond to \( \mathrm{CO}_2 \) and hydrogen ion concentration (H+) to increase ventilation; the relative contributions of these responses to this rise following the stimuli of lung or blood \( \mathrm{CO}_2/\mathrm{H}^+ \) have been studied extensively and remain controversial, especially with variations in baseline arterial blood \( \mathrm{PO}_2 \) [12, 15, 16]. Recent studies with isolated carotid sinus perfusion show that the central chemoreflex can respond to an increase in \( \mathrm{PA}_{\text{CO}_2} \) in unanesthetized dogs in 6 s, but take 11 s longer when separated from peripheral receptors [17]; this demonstrates that the gain of the central receptors is critically dependent on the peripheral ones [18]. It is often not clear whether reported time delays pertain to central and/or peripheral receptors, but the latter should respond first to the \( \mathrm{CO}_2/\mathrm{H}^+ \) signal.

Time delays result primarily from the lung-to-chemosensor circulation time. Our average time delay from first inspiration to \( 2\varphi_1/2 \) was inversely related to \( \mathrm{CO}_2 \) concentration, ranging from 25.1 to 0.5 s, for 20 and 100%, respectively. The lung to brain time delay from an increase in \( \mathrm{PA}_{\text{CO}_2} \) to affect \( \mathrm{pH} \) at the medulla oblongata in unanesthetized cats has been reported to be 5 to 7 s [19]. In humans the peripheral response to inspiring hypoxic gas has been measured at 5 s, from lung-to ear circulation time by oximetry [20]. McLean et al. [21] measured a 10 s delay to peak ventilation after a single breath of 13% \( \mathrm{CO}_2 \)-balance air in healthy humans and suggested this as a test for peripheral chemoreceptor function in patients. The time delay from infusion of \( \mathrm{CO}_2 \)-equilibrated blood into the aortic arch to increase ventilation was found to be 6.6 s in unanesthetized dogs by Sylvester et al. [22], who
concluded that the circulation time from aortic arch to aortic body, carotid body and the medulla to be 1, 3-4 and 5-6 s, respectively. Definitive time delay experiments in unanesthetized dogs were conducted by Gonzalez et al. [23]. They measured the time from injection of cold NaHCO₃ to the increase in ventilation to be 2.0 and 6.9 s, when injected into the aortic arch and superior vena cava, respectively. The corresponding times for arrival of the blood to these sites were 1.9 and 3.7 s. The time from the PAco₂ rise in the lung, induced by the NaHCO₃, to arrival at the carotid body was about 1 s, implying a lung stimulus to ventilation response time of ~3 s.

An important consideration is that a rapid response in fR and/or VT during the first inspirate will increase the rate of rise of PAco₂ to raise the alveolar/arterial blood stimulus level for the downstream arterial chemoreceptors (Fig. 2). Carboxic anhydrase, present in the interstitial lung tissue, would be expected to rapidly convert CO₂ to H+ in the pulmonary capillaries and then stimulate the downstream chemoreceptors [24, 25]. At 100% CO₂, with concurrent hypoxia, the fall in pH would be partially attenuated due to the Haldane effect [26]. Assuming that effect is negligible and with instantaneous equilibration of lung-blood PAco₂ and pH, the VT and fR measurements, and interpolating PAco₂ for the times courses in Fig. 2 at 2 s, the estimated lung tissue pH decreased 0.19, 0.41, 0.65 and 0.79 as inspired CO₂ fractions increased, respectively. With the right vagus cut the pH fell 0.59 with 100% CO₂ and to 0.34 with both cut. This is about half the increase in H+ compared to that with the vagi intact. Bartoli et al. [27] emphasized the difficulty of separating the chemoreceptors involved in responding to inhaled CO₂ vs. hypercapnic blood. They noted a vagally mediated response to inspired CO₂ on the first breath that was absent after vagotomy, similar to our results. Our responses to CO₂ in Fig. 2 at 20 and 40% in intact dogs suggest stimulation of peripheral and central chemoreceptors without an initial rapid response, as the time delays are within those reported. However, at 70 and 100% the response is faster than can be explained by those.

Our results imply that there is a third sensing site, upstream from the aortic bodies in or near the lung that is dependent on vagal afferents. We speculate that nociceptors are involved. Laryngeal CO₂ receptors have been noted in anesthetized dogs [28] and when these myelinated and unmyelinated fibers in the vagus were blocked the reflex was decreased [29]. These sensing regions are located in the trachea and larger bronchi, where they are more chemosensitive, and can stimulate ventilation. They probably add to the response of the unmyelinated C-fibers in contributing to the total reflex response [30]. There is also evidence that the J-receptors [31, 32] and vagal bronchopulmonary C-fiber sensory nerves are also involved in the rapid ventilatory responses to lung irritants and may contribute to dyspnea in patients with COPD [33, 34]. Furthermore, these C-fibers have been shown to respond rapidly in dose-related fashion to H+ induced by injections of lactic acid in anesthetized rats [35, 36]. An increase in PAco₂, acting via H+, has been shown to augment the responses of the C-fibers to chemical stimulants [37].

The estimated pH changes shown in Table 1 exceed those reported to be effective in C-fiber stimulation in anesthetized rats. The high CO₂ or H+ acting as a direct irritant, could explain our results with 70 and 100% CO₂ (Fig. 3B). Both the near instantaneous C-fiber response and part of the peripheral reflex are abolished by vagotomy, accounting for the delayed response, which then returns only from central chemoreceptors. The response by the latter is further reduced when the peripheral receptor potentiation is partially removed by cutting both vagi (Fig. 3C) and further delayed by slowing the circulation by stimulating the effenter right vagus.

**Conclusion**

Our indirect evidence for fast-acting chemoreceptors in the broncho-tracheal region to high CO₂/H+ concentrations can be criticized for having too few animals and lack of ancillary respiratory measurements. Certainly a shift in baseline acid-base status because of repeated trials with CO₂ would have an effect on the response curves. However, the time delays were carefully measured and suggest that more experiments are required to determine the contribution of airway and lung area chemoreceptor to the control of ventilation when alveolar Pco₂ is rapidly altered.

**Acknowledgements**

These experiments came about as a result of skepticism expressed in 1978 by Professor G. Bonar Sutherland, Department of Physiology and Pharmacology, University of Saskatchewan, who stated; “I’m not satisfied that current peripheral/central chemoreflex hypotheses completely explain the rapid ventilation response to CO₂.”

**References**

1. Muggenburg BA, Wolff RK, Mauderly JL, et al. Cardiopulmonary function of dogs with plutonium-induced lung injury. Rad Res 1988; 115: 314-324.
2. DuBois AB, Britt AG, Fenn WO. Alveolar CO₂ during the respiratory cycle. J Appl Physiol 1952; 4: 535-548.
3. Anrep GV, Samaan A. Double vagotomy in relation to respiration. J Physiol 1932; 77: 1-15.
4. Hirshman CA, McCullough RE, Cohen PJ, Weil JV. Hypoxic ventilatory drive in dogs during thiopental, ketamine, or pentobarbital anesthesia. Anesthesiology 1975; 43: 628-634.
5. Kashani M, Haigh AL. The effects of vagotomy on ventilation and blood gas composition in dog, sheep and rabbit. Q J Exp Physiol Cogn Med Sci 1975; 60: 285-298.
6. Cunningham DJ, Shaw DG, Lahiri S, Lloyd BB. The effect of maintained ammonium chloride acidosis on the relation between pulmonary ventilation and
alveolar oxygen and carbon dioxide in man. Q J Exp Physiol Cogn Med Sci 1961; 46: 323-334.

7. Eisele H, Eger EI (2nd), Muallem M. Narcotic properties of carbon dioxide in the dog. Anesthesiology 1967; 28: 856-865.

8. Meduna LJ. Carbon Dioxide Therapy: A Neuropsychological Treatment of Nervous Disorders. Springfield IL: Charles C Thomas;1950

9. Perna G, Battaglia M, Garberi A, Arancio C, Bertani A, Bellodi L. Carbon dioxide/oxygen challenge test in panic disorder. Psychiatry Res 1994; 52: 159-171.

10. Barcroft J, Margaria R. Some effects of carbonic acid on human respiration. J Physiol 1931; 72: 175-185.

11. Barcroft J, Margaria R. Some effects of carbonic acid in high concentrations on respiration. J Physiol 1932; 74: 156-162.

12. Dejours P. Chemoreflexes in breathing. Physiol Rev 1962; 42: 335-358.

13. Pi-sunyer A. The regulation of the respiratory movements by peripheral chemoreceptors. Physiol Rev 1947; 27: 1-38.

14. Cordier D, Heymans C. Le centre respiratoire. Ann Physiol Physiolog Biol 1935; 11: 537-771.

15. Cunningham DJ. Studies on arterial chemoreceptors in man. J Physiol 1987; 384: 1-26.

16. Lahiri S, Forster RE (II). CO2/H+ sensing: peripheral and central chemoreception. Int J Biochem Biol 2003; 35: 1413-1435.

17. Smith CA, Rodman JR, Chenuel BJ, Henderson KS, Dempsey JA. Response time and sensitivity of the ventilatory response to CO2 in unanesthetized intact dogs: central vs. peripheral chemoreceptors. J Appl Physiol 2006; 100: 13-19.

18. Blain GM, Smith A, Henderson KS, Dempsey JA. Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to CO2. J Physiol 2010; 588 (pt 13): 2455-2471.

19. Ahmad HR, Loeschcke HH. Transient and steady state responses of pulmonary ventilation to the medullary extracellular pH after approximately rectangular changes in alveolar PCO2. Pflügers Arch 1982; 395: 285-292.

20. Drysdale DB, Petersen ES. Arterial chemoreceptors, ventilation and heart rate in man. J Physiol 1977; 273: 109-120.

21. McClean PA, Phillipson EA, Martinez D, Zamel N. Single breath of CO2 as a clinical test of the peripheral chemoreflex. J Appl Physiol 1988; 64: 84-89.

22. Sylvestre JT, Whipp BJ, Wasserman K. Ventilatory control during brief infusions of CO2-laden blood in the awake dog. J Appl Physiol 1973; 35: 178-186.

23. Gonzalez F (Jr), Fordyce WE, Grodins FS. Mechanism of respiratory responses to intravenous NaHCO3, HCL, and KCN. J Appl Physiol 1977; 43: 1075-1079.

24. Iturriaga R. Carotid body chemoreception: the importance of CO2/HCO3- and carbonic anhydrase (review). Biol Res 1993; 26: 319-329.

25. Heming TA, Stabenau EK, Vanoye CG, Moghadasi H, Bidani A. Roles of intra- and extracellular carbonic anhydrase in alveolar-capillary CO2 equilibration. J Appl Physiol 1994; 77: 697-705.

26. Scotto P, Lopppky JA, Piiper J, Farhi, L.E. Acid-base status immediately following rapid changes of alveolar gas composition in awake dogs. Respir Physiol 1987; 68: 251-258.

27. Bartoli A, Cross BA, Guz A, Jain SK, Noble MI, Trenchard DW. The effect of carbon dioxide in the airways and alveoli on ventilation; a vagal reflex studied in the dog. J Physiol1974; 240: 91-109.

28. Anderson JW, Sant’Ambrogio FB, Orani GP, Sant’Ambrogio G, Mathew OP. Carbon-dioxide-responsive laryngeal receptors in the dog. Respir Physiol 1990; 82, 217-226.

29. Tatar M, Sant’Ambrogio G, Sant’Ambrogio FB. Laryngeal and tracheobronchial cough in anesthetized dogs. J Appl Physiol 1994; 76: 2672-2679.

30. Sant’Ambrogio G, Widdicombe J. Reflexes from airway rapidly adapting receptors. Respir Physiol 2001; 125: 33-45.

31. Paintal AS. Some recent advances in studies on J receptors. Adv Exp Med Biol 1995; 381: 15-25.

32. Widdicombe JG. The J reflex. J Physiol 1998; 511(pt 1): 2.

33. Burki NK, Sheatt M, Lee LY. Effects of airway anesthesia on dysnea and ventilatory response to intravenous injection of adenosine in healthy human subjects. Pulm Pharmacol Ther 2008; 21: 208-213.

34. Lee LY. Respiratory sensations evoked by activation of bronchopulmonary C-fibers. Respir Physiol Neurobiol 2009; 167: 26-35.

35. Lee LY, Morton RF, Lundberg JM. Pulmonary chemoreflexes elicited by intravenous injection of lactic acid in anesthetized rats. J Appl Physiol 1996; 81: 2349-2357.

36. Hong JL, Kwong K, Lee LY. Stimulation of pulmonary C fibres by lactic acid in rats: contributions of H+ and lactate ions. J Physiol 1997; 500 (pt 2): 310-329.

37. Gu Q, Lee LY. Alveolar hypercapnia augments pulmonary C-fiber responses to chemical stimulants: role of hydrogen ion. J Appl Physiol 2002; 93: 181-188.