Respiration Physiology (1981) 44, 365–380
Elsevier/North-Holland Biomedical Press

VENTILATORY RESPONSE TO INHALED AND INFUSED CO_2: RELATIONSHIP TO THE OSCILLATING SIGNAL

B. J. B. GRANT*, R. P. STIDWIL B. A. CROSS and S. J. G. SEMPLE

Department of Medicine, The Middlesex Hospital Medical School, London WIN 8AA, United Kingdom

Abstract: The purpose of this study was to determine the relationship between the respiratory oscillations of CO_2 and the ventilatory response to CO_2. Cats anaesthetized with chloralose and urethane were given CO_2 either by exchange transfusion into the inferior vena cava or by inhalation. Changes of amplitude of the respiratory oscillations of CO_2 (amp P_{CO_2}) were assessed from measurements of the amplitude of the arterial pH oscillations (amp pH). For short periods of CO_2 loading (2–8 min), the ventilatory response to CO_2 was greater for venous than for airway loading; this difference was statistically significant at low and intermediate loads but not at high loads. The dependence of the respiratory response to the route of administration of CO_2 could not be related to amp P_{CO_2}. For long periods of CO_2 loading (20 min or more) there was no marked change of minute ventilation when the route of administration was changed from airway to venous route, despite an increase of amp pH at both low and intermediate loads.

We suggest that the dependence of ventilatory response to CO_2 on the route of administration observed in this study was mainly due to a longer period required to attain steady state conditions for CO_2 inhalation, and that the respiratory oscillations of CO_2 do not affect the ventilatory response to CO_2.

Cats	In vivo measurement of arterial pH
Chemical control of breathing	Respiratory oscillations of CO_2

The cyclic nature of breathing generates oscillations of arterial blood gas composition about a mean level (Nims and Marshall, 1938; Honda and Veda, 1961; Purves, 1966; Band, Cameron and Semple, 1969a) and these oscillations have been shown to influence phrenic motoneurone output in the anaesthetised dog (Cross *et al.*, 1979). It is possible that CO_2 loading by the venous route will affect the oscillations differently from loads imposed *via* the airways. For example, Yamamoto (1960) has suggested that the amplitude of the oscillations will be increased with venous loads and reduced when CO_2 is administered *via* the airways. Alternatively,

Accepted for publication 5 March 1981

* Present address: University of Michigan Medical School, Pulmonary Division S11303, Box 055, 1405 E Ann Street, Ann Arbor, MI 48109, U.S.A.

changes in the phase relationship between the pH and respiratory cycles to the two forms of CO_2 loading might be different and hence affect the respiratory response (Cross *et al.*, 1979). Several investigators have compared the ventilatory response to venous and airway CO_2 loading with conflicting results. Some investigators have found a greater ventilatory response for venous CO_2 loading than for airway loading (Wasserman *et al.*, 1975; Linton, Miller and Cameron, 1976; Stremel *et al.*, 1978). Others have found no difference (Lamb, 1966; Lewis, 1975; Greco *et al.*, 1978; Ponte and Purves, 1978; Reischl *et al.*, 1979; Fordyce and Grodins, 1980). Of all these studies the only measurements of the pH oscillations were made by Ponte and Purves (1978) who found a decrease in the amplitude of the oscillations (amp pH) during venous CO_2 loading due to a concomitant rise of respiratory frequency. No measurements were recorded in this study of the phase relationships between respiratory and pH cycles. In view of the paucity of data on the effect of CO_2 loading on the oscillations in arterial P_{CO_2} (Pa_{CO_2}) we have examined the changes in the oscillations to venous and airway CO_2 loading and related them to the ventilatory response. Changes in oscillations of P_{CO_2} were judged from measurements of the oscillations of arterial pH. Preliminary reports of some of the findings included in this paper have been presented in brief (Grant and Semple, 1976a, 1977).

Methods

EXPERIMENTAL ARRANGEMENT

Anaesthesia was induced in thirty-two cats (average weight 3.4 kg, range 2.5–4.1 kg) with an intramuscular injection of ketamine hydrochloride (20 mg · kg^{-1}, Parke–Davis). Anaesthesia was maintained by intravenous injections of a solution which contained chloralose (2.5 mg · ml^{-1}, BDH Chemicals) and urethane (0.5 mg · ml^{-1}, Koch-Light Labs.) as required. The need for anaesthesia was judged primarily from a decreasing end-tidal P_{CO_2} (PA_{CO_2}) while breathing air. Body temperature was measured with a rectal thermometer and maintained at 37° ± 1 °C with a heating pad. A polyethylene catheter (1.4 mm internal diameter, 1.9 mm external diameter) was inserted into each femoral vein and positioned in the inferior vena cava so that the tip of one catheter (downstream catheter) was about 1.5 cm caudal to the xiphisternum. The tip of the other catheter (upstream catheter) was about halfway between the xiphisternum and symphysis pubis. The catheters were attached to four close-fitting 50-ml glass syringes with wide bore silicone rubber tubing (internal diameter 1.6 mm) mounted on two dual infusion/withdrawal pumps (Harvard Apparatus Co., Inc., U.S.A.). This system had a dead space of 6 ml and was used for venous CO_2 loading by exchange transfusion.

For transfusion, 100 ml of blood was infused into the downstream catheter, while the same quantity was withdrawn from the upstream catheter. This extra

volume of blood was obtained from a donor cat of similar weight which had been anaesthetized in the same way as the experimental animal. Both recipient and donor cats were heparinized (2000 units, Burroughs Wellcome Ltd.) before bleeding the donor cat. Prior to any experimental manoeuvre, the blood from the donor cat was mixed with that of the recipient animal by three exchange transfusions. Blood used for infusion was maintained at body temperature by circulating heated water through jackets around the infusion syringes. In pilot studies, exchange transfusion did not alter body temperature or the temperature of carotid artery blood. At all times during exchange transfusion care was taken to avoid air embolism and to ensure the volume of blood withdrawn was equal to the volume infused. The experiment was discarded if clots were observed in the blood at any stage. For venous CO_2 loading, the infused blood was rendered hypercapnoeic by tonometering with 92% CO_2, 8% O_2 gas mixture for 20–30 min in a water bath maintained at 37 °C. The oxygen fraction of the tonometering gas was chosen so that the oxygen tension of the infusate was close to that of the withdrawn blood. The venous CO_2 load and its duration depended on the rate of exchange transfusion. The duration of the venous CO_2 loading was 8 min at 10 ml $\cdot$ min^{-1} (low loads), 4 min at 20 ml $\cdot$ min^{-1} (intermediate loads) and 2 min at 38 ml $\cdot$ min^{-1} (high loads). Airflow was measured with a pneumotachograph (Fleish size 0) attached to the free end of a tracheal cannula and coupled to a differential pressure transducer (Mercury Electronics, Scotland). The electrical signal was modified to record inspired tidal-volume. A T-piece was attached to the free end of the pneumotachograph. The side arm of the T-piece lead to a 50-ml reservoir into which air flowed at a sufficient rate to overcome instrumental dead space and exceed the maximum inspiratory flow. For airway CO_2 loading, gas mixtures of 4–6% CO_2, 21% O_2 balance N_2 were added to the reservoir. This technique allowed the inspired CO_2 fraction (FI_{CO_2}) to be increased to any desired constant value by adjusting the flow of the hypercapnoeic gas mixture. Tracheal gas was sampled continuously with an infra-red CO_2 analyzer (LB2, Beckman) from tubing which joined the pneumotachograph to the tracheal cannula. Alveolar P_{CO_2} was judged from the end-tidal CO_2 level.

Arterial pH, P_{CO_2} and P_{O_2} were measured with a pH/blood gas analyzer (IL 413) and were corrected for body temperature. The blood was obtained from a short polyethylene catheter inserted into a femoral artery. A polyethylene loop with two sidearms was inserted into the other femoral artery. One sidearm was attached to a pressure transducer (Statham P 23 D6) to monitor arterial blood pressure. A needle which contained a fast responding pH electrode was inserted into the other sidearm. The technique for continuous recording of arterial pH and the 90% response time of the electrode system *in vitro* (40 m sec) have been described elsewhere (Band and Semple, 1967; Band, Cameron and Semple, 1969a,b, 1970; Cowell, Band, Semple, 1967). The glass electrode in the form of a closed-end capillary, fitted closely into the lumen of a short length of needle tubing. Blood flowed through the narrow annular space and then through a sidearm on the needle into a reference cell which contained saturated potassium chloride and calomel. Contact between

reference cell and blood was through a ceramic plug. Blood flowed through the system at 8 ml · min^{-1} and was returned into a superficial neck vein through a catheter. Data was collected on a photographic recorder (Electronics for Medicine Inc., model DR8).

PROTOCOL AND MEASUREMENTS

Two series of experiments were performed. In the first series, the ventilatory responses per unit change of PA_{CO_2} between airway and venous CO_2 loading were compared. In the second series, the change of ventilation was measured when CO_2 loading was altered from airway to venous routes, with PA_{CO_2} and Pa_{CO_2} held constant.

In the first series of experiments, measurements of the ventilatory response to CO_2 inhalation were made before and after measurements of the ventilatory response to venous CO_2 loading (fig. 1). For low venous CO_2 loads (exchange transfusion at 10 ml · min^{-1}), recordings were made at 4, 6 and 8 min (fig. 1a). Before and after the low venous CO_2 loads, an 8-min period of 1% CO_2 inhalation was given with recordings at 4, 6 and 8 min. For intermediate venous CO_2 loads (exchange transfusion at 20 ml · min^{-1}), recordings were made at 2 and 4 min (fig. 1b). Before and after the intermediate venous CO_2 loads, a 4-min period of 2% CO_2 inhalation and a 4-min period of 3% CO_2 inhalation were given with recordings at 2 and 4 min. For high venous CO_2 loads (exchange transfusion at 38 ml · min^{-1}), recordings were made at 2 min. Before and after the high venous loads, CO_2 inhalation was conducted in a similar manner to that described for intermediate venous CO_2 loads.

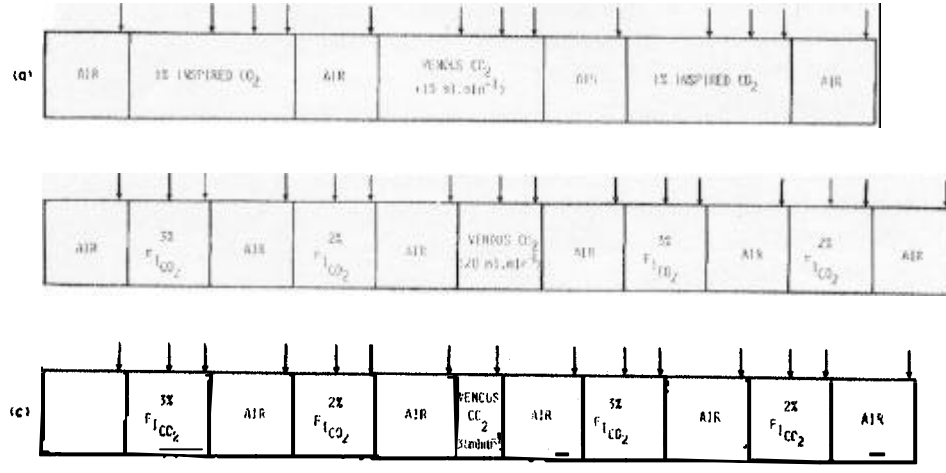

Fig. 1. Protocols used in the first series of experiments. Arrows indicate times at which measurements of minute ventilation ($\dot{V}_I$) end tidal CO_2 (PA_{CO_2}) and the amplitude (amp pH) and phase (ϕ) of the respiratory oscillations of arterial pH were made.

Control measurements with the animal breathing air, were made immediately prior (pre-control) and 4 min after (post-control) the administration of each airway and venous CO_2 load (fig. 1).

In pilot studies it was found that transient ventilatory disturbances often occurred during withdrawal of arterial blood, particularly when samples were taken rapidly. Therefore, the changes of PA_{CO_2} were used, as opposed to changes to Pa_{CO_2}, to calculate the ventilatory response. Nevertheless, measurement of arterial blood gas composition was made during control periods and CO_2 loading but these samples were limited to six in any one animal to minimize loss of blood volume.

In order to check that exchange transfusion *per se* did not provoke a ventilatory response, control exchange transfusions were carried out in a similar manner to the venous CO_2 loading. The blood for control exchange transfusions was tonometered initially with 92% CO_2, 8% O_2 and then with 6% CO_2, 8% O_2 in N_2 to bring the infused blood gas tensions close to venous blood. Exchange transfusion was conducted at all three flow rates and measurements of $\dot{V}I$ and PA_{CO_2} were made at the same intervals as described above for venous CO_2 loadings.

In the second series of experiments, CO_2 loading was continued for over 28 min (fig. 2). After 20 min of 1 or 2% CO_2 inhalation, an exchange transfusion of hypercapnoeic blood at 10 or 20 ml $\cdot$ min^{-1} was begun. During the first minute of exchange transfusion, the FI_{CO_2} was reduced (usually to zero) to maintain Pa_{CO_2} constant as judged by the mean level of arterial pH (*in vivo*) and PA_{CO_2}. When the exchange transfusion was completed, FI_{CO_2} was returned to its former level for a minimum of 4 min. Recordings were made and arterial blood samples were taken immediately prior to, just before the end of, and 4 min after each exchange transfusion.

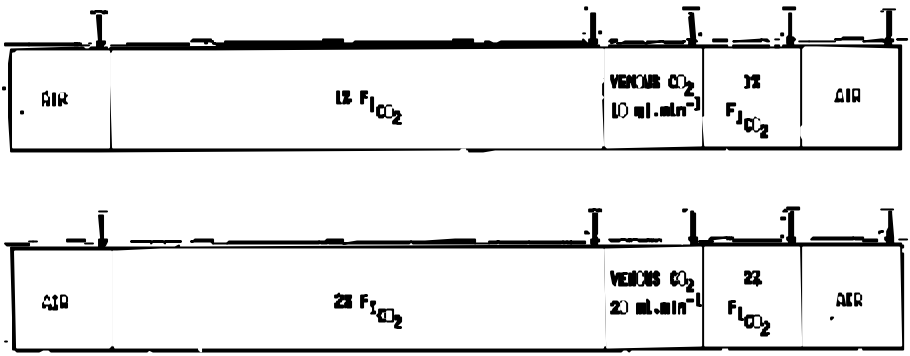

Fig. Protocols used in the second series of experiments. Arrows indicate times at which measurements of $\dot{V}I$, PA_{CO_2}, amp pH, ϕ, Pa_{CO_2} Pa_{O_2} and mean arterial pH were made.

CALCULATIONS AND RESULTS

At each recording, inspired minute ventilation ($\dot{V}I$) was calculated from the average inspired tidal volume and respiratory frequency over five consecutive breaths, together with the average PA_{CO_2} and amp pH. Amp pH was assessed from the peak to trough of the respiratory oscillations of arterial pH measured *in vivo*. Because we have demonstrated that there is a dependence of phrenic motoneurone output on its relationship to the respiratory oscillations of arterial pH (Cross *et al.*, 1979), we measured the phase relationship between ventilation and the respiratory oscillations of arterial pH (ϕpH) by a similar method. The interval between end-inspiration and the moment of maximal acidity immediately preceding it was divided by the pH cycle time. The resulting values for five consecutive breaths were averaged.

In the first series of experiments, the slope of the ventilatory response to CO_2 was calculated as the change of $\dot{V}I$ ($ml \cdot min^{-1}$) divided by the change of PA_{CO_2} (mm Hg) from the respective average of pre- and post-control periods.

In the second series of experiments, we compared the change of $\dot{V}I$ with the changes of PA_{CO_2}, pHa, Pa_{CO_2}, Pa_{O_2}, amp pH and ϕpH that occurred at 4 min of exchange transfusion from the corresponding values obtained during CO_2 inhalation. These CO_2 inhalation values were the averages of those obtained immediately prior to and 4 min after exchange transfusion.

Results were excluded when (a) there was a failure to withdraw and infuse equal volumes of blood during exchange transfusion, (b) mean arterial blood pressure fell below 90 mm Hg and (c) there was a difference of PA_{CO_2} greater than 2 mm Hg between pre- and post-control periods. These criteria for exclusion were used to avoid spurious results due to haemodynamic changes and variation in the level of anaesthesia between control and experimental measurements during CO_2 loading. Statistical significance was accepted at the 5% level with either the Wilcoxon test for matched pairs, or the Mann-Whitney U test for unpaired data (Siegel, 1956).

Results

CONTROL MEASUREMENTS

The levels of $\dot{V}I$ and PA_{CO_2} obtained while the animal was breathing air before (pre-control) and after (post-control) each procedure were compared. No significant differences were found in either series of experiments, between pre- and post-control values of either $\dot{V}I$ or PA_{CO_2}. Control exchange transfusions failed to produce any significant changes of $\dot{V}I$ or PA_{CO_2} (table 1).

The mean amp pH of animals in both series of experiments while breathing air was 0.013 ± 0.003 SD pH units.

TABLE I
Control exchange transfusion data

Flow rate ($ml \cdot min^{-1}$)	Duration (min)	Control $\dot{V}_I$ ($ml \cdot min^{-1}$)	Control $P_{A_{CO_2}}$ (mm Hg)	$\Delta\dot{V}_I$ ($ml \cdot min^{-1}$)	$\Delta P_{A_{CO_2}}$ (mm Hg)	n
10	4–8	886 (188)	25.3 (3.7)	+47 (76)	0.0 (0.6)	4
20	4	805 (281)	29.1 (3.6)	−15 (72)	−0.4 (0.9)	5
38	2	674 (150)	29.7 (4.5)	−11 (94)	−0.1 (1.6)	6

Control $\dot{V}_I$ and control $P_{A_{CO_2}}$ were calculated from the average of pre- and post-control values for each run while breathing air. $\Delta\dot{V}_I$ and $\Delta P_{A_{CO_2}}$ are the changes of $\dot{V}_I$ and $P_{A_{CO_2}}$ from control values during exchange transfusions of given flow rate and duration. n is the number of trials. All results are expressed as mean (± 1 SD).

First series of experiments

Four comparisons of the ventilatory response to CO_2 loading by airway and venous routes were made; results are shown in table 2. In each comparison there were no significant differences in the control values of $\dot{V}_I$ or $P_{A_{CO_2}}$ between airway and venous CO_2 loading. The response to CO_2 loading by the airways was always hypercapnoeic as was the response to the intermediate (20 $ml \cdot min^{-1}$) and high (38 $ml \cdot min^{-1}$) loads via the venous route. The response to the low venous loads (10 $ml \cdot min^{-1}$) was usually, but not always, hypercapnoeic. At the end of the 8-min infusion $\Delta P_{A_{CO_2}}$ was negative in three of the seven runs. In one of the three runs $\Delta P_{A_{CO_2}}$ was consistently negative from the 4th min onwards whilst in the other two runs $\Delta P_{A_{CO_2}}$ was negative at 8 min only, being positive at 2 and 4 min.

At low loads, the changes of $\dot{V}_I$ and $P_{A_{CO_2}}$ at 4, 6 and 8 min for each method of CO_2 loading were not significantly different, therefore average values for each period of CO_2 loading were used in the calculations. The average values of $\Delta\dot{V}_I$ and of $\Delta P_{A_{CO_2}}$ before and after the low venous CO_2 load were compared with the corresponding values obtained during the low venous CO_2 load. In each of the seven comparisons, $\Delta\dot{V}_I$ was greater and $\Delta P_{A_{CO_2}}$ was less for venous CO_2 loading than for CO_2 inhalation. Consequently, the ventilatory response to CO_2 ($\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$) was greater for venous loading than for CO_2 inhalation (table 2).

For comparison with the intermediate and high venous CO_2 loads, CO_2 inhalations of 2% and 3% respectively were used. On the intermediate load there was no significant difference between $\Delta\dot{V}_I$ for CO_2 inhalation and infusion but $\Delta P_{A_{CO_2}}$ was significantly lower during infusion, both at 2 and 4 min. Consequently the ventilatory response to CO_2 ($\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$) was greater for venous loading than for CO_2 inhalation on intermediate CO_2 loads although only significantly so at 4 min (see table 2). At the high CO_2 loads there was no significant difference in $\Delta\dot{V}_I$, $\Delta P_{A_{CO_2}}$ or $\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$ between airway and venous CO_2 loading, although $\Delta P_{A_{CO_2}}$ was smaller and $\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$ was greater for the venous loads in five out of the seven comparisons.

TABLE 2

Comparison of ventilatory response to CO_2 by different routes

Degree of load	Route of administration	CO_2 load	Duration (min)	Control $\dot{V}_I$ ($ml \cdot min^{-1}$)	Control $P_{A_{CO_2}}$ (mm Hg)	$\Delta\dot{V}_I$ ($ml \cdot min^{-1}$)	p	$\Delta P_{A_{CO_2}}$ (mm Hg)	p	n	$\Delta\dot{V}_I / \Delta P_{A_{CO_2}}$ ($ml \cdot min^{-1}$/mm Hg)	p
Low	Airway	1%	4–8	750 (228)	28.6 (3.4)	149 (70)	<0.05	1.6 (0.7)	<0.05	7	*99 (–)	<0.05
	Venous	10 ml · min^{-1}		697 (139)	27.8 (3.7)	241 (86)		0.7 (1.2)			*326 (–)	
Intermediate	Airway	2%		747 (119)	26.9 (2.9)	410 (102)	NS	4.8 (1.8)	<0.05	6	95 (39)	NS
	Venous	20 ml · min^{-1}		741 (162)	26.8 (3.0)	357 (61)		2.4 (1.1)			191 (106)	
	Airway	2%	4	747 (119)	26.9 (2.9)	402 (96)	NS	4.0 (1.3)	<0.05	6	106 (40)	<0.05
	Venous	20 ml · min^{-1}		741 (162)	26.8 (3.0)	358 (46)		1.8 (0.6)			244 (118)	
High	Airway	3%		624 (162)	27.5 (3.7)	536 (234)	NS	8.3 (2.7)	NS	6	68 (32)	NS
	Venous	38 ml · min		676 (179)	26.8 (3.7)	528 (112)		7.4 (1.7)			78 (36)	
	Airway	3%	4	624 (162)	27.5 (3.7)	579 (197)		7.0 (1.7)		6	87 (38)	

Control $\dot{V}_I$ and control $P_{A_{CO_2}}$ were calculated from the average of pre- and post-control values for each run while breathing air. The ventilatory response to CO_2 ($\Delta\dot{V}_I / \Delta P_{A_{CO_2}}$) is expressed in absolute units, p is the probability of a statistically significant difference; n is the number of paired comparisons. Mean values are shown with ± 1 SD in parenthesis. Measurements were made on 6 cats at the low and high loads and on 5 cats at the intermediate load.

* On one of the runs of the low intravenous CO_2 load $\Delta P_{A_{CO_2}}$ was negative so $\Delta\dot{V}_I / \Delta P_{A_{CO_2}}$ was negative and hence the CO_2 sensitivity was infinite. We therefore used an arc tangent transformation of the data to obtain the mean values for airway and venous loading. (A similar method for expressing CO_2 sensitivity has been used by Ponte and Purves, 1978 and Fordyce and Grodins, 1980). We did not include values for the standard deviations because of the alinear and discontinuous form of the tangent function which does not permit the use of standard or directional statistics (K.V. Mardia, personal communication).

TABLE 3

Changes of ventilation and mean levels of arterial blood gas composition associated with increasing amplitude of pH oscillations

	Experimental condition	Baseline $\dot{V}_I$ ($ml \cdot min^{-1}$)	Baseline $P_{A_{CO_2}}$ (mm Hg)	$\Delta\dot{V}_I$ ($ml \cdot min^{-1}$)	Δamp pH (%)	$\Delta P_{A_{CO_2}}$ (mm Hg)	ΔpHa	ΔPa_{CO_2} (mm Hg)	ΔPa_{O_2} (mm Hg)
A	Mean	1051	29.4	+66	+18	−0.6	−0.008	+0.1	+1.4
	SD	(332)	(2.4)	(15)	(9)	(0.4)	(0.007)	(0.8)	(3.2)
B	Mean	1206	30.1	+41	+80	−0.7	−0.003	+0.3	−2.9
	SD	(54)	(2.2)	(132)	(25)	(0.8)	(0.007)	(0.6)	(2.5)

Condition A is an exchange transfusion of hypercapnoeic blood at 10 $ml \cdot min^{-1}$ following 1% CO_2 inhalation for 20 min and Condition B is an exchange transfusion at 20 $ml \cdot min^{-1}$ following 2% CO_2 inhalation. $\dot{V}_I$ is minute inspired ventilation, $P_{A_{CO_2}}$ is alveolar P_{CO_2}, amp pH is the amplitude of pH oscillation measured *in vivo*, pHa is arterial pH measured *in vitro*, Pa_{CO_2} and Pa_{O_2} are arterial P_{CO_2} and P_{O_2} respectively, n is the number of trials. 'Baseline' values express the average values of $\dot{V}_I$ or $P_{A_{CO_2}}$ measured immediately before and 4 min after exchange transfusion during inhalation. Δ represents the change of corresponding variable during exchange transfusion, from the baseline values during CO_2 inhalation.

TABLE 3

Changes of ventilation and mean levels of arterial blood gas composition associated with increasing amplitude of pH oscillations

	Experimental condition	Baseline $\dot{V}_I$ ($ml \cdot min^{-1}$)	Baseline $P_{A_{CO_2}}$ (mm Hg)	$\Delta\dot{V}_I$ ($ml \cdot min^{-1}$)	Δamp pH (%)	$\Delta P_{A_{CO_2}}$ (mm Hg)	ΔpHa	ΔPa_{CO_2} (mm Hg)	ΔPa_{O_2} (mm Hg)	
A	Mean	1051	29.4	+66	+18	−0.6	−0.008	+0.1	+1.4	5
	SD	(332)	(2.4)	(15)	(9)	(0.4)	(0.007)	(0.8)	(3.2)	
B	Mean	1206	30.1	+41	+80	−0.7	−0.003	+0.3	−2.9	
	SD	(54)	(2.2)	(132)	(25)	(0.8)	(0.007)	(0.6)	(2.5)	

Condition A is an exchange transfusion of hypercapnoeic blood at 10 $ml \cdot min^{-1}$ following 1% CO_2 inhalation for 20 min and Condition B is an exchange transfusion at 20 $ml \cdot min^{-1}$ following 2% CO_2 inhalation. $\dot{V}_I$ is minute inspired ventilation, $P_{A_{CO_2}}$ is alveolar P_{CO_2}, amp pH is the amplitude of pH oscillation measured *in vivo*, pHa is arterial pH measured *in vitro*, Pa_{CO_2} and Pa_{O_2} are arterial P_{CO_2} and P_{O_2} respectively, n is the number of trials. 'Baseline' values express the average values of $\dot{V}_I$ or $P_{A_{CO_2}}$ measured immediately before and 4 min after exchange transfusion during inhalation. Δ represents the change of corresponding variable during exchange transfusion, from the baseline values during CO_2 inhalation.

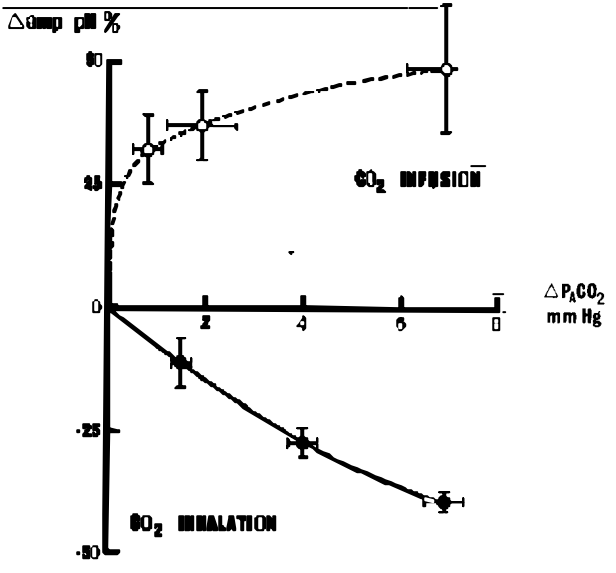

Fig. 3. Percentage changes of the amplitude of the respiratory oscillations of arterial pH (Δamp pH%, ordinate) plotted against the change of alveolar P_{CO_2}, (ΔP_{ACO_2} mm Hg, abscissa) during CO_2 loading from control values while breathing air. The data points correspond to conditions described in fig. 1, the bars represent ± 1 SEM.

The differences in ventilatory response produced by the two methods of CO_2 loading could not be explained in terms of the difference in the changes of amp pH. This is illustrated in fig. 3, where the percentage change of amp pH from the average of pre- and post-control values is plotted against P_{ACO_2} for the different CO_2 loads. With increasing CO_2 loads there is a progressive increase of amp pH for venous loading and a progressive decrease for airway loading. In contrast, we found that the ventilatory response was not significantly greater for venous than airway CO_2 administration at high CO_2 loads. Furthermore, the differences of ventilatory responses observed between the two methods of CO_2 administration at low and at intermediate venous CO_2 loads did not correlate with the corresponding changes of amp pH. In the majority of experiments ϕpH did not change during airway or venous CO_2 administration. Where changes of ϕpH did occur, they were small and inconsistent in direction.

For a given ΔP_{ACO_2}, the increases in ventilation produced by 4 min of low and intermediate venous CO_2 loads was greater than that produced by 4 min of 1% and 2% CO_2 inhalation. The data suggests that this difference is not related to differences in the oscillating signal but does not exclude the possibility that the period of CO_2 inhalation was insufficient to obtain a steady state response. In order to clarify this issue, a second series of experiments were conducted.

SECOND SERIES OF EXPERIMENTS

In this series of experiments, we measured the change of $\dot{V}I$ which occurred during venous CO_2 loading after a prolonged period of CO_2 inhalation (20 min) while PA_{CO_2} was held constant by reducing FI_{CO_2}. We used an exchange transfusion rate of 10 ml · min^{-1} with 1% CO_2 inhalation (condition A) and an exchange transfusion rate of 20 ml · min^{-1} with 2% CO_2 inhalation (condition B).

The mean $\dot{V}I$ and PA_{CO_2} while breathing air were 793 ± 149 SD ml · min^{-1} and 29.3 ± 2.5 SD mm Hg respectively for five trials in four animals with low venous CO_2 loads, and 710 ± 174 SD ml · min^{-1} and 27.2 ± 2.3 mm Hg respectively for five trials in four animals with intermediate venous CO_2 loads. These values are similar to those obtained in the first series of experiments (table 2). The changes of $\dot{V}I$ and amp pH are shown in table 3 and an example of the recordings made is shown in fig. 4. Under both experimental conditions arterial blood gas composition and PA_{CO_2} did not change but in every trial amp pH increased during exchange transfusion. There were no significant changes of ϕpH (+0.03 ± 0.004 SD for condition A and −0.03 ± 0.03 SD for condition B). $\dot{V}I$ marginally increased by 6.9% ± 3.2 SD while amp pH increased by 18% (condition A) but there was no systematic change of $\dot{V}I$ associated with an 80% increase of amp pH (condition B).

The second series of experiments was undertaken to see if the period of CO_2 inhalation was insufficient to obtain a steady state response. We have therefore compared $\Delta\dot{V}I$ with ΔPA_{CO_2} on 1% CO_2 inhalation at 4 and 20 min. $\Delta\dot{V}I$ was 214 ± 51 SD ml · min^{-1} and 326 ± 149 SD ml · min^{-1} at 4 and 20 min, respectively while the corresponding figures for ΔPA_{CO_2} were 1.9 ± 1.5 SD mm Hg and 0.3 ± 1.1 SD mm Hg. The difference in ΔPA_{CO_2} between the 4th and 20 th min is significant but not that for $\Delta\dot{V}I$.

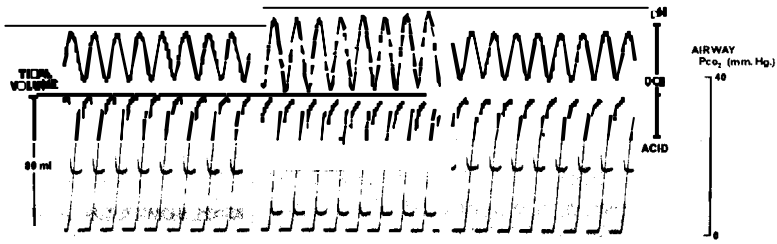

Fig. 4. A recording which shows the increased amplitude pH not associated with any marked change of minute ventilation. 2% CO_2 inhalation had been continued for 20 min prior to the recording on the left. In the middle section is a recording made after 4 min of exchange transfusion of hypercapnoeic blood at 20 ml · min^{-1}. On the right is a recording made 4 min after completion of exchange transfusion when 2% CO_2 inhalation was in progress. From the top, the tracings are arterial pH measured continuously *in vivo*, tracheal CO_2, inspired tidal volume and a 1 sec time marker.

Discussion

CRITICISM OF METHODS

In our studies, venous CO_2 loading was achieved by exchange transfusion rather than the more commonly used extracorporeal circulation. Exchange transfusion has the advantage that the CO_2 load is presented abruptly and any changes in the animal's blood volume due to this technique are readily apparent from inspection of the infusion/withdrawal syringes. In addition, there is no ventilatory drive from our exchange transfusion which is independent of a CO_2 load (see control infusions in table 1) whereas there is such an independent drive to ventilation from the use of an extracorporeal circulation (Greco *et al.*, 1978). Control exchange transfusions with isocapnoeic blood demonstrate that our results cannot be related to any haemodynamic disturbance or physio-chemical properties of the infused blood.

In the first series of experiments, $P_{A_{CO_2}}$ rather than $P_{a_{CO_2}}$ was used to determine the ventilatory response to CO_2 for reasons described above (see Methods). A comparison between changes of $P_{A_{CO_2}}$ and $P_{a_{CO_2}}$ yielded a correlation coefficient of 0.95 ($n = 37$). Likewise, in the second series of experiments, changes of $P_{A_{CO_2}}$ proved to be an accurate indicator of $P_{a_{CO_2}}$ changes.

The rapidly responding pH electrode system was sited in the femoral artery rather than the carotid artery in order to avoid impairment of carotid body function. The carotid body can be damaged by temporary impairment of blood flow during cannulation and by platelet emboli from disrupted endothelium or from foreign material in the carotid arterial blood stream. It is possible that the magnitude and phase relationship of the pH cycle to breathing may have been distorted compared with events at the peripheral chemoreceptors. Nevertheless, the site of measurement would not affect our ability to measure proportional changes of amp pH or shifts of ϕpH.

Fordyce and Grodins (1980) have suggested that there are two important defects in experimental design which should be excluded or allowed for when studying the effects of CO_2 loading if errors of interpretation are to be avoided. The first defect consists of a failure to allow for time-dependent hyperventilation due to a shifting anaesthetic level. The second defect in design is the use of low CO_2 loads when it may not be possible to establish with certainty whether a response is hypercapnoeic or isocapnoeic because the change in $P_{A_{CO_2}}$ due to the load may be less than changes from anaesthetic level, body temperature, blood pressure, etc.

From the first publication of our results on experiments relating ventilation to amp pH (Grant and Semple, 1976b) we have consistently allowed for time-dependent hyperventilation by (a) bounding all CO_2 loads, whether administered by airway or vein, by a control period breathing air and excluding any run where pre- and post-control values of $P_{A_{CO_2}}$ differed by 2 mm Hg or more and (b) expressing changes in $\dot{V}_I$ and $P_{A_{CO_2}}$ in relation to the mean of the corresponding pre-

and post-control values. By excluding from analysis those experiments where the difference in ΔPA_{CO_2} between pre- and post-control was greater than 2 mm Hg we have ensured that changes in $\dot{V}I$ and PA_{CO_2} between the two control periods were small. As a result we found no advantage in using a linear extrapolation of changes in $\dot{V}I$ and PA_{CO_2} between control periods to determine the effect of time-dependent hyperventilation on the experimental results as used by Fordyce and Grodins (1980).

The use of a low CO_2 load (1%) via the airways did not lead to any uncertainty in showing that in all runs the response was hypercapnoeic over a time period of 8 min. Our finding that the response to low CO_2 loads was hypercapnoeic over 8 min is in agreement with the findings of Fordyce, Knuth and Bartlett (1980) in awake cats but not those of Orr and Busija (1979) in awake ponies. The response to the low loads of CO_2 (10 ml $\cdot$ min^{-1}) by the venous route was usually but not always hypercapnoeic (ΔPA_{CO_2} being positive at 8 min in four out of seven runs). This result, taken with the consistently hypercapnoeic response to the intermediate load, suggests that the response to low loads of CO_2 is hypercapnoeic as with airway loading. Nevertheless, as predicted by Fordyce and Grodins (1980), it was not possible to consistently demonstrate this, probably because of variation in anaesthetic level and other factors not directly related to the CO_2 loading.

COMPARISON OF HE VENTILATORY RESPONSE TO CO_2 OADING BY DIFFERENT ROUTES

Our results do not suggest that there is any fundamental difference in the chemical stimulus to ventilation when CO_2 is administered by the airway or venous route. Although we found that there was a greater ventilatory response to venous than to airway CO_2 loading on low and intermediate loads of short duration, no marked differences in ventilatory response occurred in the second series of experiments where CO_2 loading was continued for 28 min. From the results of the first series of experiments we might have expected to double minute ventilation when CO_2 loading was switched from airway to venous route in the second series of experiments, in fact minute ventilation increased by less than 10%. This result is in agreement with the findings of Cropp and Comroe (1961) who employed a similar technique.

The discrepancy between the two series of experiments may be due to differences in the time required to attain a steady state response. When CO_2 is administered by the venous route with an exchange transfusion, the load is imposed as a step input. Airway CO_2 loading may take longer because the CO_2 load to the lungs increases as ventilation increases. Therefore differences in the ventilatory response between airway and venous CO_2 loading in the first series of experiments may occur not because of a fundamental difference of ventilatory response to the chemical stimulus, but because the measurements were obtained in the transient phase.

Comparison of the response to inhaled CO_2 between the 1st and 2nd series of experiments support the contention that the difference in ventilatory response

between infused and inhaled CO_2 seen in the first series of experiments is due to measurements being made in the transient state. Both for 1% and 2% CO_2 inhalation the mean results show that at 20 min $\Delta\dot{V}I$ is greater and ΔPA_{CO_2} smaller than the corresponding values at 4 and 8 min obtained in the first series of experiments. It is likely therefore that if CO_2 administration via the airways had been continued to 20 min in the first series, then the values for $\Delta\dot{V}I/\Delta PA_{CO_2}$ would have been greater.

Respiratory oscillations of arterial pH during CO_2 loading

In contrast to Ponte and Purves (1978) but in agreement with predictions of Yamamoto (1960), we found that amp pH did increase with venous CO_2 loading and decreased with CO_2 inhalation. In the study of Ponte and Purves there was a marked increase in respiratory rate during the venous CO_2 load which is not a feature of our experiments or those on the awake dog (Greco *et al.*, 1978). Presumably it is this difference in respiratory frequency response which accounts for the different findings of Ponte and Purves with regard to the amp pH though the relatively slow frequency response of their pH electrode may have contributed to the result (Cragg, Patterson and Purves, 1977).

Despite our findings, it is difficult to relate differences of the ventilatory responses between airway and venous CO_2 loading to changes of the oscillations of pH in the first series of experiments for the following reasons. First, the greatest differences of amp pH between airway and venous CO_2 loading occurred with high venous CO_2 loads where the difference in the ventilatory response to CO_2 was smaller and not statistically significant. Second, at low and intermediate venous CO_2 loads, where differences of the ventilatory response to CO_2 were greater and statistically significant, there was no correlation between the changes of amp pH and the discrepancy between the ventilatory responses to CO_2 assessed by the two routes. Third, we found that there was no significant changes of ϕpH for airway or venous CO_2 loading.

The results of the second series of experiments support the view that changes of amp pH do not have an effect on minute ventilation. In these experiments, increases of amp pH by 80% without changing the mean level of Pa_{CO_2} did not alter minute ventilation.

In both this study and in an earlier study (Grant and Semple, 1976b) where the amplitude of respiratory oscillations of pH have been increased and decreased respectively while the mean level of arterial blood gas composition was held constant, no marked change of minute ventilation occurred.

In these experiments, where we have taken oscillations of pH to represent those of P_{CO_2}, we have found no evidence to suggest that the oscillatory signal contributes to the ventilatory response to CO_2.

References

Band, D. M. and S. J. G. Semple (1967). Continuous measurement of blood pH with an indwelling glass electrode. *J. Appl. Physiol.* 22: 854–857.

Band, D. M., I. R. Cameron and S. J. G. Semple (1969a). Oscillations in arterial pH with breathing in the cat. *J. Appl. Physiol.* 26: 261–267.

Band, D. M., I. R. Cameron and S. J. G. Semple (1969b). Effect of different methods of CO_2 administration on oscillations of arterial pH in the cat. *J. Appl. Physiol.* 26: 268–273.

Band, D. M., I. R. Cameron and S. J. G. Semple (1970). The effect on respiration of abrupt changes in carotid artery pH and P_{CO_2} in the cat. *J. Physiol. (London)* 211: 479–494.

Cowell, T. K., D. M. Band and S. J. G. Semple (1967). A fast response pH meter. *J. Appl. Physiol.* 22: 858–860.

Cragg, P., L. Patterson and M. J. Purves (1977). The pH of brain extracellular fluid in the cat. *J. Physiol. (London)* 272: 137–166.

Cross, B. A., B. J. B. Grant, A. Guz, P. W. Jones, S. J. G. Semple and R. P. Stidwill (1979). Dependence of phrenic motoneurone output on the oscillatory component of arterial blood gas composition. *J. Physiol. (London)* 290: 163–184.

Cropp, G. J. A. and J. H. Comroe (1961). Role of mixed venous blood P_{CO_2} in respiratory control. *J. Appl. Physiol.* 16: 1029–1033.

Fordyce, W. E. and F. S. Grodins (1980). Ventilatory response to intravenous and airway CO_2 administration in anaesthetised dogs. *J. Appl. Physiol.* 48: 337–346.

Fordyce, W. E., S. L. Knuth and D. Bartlett (1980). Ventilatory responses to low levels of CO_2 inhalation in the cat. *Proc. 28th Union Physiol. Sci.*, Budapest, 14: 414.

Grant, B. J. B. and S. J. G. Semple (1976a). Comparison of the ventilatory response to carbon dioxide administration by intravenous infusion in the cat. *J. Physiol. (London)* 258: 110–111P.

Grant, B. J. B. and S. J. G. Semple (1976b). Mechanisms whereby oscillations in arterial carbon dioxide tension might affect pulmonary ventilation. In: Morphology and Mechanisms of Chemoreceptors, edited by A. S. Paintal. Delhi, Vallabhbhai Patel Chest Institute, University of Delhi, pp. 191–196.

Grant, B. J. B. and S. J. G. Semple (1977). Effect of increasing the amplitude of the respiratory oscillations of arterial P_{CO_2} on ventilation of anaesthetised cats. Proc. 27th Union Physiol. Sci. (Paris), 13: 279.

Greco, E. C., W. E. Fordyce, F. Gonzalez, P. Reischl and F. S. Grodins (1978). Respiratory responses to intravenous and intrapulmonary CO_2 in awake dogs. *J. Appl. Physiol.* 45: 109–114.

Honda, Y. and M. Veda (1961). Fluctuations of arterial pH with the respiratory cycle in dogs. *Jap. J. Physiol.* 11: 223–228.

Lamb, T. W. (1966). Ventilatory response to intravenous and inspired carbon dioxide in anaesthetised cats. *Respir. Physiol.* 2: 99–104.

Lewis, S. M. (1975). Awake baboon's ventilatory response to venous and inhaled CO_2 breathing. *J. Appl. Physiol.* 39: 417–422.

Linton, R. A. F., R. Miller and I. R. Cameron (1976). Ventilatory response to CO_2 inhalation and intravenous infusion of hypercapnoeic blood. *Respir. Physiol.* 26: 383–394.

Nims, L. F. and C. Marshall (1938). Blood pH *in vivo*. I. Changes due to respiration. *Yale J. Biol. Med.* 10: 445–448.

Orr, J. A. and D. W. Busija (1979). Isocapnoeic hyperpnoea in awake ponies during inspiration of 4% CO_2. *J. Appl. Physiol.* 47: 445–452.

Ponte, J. and M. J. Purves (1978). Carbon dioxide and venous return and their interaction as stimuli to ventilation in the cat. *J. Physiol. (London)* 274: 455–475.

Purves, M. J. (1966). Fluctuations of arterial oxygen tension which have the same period as respiration. *Respir. Physiol.* 1: 281–286.

Reischl, P., F. Gonzalez, E. C. Greco, W. E. Fordyce and F. S. Grodins (1979). Arterial P_{CO_2} response to intravenous CO_2 in awake dogs unencumbered by external breathing apparatus. *J. Appl. Physiol.* 47: 1099–1104.

Siegel, S. (1956). Nonparametric statistics for the behavioural sciences. Kogakusha, McGraw-Hill.

Stremel, R. W., D. J. Huntsman, R. Casaburi, B. J. Whipp and K. Wasserman (1978). Control of ventilation during intravenous CO_2 loading in the awake dog. *J. Appl. Physiol.* 44: 311–316.

Wasserman, K., B. J. Whipp, R. Casaburi, D. J. Huntsman, J. Castagna and R. Lugliani (1975). Regulation of arterial P_{CO_2} during intravenous CO_2 loading. *J. Appl. Physiol.* 38: 651–656.

Yamamoto, W. S. (1960). Mathematical analysis of the time course of alveolar CO_2. *J. Appl. Physiol.* 15: 215–219.