Journal of Physiology (1992), **445**, *pp.* 487–498
With 4 figures
Printed in Great Britain

CONVENTIONAL *VERSUS* SLUG CO_2 LOADING AND THE CONTROL OF BREATHING IN ANAESTHETIZED CATS

BY C. P. M. VAN DER GRINTEN*, E. SCHOUTE†, W. R. DE VRIES† AND S. C. M. LUIJENDIJK*

*From the *Department of Pulmonology, University Hospital Maastricht, University of Limburg, PO Box 1918, 6201 BX Maastricht, The Netherlands and the †Department of Medical Physiology and Sports Medicine, University of Utrecht, Vondellaan 24, 3521 GG Utrecht, The Netherlands*

(*Received 8 March 1991*)

SUMMARY

1. Conventional inspiratory CO_2 loading (CCL) is accomplished by having the subject breathe CO_2-enriched air. An alternative method of CO_2 loading is to inject a bolus of CO_2 at the start of each inspiration into the inspired air: slug CO_2 loading (SCL). During SCL P_{CO_2} in the conducting airways declines quickly towards 0 kPa in the course of inspiration, whereas P_{CO_2} remains at a constant value equal to the inspiratory P_{CO_2} during CCL. Therefore, CCL and SCL may stimulate the respiratory controller differently.

2. We compared the ventilatory responses to SCL and CCL in fourteen anaesthetized, spontaneously breathing cats. In each experimental animal we applied, in a fixed randomized order, five CCL experiments (fractional inspiratory CO_2, $F_{I,CO_2} = 0{\cdot}01$–$0{\cdot}05$), six SCL experiments (slugs of 50% CO_2 ranging from 0·5 to 6 ml) and three control experiments in which no CO_2 was loaded. Partial pressure of CO_2 in arterial blood was determined from small blood samples (0·14 ml). In three cats we repeated the experiments after bilateral transection of the cervical vagi to evaluate the contribution of vagal receptors to the responses observed.

3. The average slope of the CO_2 response curves for SCL was 2 times steeper than that for CCL ($P < 0{\cdot}01$). The larger minute ventilation for SCL for a particular value of arterial P_{CO_2} (P_{a,CO_2}) could not be attributed exclusively to the increased breathing frequency or the increased tidal volume ($P > 0{\cdot}10$). Further, mean inspiratory flow (V_I) was larger for SCL than for CCL at the same P_{a,CO_2} ($P < 0{\cdot}01$), also because the ratio T_I/T_E (inspiratory duration/expiratory duration) was smaller ($P < 0{\cdot}01$). After vagotomy the difference between SCL and CCL response curves was much reduced.

4. It is concluded that SCL and CCL affect the respiratory controller in a different way causing differences in breathing pattern and CO_2 sensitivity between the two methods. Evidently, a mechanism based on CO_2 sensitivity of pulmonary receptors is involved in the responses observed.

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INTRODUCTION

During conventional CO_2 loading (CCL) gas mixtures are inspired with a constant concentration of CO_2. An alternative method has been suggested by Fenn & Craig (1963), in which CO_2 is loaded by adding a bolus or 'slug' of CO_2 to inspired air at the start of each inspiration: slug CO_2 loading (SCL) (Swanson, 1978). This method was originally designed to gain better control of the CO_2 load imposed upon the lungs, thus overcoming the problem of inspiring increasing amounts of CO_2 when ventilation is increased. These two techniques produce different levels of P_{CO_2} in the conducting airways during the course of inspiration. For CCL P_{CO_2} remains at inspiratory level during the entire inspiration, whereas it decreases to 0 kPa for SCL. We tested the hypothesis that this difference in P_{CO_2} is important for the control of breathing. We tried to maximize the difference between SCL and CCL by injecting a small bolus (0·5–6 ml) of 50 % CO_2 in air directly into the tracheal cannula starting a few milliseconds after the start of each inspiration. During CCL a constant fraction of CO_2 was inspired (1–5 %).

Cummin, Patil, Jacobi & Saunders (1987) compared minute ventilation ($\dot{V}_E$) obtained by CCL to $\dot{V}_E$ obtained by the Fenn & Craig (1963) technique at the same CO_2 load in man and found a higher $\dot{V}_E$ for the latter, which they ascribed to differences in dead space CO_2 concentration. We followed a different approach. We did not try to obtain comparable CO_2 loads for SCL and CCL, but measured $\dot{V}_E$ and arterial P_{CO_2} at several different CO_2 loads in the same animal and compared the slopes of the response curves obtained during SCL and CCL for each animal. For three cats we repeated the experiments after vagotomy to find out whether the responses depended on vagal receptors.

The differences in the responses between SCL and CCL are discussed in the light of the CO_2 sensitivity of pulmonary receptors and the oscillations of CO_2 in arterial blood.

METHODS

Experimental set-up

The experiments were carried out in fourteen spontaneously breathing adult cats (mean body mass 3·5 ± 0·87 kg). Cats were anaesthetized with an initial dose of ketamine (10 mg kg^{-1} I.M.) followed by intravenous administration of a mixture of urethane (100 mg kg^{-1}) and chloralose (20 mg kg^{-1}). Small supplemental doses of this mixture were given when required. The cats were placed on an operating table in the supine position. The left femoral vein and artery were cannulated and a tracheal cannula was inserted just below the larynx.

The tracheal cannula was connected to the flow pressure transducer (Fleisch No. 0) of a pneumotachograph (Gould). The other side of the Fleisch head was connected to a main tube by means of a T-piece. Through this main tube a constant bias flow of 18 l min^{-1} was directed, which was sufficient to prevent rebreathing of expired gas. For CCL, CO_2 (1–5 vol %) was added to this bias flow. For SCL, a bolus of CO_2 (50 vol % in air) was injected into the trachea at the distal end of the tracheal cannula using a fast-opening (< 5 ms) electromagnetic valve. This valve was triggered by the 'zero crossing flow' of the pneumotachograph. The duration of the injection was set to 50 ms and the pressure used to inject the bolus was adjusted to obtain different levels of SCL. The injection was completed within 60 ms after the onset of inspiration.

The equipment dead space had a volume of 8·5 ml and a flow resistance of 0·6 kPa s l^{-1}. Body temperature was measured with a rectal thermistor (Newport) and kept between 37·5 and 38·5 °C by heating the operating table.

To evaluate steady-state conditions of ventilation and circulation the partial pressure of carbon dioxide in inspired and expired gas was measured by a mass spectrometer (Balzers, QMG511) or

a capnograph (Nellcor, N1000). Arterial blood pressure was measured with a differential pressure transducer (Statham, P23). Systolic, diastolic and mean values as well as heart rate were displayed continuously.

The signals were A–D converted by a computer. Data were stored on floppy disc for off-line analysis. To allow on-line inspection of the data, most of the signals were also recorded on an 8-channel ink recorder (Elema-Schoenander).

From the arterial cannula small blood samples (0·14 ml) were collected in heparin-coated glass capillaries (Corning) and immediately analysed in duplicate at 37 °C for pH, P_{O_2} and P_{CO_2} (IL413 or ABL3). The values were corrected to body temperature (Ashwood, Kost & Kenny, 1983). The total volume of blood collected during the entire experiment was less than 5% of the animal's blood volume.

Experimental procedure

During each experimental run of CO_2 loading one data file and two pairs of blood samples were collected as follows. When the cat was in a stable condition regarding ventilation, end-tidal P_{CO_2} and blood pressure, a duplicate blood sample was drawn from the arterial cannula for control pH, P_{O_2} and P_{CO_2}. Then a CCL or SCL was started. After 3 min, data sampling was started and it was continued for at least 1 min. Thereafter, another duplicate blood sample was collected and the CO_2 load was ended. At least 5 min were allowed for full recovery before the next run was started. In this way six SCL runs (0·5–6 ml of 50% CO_2 in air) and five CCL runs (1–5% of CO_2 in air) were carried out in each cat. Control arterial P_{CO_2} (P_{a,CO_2}) levels for two consecutive runs seldom differed by more than 0·15 kPa.

The procedure of the three control runs was simple: in a steady-state condition (without CO_2 loads) a data file was gathered, whereafter two blood samples were taken. The runs were generally performed in a fixed randomized order. A control measurement preceded each sequence of four runs with CO_2 loading.

In three cats we repeated the entire sequence of SCL, CCL and control measurements after bilateral transection of the cervical vagi. Because breathing frequency (f) was lower after vagotomy, we used slugs of 100% CO_2 instead of 50% in air to obtain comparable CO_2 loads.

Data processing and statistics

From the flow signal tidal volume (V_T), $\dot{V}_E$, f and the durations of inspiration (T_I) and expiration (T_E) were determined. For all these quantities we used the mean value obtained from the last thirty breaths of each data file. From the arterial P_{CO_2} (P_{a,CO_2}) obtained from the blood samples just before and at the end of a CCL or SCL run we calculated $\Delta P_{a,CO_2}$ for that run. $\Delta \dot{V}_E$ was derived from the difference in $\dot{V}_E$ between a CO_2-loaded run (SCL or CCL) and the preceding run under control conditions.

For each cat separately the slopes of CO_2 response curves ($\Delta \dot{V}_E$ as a function of $\Delta P_{a,CO_2}$) for CCL and for SCL were estimated by $\Sigma y(\Sigma x)^{-1}$ (Mardia, Bogle & Edwards, 1983). For other parameters we used normal linear regression analysis. Wilcoxon's signed-rank test for paired observations was used to ascertain the statistical significance of the differences in the slopes of the regression lines for CCL and SCL.

RESULTS

Figure 1 displays $\Delta \dot{V}_E$ as a function of $\Delta P_{a,CO_2}$, separately for CCL and SCL, and for each cat a separate panel is used. All data points are indicated. In all of the cats the lines fitted through the data points for SCL (dashed lines) are steeper than the lines through the data points for CCL (continuous lines). The average slope of the lines for SCL was 2·06 times larger than that for CCL. The differences between the SCL and CCL slopes were significant ($P < 0·01$). In five cats hypocapnic responses were sometimes observed during the SCL experiments (Fig. 1, cats 2, 5, 7, 8 and 14) and in one cat (7) also during CCL. The ratio between the slopes for SCL and CCL showed some scatter between the cats. The largest value was 8·65 for cat 13 and the smallest was 1·10 for cat 14, and on average the ratio was 2·06 ($\pm$2·88). The apnoea

points calculated from the $\dot{V}_E$–P_{a,CO_2} plots were 3·22 (±0·29) and 3·33 (±0·43) kPa for CCL and SCL, respectively, and they were not significantly different ($P > 0·10$).

Besides the response of $\dot{V}_E$ to P_{a,CO_2}, we analysed several other relationships between respiratory variables. The results are shown in Table 1 in terms of the

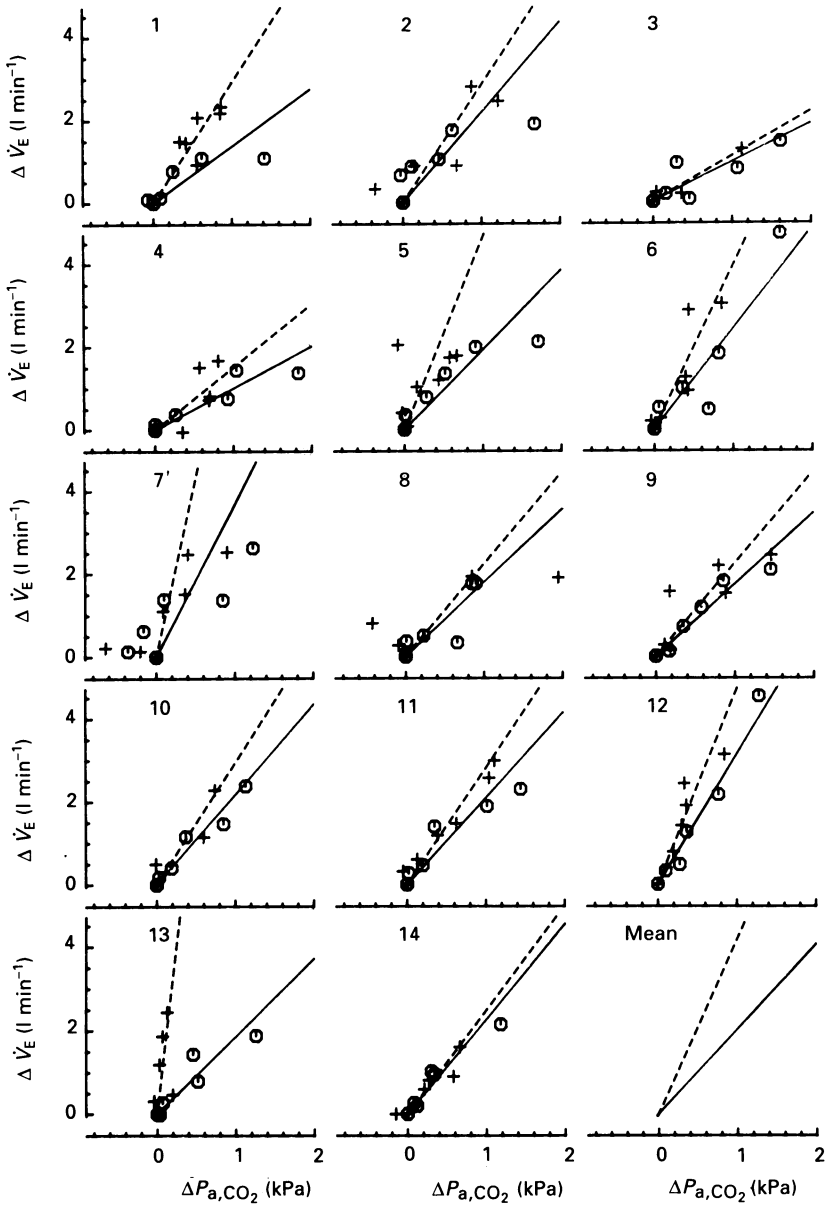

Fig. 1. CO_2 response curves of fourteen cats, separately for CCL (○——○) and SCL experiments (+---+). The right-hand bottom panel displays the average response curves for all cats for SCL and CCL.

TABLE 1. Mean regression coefficients (±S.D.) for CCL and SCL experiments. The last column gives the P values of Wilcoxon's signed-rank test for the fourteen paired values of the slopes for SCL and CCL. No significant differences were found between the intercepts for SCL and CCL in any of these relations

Variables		CCL		SCL		
Dependent	Independent	Slope	Intercept	Slope	Intercept	P
$\Delta\dot{V}_E$ (ml min^{-1})	$\Delta P_{a,CO_2}$ (kPa)	2032 (728)	—	4188 (3861)	—	< 0·01
V_T (ml)	$\Delta P_{a,CO_2}$ (kPa)	44·5 (21·5)	40·3 (11·5)	54·1 (30·8)	43·9 (16·2)	n.s.
f (min^{-1})	$\Delta P_{a,CO_2}$ (kPa)	2·9 (3·2)	30·5 (7·8)	5·7 (8·5)	31·4 (7·5)	n.s.
T_I (s)	$\Delta P_{a,CO_2}$ (kPa)	−0·17 (0·15)	1·09 (0·32)	−0·28 (0·26)	1·05 (0·20)	< 0·05
T_E (s)	$\Delta P_{a,CO_2}$ (kPa)	−0·08 (0·10)	1·02 (0·23)	−0·03 (0·33)	0·98 (0·21)	n.s.
T_I/T_E	$\Delta P_{a,CO_2}$ (kPa)	−0·08 (0·12)	1·08 (0·20)	−0·26 (0·33)	1·08 (0·21)	< 0·01
$\dot{V}_I$ (ml s^{-1})	$\Delta P_{a,CO_2}$ (kPa)	56·0 (26·2)	38·2 (9·9)	79·1 (28·7)	43·9 (16·3)	< 0·01
$\dot{V}_E$ (ml min^{-1})	V_T (ml)	37·6 (5·2)	−332 (323)	43·3 (9·6)	−479 (494)	< 0·02

regression parameters slope and intercept. The differences between the values of the intercepts for SCL and CCL were on average small (Table 1), and none was significant. Therefore, we will now focus on the difference between the slopes of the regression lines for SCL and CCL.

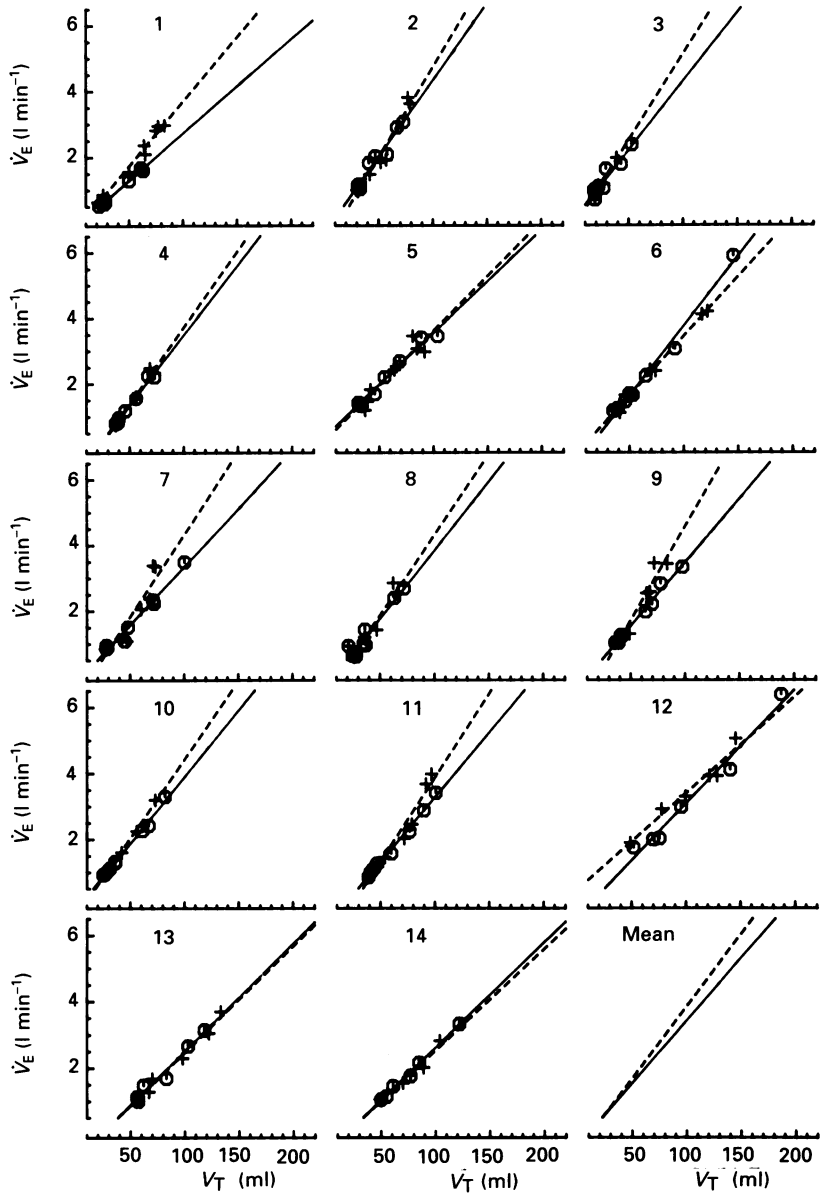

Fig. 2. Hey-plots ($\dot{V}_E$ as a function of V_T) for CCL and SCL experiments for each cat separately. For further explanation see Fig. 1.

The 'Hey-plots' ($\dot{V}_E$ as a function of V_T) are given in Fig. 2. Except for cats 6, 13 and 14, the regression lines for SCL are steeper than for CCL. The larger values for the slopes in the Hey-plots ($P < 0{\cdot}02$) were the result of higher f for SCL than for CCL

experiments at the same P_{a,CO_2} (Table 1). The difference between the slopes for SCL and CCL for the latter relationship was, however, not significant ($P > 0{\cdot}10$). Neither was the difference between the slopes of V_T as a function of $\Delta P_{a,CO_2}$ for SCL and CCL ($P > 0{\cdot}10$). The larger values for f during SCL compared to CCL were due to

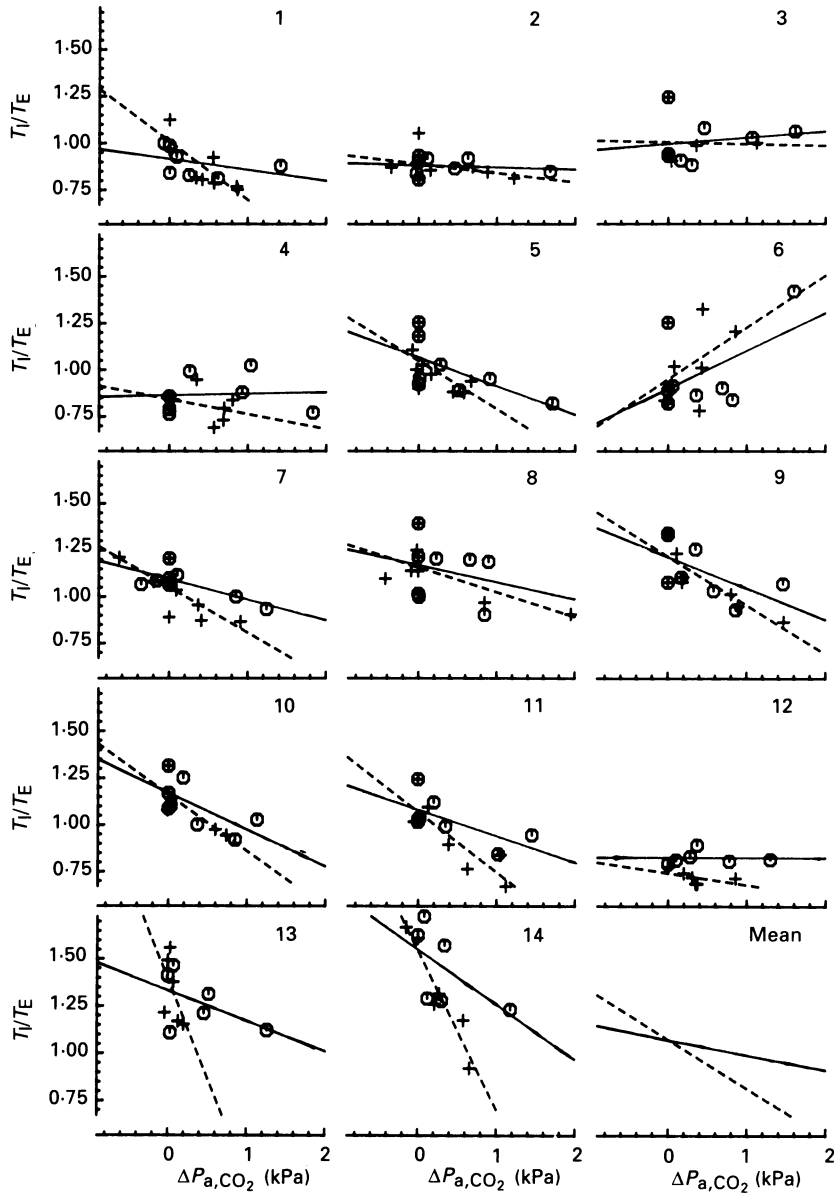

Fig. 3. The ratio T_I/T_E as a function of $\Delta P_{a,CO_2}$ for CCL and SCL experiments for each cat separately. For further explanation see Fig. 1.

decreased duration of inspiration (T_I). The values for the slopes for T_I as a function of $\Delta P_{a,CO_2}$ were significantly different between SCL and CCL ($P < 0{\cdot}05$), while they were not for expiratory duration (T_E) as a function of $\Delta P_{a,CO_2}$ ($P > 0{\cdot}10$). Hence, the

slopes for T_I/T_E as a function of $\Delta P_{a,CO_2}$ for SCL were generally smaller than those for CCL (Fig. 3, Table 1, $P < 0{\cdot}01$). Mean inspiratory flow ($\dot{V}_I$) is equal to $\dot{V}_E/60(1 + T_E/T_I)$. Accordingly, the slopes for $\dot{V}_I$ as a function of $\Delta P_{a,CO_2}$ were larger for SCL than for CCL ($P < 0{\cdot}01$). The average ratio of these slopes (SCL/CCL) was 1·56 (range 0·68–3·30).

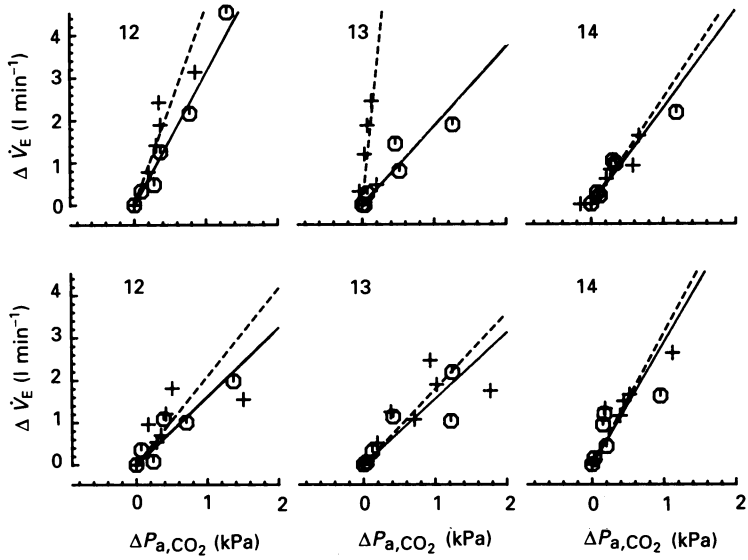

Fig. 4. CO_2 response curves for three cats before (upper panels) and after vagotomy (lower panels). For further explanation see Fig. 1.

Figure 4 displays the response curves for three cats before and after vagotomy. The differences between the response curves for SCL and CCL before vagotomy are representative for the whole group (Fig. 1): cat 13 had the largest ratio of all fourteen cats between the slopes for SCL and CCL (8·65), and cat 14 had the smallest value (1·10). For cat 12 the ratio (1·49) was somewhat below average (2·06). After vagotomy the ratio between SCL and CCL slopes was strongly reduced for cat 13 (1·15) and also the other two cats showed somewhat lower values (1·29 and 1·08 for cats 12 and 14, respectively). The mean values for the slopes after vagotomy (2·0 and 2·3 l min^{-1} kPa^{-1}, for CCL and SCL respectively) were comparable to the CCL slopes before vagotomy (2·4 l min^{-1} kPa^{-1}). This means that vagotomy reduced the response to SCL, whereas a vagal mechanism did not contribute importantly for CCL. For the small difference between SCL and CCL which remained after vagotomy, an extra-vagal mechanism might be considered.

DISCUSSION

Critique of methods

The slopes of the CO_2 response curves for SCL were 2 times larger than the slopes for CCL (Table 1). The slopes of the CO_2 response curves for CCL (2·0 l min^{-1} kPa^{-1})

were steeper than those described by Gautier (1976) for awake cats, i.e. about 1·1 l min^{-1} kPa^{-1}. We used bigger cats in our study and, further, we estimated the slopes of the response curves according to Mardia *et al.* (1983), both of which may lead to an increase in the values for the slopes.

We did the following tests to ascertain that our results were not influenced by unintended effects during the experiments and subsequent data analysis. Firstly, we introduced slugs of N_2, O_2 or air into the trachea which had no effect on $\dot{V}_E$ or the breathing pattern, suggesting that there was no mechanical stimulus involved in SCL. Further, the blood gas values obtained from the low-volume capillaries (0·14 ml) were not systematically different from those of simultaneously obtained larger blood samples in heparinized glass syringes of 1·5 ml. The small size of the samples allowed us to obtain a value for P_{a,CO_2} before and after each SCL or CCL experiment, without depriving the experimental animal of more than 5 % of its blood volume. Calculating $\Delta P_{a,CO_2}$ instead of using P_{a,CO_2} directly, corrected for gradual changes in P_{a,CO_2} during the day e.g. resulting from small changes in the depth of anaesthesia. To ascertain that measurements were done in a steady-state condition we examined each data file for a trend in the breath-to-breath calculated value for $\dot{V}_E$, which was absent. The P_{a,CO_2} levels obtained before each experimental run seldom differed by more than 0·15 kPa between consecutive runs, suggesting that all runs were started from a steady-state condition.

Techniques for loading CO_2 at the start of inspiration

Loading the respiratory system with CO_2 at the start of inspiration mimics metabolic CO_2 loading more closely than CCL does (Swanson, 1978). This has been done basically by two methods: the Fenn & Craig technique (1963) and the tube-breathing technique. Fenn & Craig (1963) added a constant flow of CO_2 to the inspiratory limb of a valve system. During expiration CO_2 accumulates in front of the inspiratory valve, and the next inspiration starts with a high CO_2 content in inspired air, declining in the course of inspiration towards lower levels, although never reaching zero value. End-inspiratory CO_2 concentrations may be 0·5–1·0 % (Cummin, Iyawe, Jacobi, Patil & Saunders, 1984; Cummin *et al.* 1987).

With the tube-breathing technique, an external dead space is added. At the start of inspiration the content of the external dead space, i.e. alveolar gas, is inspired first, followed by fresh air. In fact this is a kind of rebreathing technique, causing not only an increase in P_{a,CO_2} but also a decrease in P_{a,O_2}. Neither of these techniques, however, may be very effective in mimicking metabolic loading: longitudinal dispersion of a gas bolus in the airways is considerable, especially when the mouth and larynx are not bypassed (Ultman & Thomas, 1979). During tube breathing, the volume of the tube is larger than half the V_T (Masuyama & Honda, 1984). For both these techniques the difference with CCL is, therefore, likely to be small.

Concerning our method CO_2 is loaded as early in inspiration as possible. CO_2 is injected directly into the tracheal cannula, thus bypassing the Fleisch head and other 'mixing chambers' in the breathing circuit. The bolus may spread over the tracheal volume and enter in part the equipment dead space. This is, however, unlikely since the largest flow used to inject a bolus was 120 ml s^{-1}, which is a normal inspiratory flow for a SCL experiment with a 6 ml bolus. In conclusion, the technique

for SCL used in our study may be much more effective in mimicking a metabolic CO_2 load of breathing than the methods used so far.

The effect of different loading techniques on ventilatory parameters

Our results show larger $\dot{V}_E$ for SCL compared to CCL. Using tube breathing, Masuyama & Honda (1984) found comparable increases (about 100%) in the slopes of the CO_2 response curve in man. They only found significant differences in V_T, whereas f was fully unaffected. In a more recent paper of the same group (Maruyama, Masuyama, Tanaka, Nishibayashi & Honda, 1988) these results could apparently not be reproduced: a small difference in the slope of the CO_2 response curve was found only in hypoxia. Studies in human subjects comparing different methods for CO_2 loading often require a background of hypoxia to obtain different results for the two methods (see Cummin *et al.* 1987). Our results were obtained in normoxaemia: P_{a,O_2} ranged between 11·2 kPa for a control experiment up to 16·5 kPa for the highest CO_2 loads. Also in cats a small increase in $\dot{V}_E$ (11%) has been reported during tube breathing as compared to CCL, and these results were also obtained in normoxia (Marsh & Nye, 1982).

Mechanisms for the control of ventilation during CCL and SCL

Marsh & Nye (1982), comparing tube breathing to CCL at the same CO_2 load, obtained about the same increases in $\dot{V}_E$ before and after vagotomy, whereas the differences between CCL and SCL had largely disappeared after section of the sinus nerves. From these findings they concluded that oscillations in arterial P_{CO_2} were the most important mechanism causing differences between CCL and tube breathing. In our experiments the slopes of the response curves for SCL were diminished after vagotomy (Fig. 4). The magnitude of the change in $\dot{V}_E$ obtained in their experiments (11%) is, however, comparable to the mean change between SCL and CCL slopes we obtained after vagotomy (17%). This brings us to the following hypothesis: tube breathing is hardly capable of stimulating a pulmonary mechanism and most of the effects on $\dot{V}_E$ are caused by oscillations of CO_2 in arterial blood. Our technique may deliver comparable oscillations in arterial P_{CO_2} and their effects on $\dot{V}_E$ may be estimated from the difference in the response curves for SCL and CCL obtained after vagotomy. Evidently, the larger part of the difference in $\dot{V}_E$ between SCL and CCL in our experiments may be caused by a vagal mechanism. Why this mechanism is not active during tube breathing will be discussed below.

Vagal mechanisms for the control of breathing during SCL

During the larger part of inspiration the P_{CO_2} level at the pulmonary receptor site is different between SCL and CCL. During SCL, P_{CO_2} in the conducting airways rapidly decreases to 0 kPa, whereas during CCL P_{CO_2} equals inspiratory levels. The tissue P_{CO_2} at the receptor site may follow the variations in airway P_{CO_2} rapidly (Luijendijk, 1983) and lung receptors in cats are sensitive to changes in airway CO_2 (Kunz, Kawashiro & Scheid, 1976). The activity of both slowly and rapidly adapting receptors is reduced by increasing airway CO_2, especially in the range between 0 and 3 kPa (Coleridge, Coleridge & Banzett, 1978). Hence, the variations in airway P_{CO_2} may modulate the firing pattern of the receptor. During tube breathing the firing

pattern of the receptor may not be very different from that during CCL, for the CO_2-free air reaches the receptor site only at the end of inspiration. Inhaling CO_2 decreases the activity of slowly adapting stretch receptors (Bradley, Noble & Trenchard, 1976). As a result, T_I may increase and f decrease. Accordingly, we found higher slopes for T_I as a function of $\Delta P_{a,CO_2}$ for CCL than for SCL experiments, whereas slopes for f as a function of $\Delta P_{a,CO_2}$ were lower, though the difference for this last relationship was not significant. The question of how this mechanism causes increases in $\dot{V}_I$ during SCL remains to be solved.

Besides a direct influence of CO_2 on lung receptor output, there may also exist an indirect influence due to CO_2-induced bronchodilatation. During SCL bronchodilatation may be less than during CCL, because in the former case airway CO_2 is low during the larger part of inspiration. However, this effect is generally thought to be negligible (Coleridge *et al.* 1978; Sant'Ambrogio, 1987). The bronchoconstriction that is caused by stimulation of peripheral chemoreceptors by increased P_{a,CO_2} and which increases slowly adapting stretch receptor activity (Fisher, Sant'Ambrogio & Sant'Ambrogio, 1983), is likely to be the same for SCL and CCL.

SCL mimics a metabolic CO_2 load more closely than CCL does (Swanson, 1978). Accordingly, we found a more isocapnic response for SCL compared to CCL. The same vagal mechanism stimulating ventilation may be underlying the observation in the experiments of Sheldon & Green (1982). They found in dogs in which the systemic and pulmonary circulation had been completely separated that increases in pulmonary arterial CO_2 content increased ventilation, while systemic conditions were kept constant. After vagotomy this response disappeared. It has been demonstrated that in response to changes in pulmonary arterial P_{CO_2} the activity of slowly adapting stretch receptors could be influenced in this kind of experiment (Green, Schertel, Coleridge & Coleridge, 1986).

In conclusion, we found 2 times higher slopes for the CO_2 response curve for SCL compared to CCL during spontaneous, normoxic breathing. These differences were due to higher V_T and higher f for SCL. The increases in f were predominately caused by shortening of inspiratory time. It is shown that the differences in $\dot{V}_E$ are largely caused by a vagal mechanism. The way in which slug CO_2 loading is implemented may be important to stimulate this vagal mechanism. Further studies in conscious man injecting CO_2 at the start of inspiration directly into the naso-pharynx should elucidate the importance of our findings for human respiratory control, whereas animal studies may further clarify the underlying vagal mechanisms.

Dr A. Berkenbosch and Dr J. DeGoede are kindly acknowledged for their valuable discussions. This study was supported by the Netherlands Organisation for Scientific Research, NWO.

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