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ON A PSEUDO-REBREATHING TECHNIQUE TO ASSESS THE VENTILATORY SENSITIVITY TO CARBON DIOXIDE IN MAN

BY ALBERT DAHAN, AAD BERKENBOSCH, JACOB DEGOEDE, IDA C. W. OLIEVIER AND JIM G. BOVILL

From the Departments of Anaesthesiology and Physiology, University of Leiden, PO Box 9604, 2300 RC Leiden, The Netherlands

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SUMMARY

1. The ventilatory sensitivity to carbon dioxide obtained from a step-ramp CO_2 challenge was compared to the CO_2 sensitivity from the steady-state method.

2. Experiments were performed in nine healthy male subjects against a background of hyperoxia and in two subjects against a background of normoxia.

3. In each subject experiments were performed in which the stepwise increase in end-tidal P_{CO_2} above its resting value (A) was varied (range 0–2 kPa) and the subsequent rate of rise of end-tidal P_{CO_2} in time (R) kept constant at 0·6 or 0·8 kPa min^{-1}.

4. The results of the hyperoxic experiments show that the slope of the non-steady-state ventilatory response to CO_2 (S_n) is greatly influenced by the magnitude of A. An increase of A of 1 kPa results in a 54% increase of the ratio non-steady-state ventilatory CO_2 sensitivity to steady-state ventilatory CO_2 sensitivity (S_s). The magnitude of R plays a minor role in determining S_n. The normoxic experiments gave similar results.

5. In experiments performed during hyperoxia S_n approximates S_s when the magnitude of A is 0·5 kPa.

6. The results are discussed and related to a physiological model. Simulations with representative values for the model parameters are in fair agreement with experimental values.

INTRODUCTION

In a previous study we compared the ventilatory response to changes in end-tidal carbon dioxide tension (P_{ET,CO_2}) during hyperoxia, obtained with Read's rebreathing method (Read, 1967) and a steady-state method (Berkenbosch, Bovill, Dahan, DeGoede & Olievier, 1989). In ten subjects we found that the CO_2 sensitivity from rebreathing largely overestimated the steady-state CO_2 sensitivity. In his original experiments Read (1967) found good agreement between the ventilatory CO_2 sensitivities from the steady-state and rebreathing methods. It is possible that the differences found in our experiments and those of Read are due to differences in the step increase of P_{ET,CO_2} (A) and the rate of rise of P_{ET,CO_2} in time (R). Our rebreathing

experiments yielded a mean value for A and R of 1·5 kPa and 0·53 kPa min^{-1}, respectively. Read did not mention his values for A but an estimate of 1·2 kPa can be made. The mean value of R was approximately 0·8 kPa min^{-1}.

To get some insight into the effect of changes in A and R on the non-steady-state CO_2 sensitivity we performed experiments using a pseudo-rebreathing technique (Berkenbosch, DeGoede, Olievier & Schuitmaker, 1986). In a first approach we restricted ourselves to changing the step A, keeping R close to the values used by Read (1967). Furthermore, we explored whether there are conditions on the step-ramp input in P_{ET,CO_2} in which the non-steady-state CO_2 sensitivity (S_n) approximates the steady-state CO_2 sensitivity (S_s).

METHODS

Physiological model

To have a framework in which we can conveniently discuss our experimental results we use a physiological model which is essentially the same as that described by Read & Leigh (1967). We start writing the mass balance for CO_2 of a brain compartment as (Berkenbosch *et al.* 1989)

$$\frac{d}{dt}P_{t,CO_2} = \frac{l_a}{l_t}\dot{Q}(P_{a,CO_2} - P_{t,CO_2}) + \frac{(1-\gamma)}{l_t}(\dot{M} - h), \tag{1}$$

where P_{t,CO_2} is the brain tissue P_{CO_2}, P_{a,CO_2} the arterial P_{CO_2}. $\dot{Q}$ and $\dot{M}$ are the brain blood flow density and brain metabolism density. In deriving eqn (1) we have used linear approximations to the blood and brain tissue CO_2 dissociation curve, viz. l_t is the slope of the brain tissue and l_a the slope of the blood CO_2 dissociation curve. The Haldane parameter h equals $(b_v - b_a)\dot{Q}$, in which b_v and b_a are the intercepts of the venous and arterial CO_2 dissociation curves, respectively. The parameter γ 'locates' P_{t,CO_2} between P_{a,CO_2} and the cerebral venous P_{CO_2} (P_{cv,CO_2}) in the steady state ($0 < \gamma < 1$).

The cerebral blood flow $\dot{Q}$ is assumed to be coupled to P_{t,CO_2} in a hyperbolic fashion:

$$\dot{Q} = \frac{a}{b - P_{t,CO_2}}. \tag{2}$$

This implies that in the steady state there also is a hyperbolic relationship between $\dot{Q}$ and P_{a,CO_2}. In the cat there is some evidence for such a relationship (VanBeek, 1983).

During hyperoxia the peripheral chemoreceptors do not contribute to ventilation in human subjects and we assume that the ventilation ($\dot{V}_E$), due to the central chemoreceptors, is instantaneously and linearly related to P_{t,CO_2} so that

$$\dot{V}_E = S_t P_{t,CO_2} + k_t. \tag{3}$$

In eqn (3) S_t is the CO_2 sensitivity 'at the site' of the central chemoreceptors and k_t a constant.

If we apply a step increase A in P_{a,CO_2} from the initial value P^0_{a,CO_2} followed by a linear rate of rise R we may write

$$P_{a,CO_2}(t) = P^0_{a,CO_2} + (A + Rt)\,U(t), \tag{4}$$

in which the unit step function is defined by

$$U(t) = 0 \quad t < 0,$$

and

$$U(t) = 1 \quad t > 0.$$

We now look for responses of P_{t,CO_2} which are of the form

$$P_{t,CO_2} = P^0_{t,CO_2} + \lambda R t U(t), \tag{5}$$

with λ a (positive) constant and P^0_{t,CO_2} the initial tissue P_{CO_2}.

By substituting eqns (4) and (5) into eqn (1) we find that the condition for a response given by eqn (5) is

$$\frac{A}{R} = \tau_t^0 \left[1 + \frac{l_t}{a l_a}\left(m - \frac{A}{\tau_\tau^0}\right)\right]^{-1}, \tag{6}$$

where we have introduced the abbreviations

$$m = \frac{(1-\gamma)(\dot{M}-\dot{h})}{l_t}, \tag{7}$$

and

$$\tau_t^0 = \left(\frac{l_a}{l_t}\dot{Q}_0\right)^{-1}. \tag{8}$$

In eqn (8) $\dot{Q}_0$ is the blood flow just before the applied step A.

For λ we find

$$\lambda = \frac{A}{\tau_t^0 R}. \tag{9}$$

Therefore, given a step change A in P_{a,CO_2}, one can always find a rate of rise R from eqn (5) and a λ from eqn (9) such that P_{a,CO_2} changes according to eqn (4) and P_{t,CO_2} changes according to eqn (5). Consequently the ventilation ($\dot{V}_E$) also changes linearly with a rate of rise $\lambda R S_t$ (see eqn (3)). We express this by saying that R is matched to A. After the step change A, the relationship between $\dot{V}_E$ and P_{a,CO_2} is linear with slope S_n given by

$$S_n = S_t \left[1 + \frac{l_t}{a l_a}\left(m - \frac{A}{\tau_t^0}\right)\right]^{-1}. \tag{10}$$

From eqns (1) and (3) the steady-state CO_2 sensitivity S_s, setting $\mathrm{d}/\mathrm{d}t\, P_{t,CO_2}$ equal to zero, is

$$S_s = S_t \left(1 + \frac{m l_t}{a l_a}\right)^{-1}. \tag{11}$$

We now write

$$\frac{S_n}{S_s} = \left(1 + \frac{m l_t}{a l_a}\right)\left[1 + \frac{l_t}{a l_a}\left(m - \frac{A}{\tau_t^0}\right)\right]^{-1}. \tag{12}$$

From eqn (10) we see that S_n equals S_t if

$$A = \tau_t^0 m, \tag{13}$$

which together with

$$R = m \tag{14}$$

we call the Read conditions (Berkenbosch *et al.* 1989).

We emphasize that there is an infinite number of combinations of A and R such that the relationship between $\dot{V}_E$ and P_{a,CO_2} is linear. However, the slopes of these linear relations are all different. If A and R obey the Read conditions S_n equals S_t. From eqns (6) and (12) we see that S_n equals S_s if A and R go to zero in such a way that A/R goes to the limiting time constant

$$\tau_t^1 = \tau_t^0 \left[1 + \frac{m l_t}{a l_a}\right]^{-1}. \tag{15}$$

The latter result suggests that there are small but finite values of A and R which give rise to an S_n close to S_s especially if R is not exactly matched to A. After some reflexions it becomes clear that when R is larger than the matched value a convex respiratory response will be obtained and if R is smaller than the matched value a concave curve will be obtained (see simulations).

Finally, we point out that elimination of P_{t,CO_2} from eqns (1) and (3), also using eqn (2), leads to the differential equation

$$\tau_t \frac{\mathrm{d}\dot{V}_E}{\mathrm{d}t} + \dot{V}_E = S_s (P_{a,CO_2} - B), \tag{16}$$

in which $$\tau_t = l_t\left[l_a\dot{Q}\left(1+\frac{ml_t}{al_a}\right)\right]^{-1} \quad (17)$$

and B a constant.

Simulations

To determine the influence of A and R on the slope of the non-steady-state response we made a number of simulations using the above equations. We took parameter values that we used before (Berkenbosch *et al.* 1989): $l_t = 2{\cdot}25 \times 10^{-2}$ ml ml^{-1} kPa^{-1}, $l_a = 3{\cdot}22 \times 10^{-2}$ ml ml^{-1} kPa^{-1}, $\gamma = 0$,

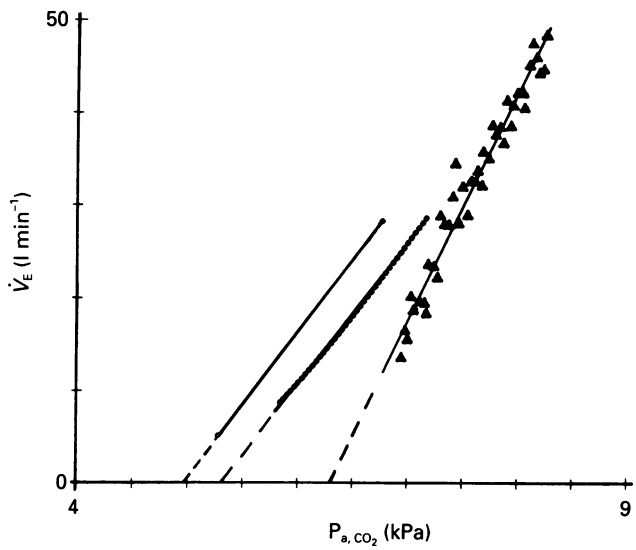

Fig. 1. Simulations of the ventilatory response to CO_2. Continuous line: steady-state response with a slope of 14·5 l min^{-1} kPa^{-1}. •••, non-steady-state response for values of A of 0·4 kPa and R of 0·5 kPa min^{-1}. Linear regression yielded a slope of 15·0 l min^{-1} kPa^{-1}. ▲, non-steady-state response for values of A of 1·5 kPa and R of 0·5 kPa min^{-1}. Linear regression yielded a slope of 24·2 l min^{-1} kPa^{-1}. Note that in this last simulation white noise was added to the data.

$\dot{M} = 5 \times 10^{-4}$ ml ml^{-1} s^{-1}, $h = 1{\cdot}83 \times 10^{-4}$ ml ml^{-1} s^{-1}, $S_t = 20$ l min^{-1} kPa^{-1}, $k_t = -128$ l min^{-1}, $a = 0{\cdot}025$ kPa s^{-1} and $b = 9{\cdot}5$ kPa. The values for a and b were estimated from data of Kety & Schmidt (1948) using eqns (1) and (2).

As input of the model we used step-ramp inputs. All simulations were made with an initial P_{a,CO_2} of 5·3 kPa. The value of A ranged between 0 and 2 kPa, and the value of R ranged between 0·5 and 1·0 kPa min^{-1}. In our model we did not take into account the transport delay from lung to intracranial chemosensitive structure of about 12 s (Ward & Bellville, 1983). Furthermore, to make the simulations comparable to the experiments of Read (1967) and ours in which the first 30 s of the data after the initiation of rebreathing are omitted we deleted the first 18 s of the simulated data. To make the simulated data somewhat realistic white noise was added to the data of some simulations.

Figure 1 shows the results of two simulations. $A = 0{\cdot}4$ kPa and $R = 0{\cdot}5$ kPa min^{-1} (•••) results in a convex non-steady-state response curve. Linear regression of the data points yields a slope close to the slope of the steady-state response (15·0 *vs.* 14·5 l min^{-1} kPa^{-1}). $A = 1{\cdot}5$ kPa and $R = 0{\cdot}5$ kPa min^{-1} (▲) results in a concave response curve. Linear regression yields a slope of 24·2 l min^{-1} kPa^{-1}. As illustrated in Fig. 1 the curvilinearity of the response is markedly masked when the data are corrupted by noise. The P_{CO_2} range of the steady-state response is 1·5 kPa. This is the range normally used in steady-state experiments to avoid discomfort for the subject. The

same P_{CO_2} range is covered by the non-steady-state curve with a small A. Note that the ventilation range of the non-steady-state response curve with a large A is much larger than the range covered in the steady-state curve and the non-steady-state curve with a small A.

In Fig. 2 different values of A at different values of R are plotted against S_n/S_s. The range of R found by Read (1967) in his experiments is 0·5–0·8 kPa min^{-1}. It is clear that at all rates of rise between 0·5 and 1·0 kPa min^{-1} a step A of approximately 0·4 kPa is necessary to obtain the steady-state response slope with the non-steady-state method using the above mentioned values for the parameters. The deviation from the steady-state slope for a change in A of 1 kPa is 61 % for an R of 0·5 kPa min^{-1}, 48 % for an R of 0·75 kPa min^{-1} and 42 % for an R of 1·0 kPa min^{-1}. This illustrates that the effect of changes in R on the ratio S_n/S_s is much less pronounced than changes in A.

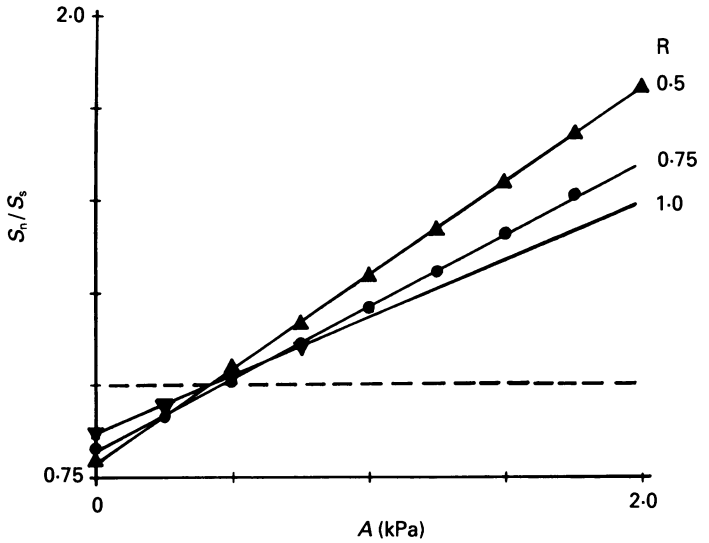

Fig. 2. Simulations of the influence of A on the ratio non-steady-state slope over steady-state slope (S_n/S_s) at different values of R. Note that for all values of R the ratio $S_n/S_s = 1$ at an $A \approx 0{\cdot}4$ kPa. R values are in kPa min^{-1}.

To demonstrate the influence of metabolism and brain blood flow on S_n/S_s simulations were performed with different values for metabolism ($\dot{M}$), initial brain blood flow at a P_{a,CO_2} of 5·3 kPa ($\dot{Q}_0$) and the location of the asymptote (b). This was done by varying A and keeping R constant at 0·5 kPa min^{-1}. The results are shown in Figs 3 and 4. In Fig. 3 metabolism was kept constant at $5{\cdot}0 \times 10^{-4}$ ml ml^{-1} s^{-1}. Changing initial brain blood flow at a P_{a,CO_2} of 5·3 kPa or the asymptote b does not change the influence of A on S_n/S_s much. An A of approximately 0·4 kPa approximates the steady-state slope. Only in the case of a very high $\dot{Q}_0$ is a much smaller A necessary and the slope of the S_n/S_s–A relation diminished.

Figure 4 shows that changing metabolism and asymptote b has minimal influence on the slope of the S_n/S_s–A relation or the value of A at which the steady-state is approximated. Only when $\dot{M}$ is lowered and the $\dot{Q}_0$ is matched to keep the brain tissue P_{CO_2} constant in the initial state is a much larger A necessary to attain the steady-state slope.

Subjects

The same subject group who performed in a previous study (Berkenbosch *et al.* 1989) plus one extra subject (in total eleven subjects) took part in the experimental protocol approved by the Leiden University Ethics Committee. They all gave an informed consent to the protocol. The subjects were naive to respiratory physiology, with the exception of subjects A. B. E. and A. D. A. None was a smoker or had any history of chronic obstructive pulmonary disease. Each subject was familiarized with the experimental procedure on the day before testing. All subjects refrained from stimulant and depressant substances 12 h prior to the experiment.

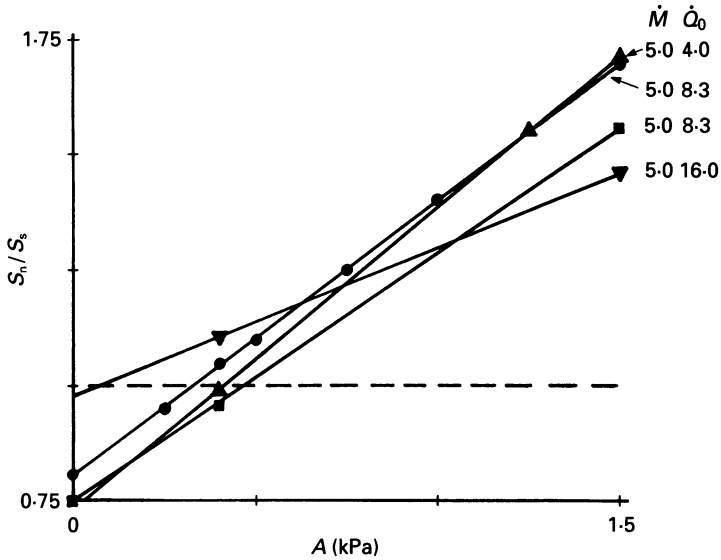

Fig. 3. Simulations of the influence of A on S_n/S_s at a constant R and different values for the initial brain blood flow ($\dot{Q}_0$) and location of the asymptote b. Values of $\dot{M}$ are in 10^{-4} ml ml^{-1} s^{-1} and for $\dot{Q}_0$ in 10^{-3} ml ml^{-1} s^{-1}. ▲, ●, ▼: $a = 0{\cdot}025$ kPa s^{-1} and $b = 9{\cdot}5$ kPa; ■: $a = 0{\cdot}025$ kPa s^{-1} and $b = 12{\cdot}5$ kPa.

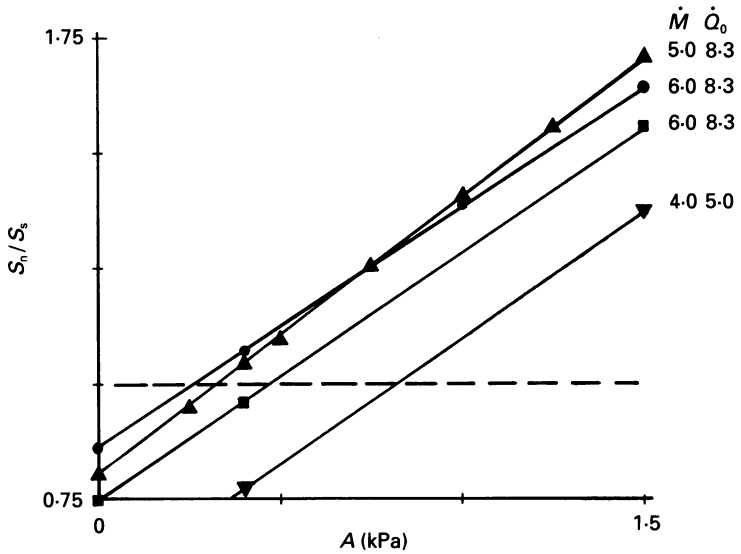

Fig. 4. Simulations of the influence of A on S_n/S_s at a constant R and different values for the metabolism ($\dot{M}$), the initial brain blood flow ($\dot{Q}_0$) and location of the asymptote b. $\dot{M} = 4$ and $\dot{Q}_0 = 5$ give a matched brain tissue P_{CO_2} with control. ▲, ●: $a = 0{\cdot}025$ kPa s^{-1} and $b = 9{\cdot}5$ kPa; ▼: $a = 0{\cdot}028$ kPa s^{-1} and $b = 12{\cdot}5$ kPa; ■: $a = 0{\cdot}025$ kPa s^{-1} and $b = 12{\cdot}5$ kPa. Values for $\dot{M}$ are in 10^{-4} ml ml^{-1} s^{-1} and for $\dot{Q}_0$ in 10^{-3} ml ml^{-1} s^{-1}.

Measurements and procedures

An oronasal face mask was fitted and the subjects were instructed to breathe through their mouths to prevent a change in airway resistance during the experiment. The airway gas flow was measured with a Fleisch No. 3 pneumotachograph connected to a differential pressure transducer

(Hewlett–Packard model 270, USA) and electronically integrated (Drummond & Goodenough, 1977) to yield a volume signal. The volume was calibrated with a motor-driven piston pump (stroke volume 1·0 l, with a frequency of 20 min^{-1}). Corrections were made for changes in gas viscosity due to changes in gas composition of the expired gas mixture. The CO_2 concentrations of the inspired and expired gases were measured with a fast-response infra-red analyser (Gould Godart MK2 capnograph, The Netherlands) and the O_2 concentrations with a fast-response zirconium oxide cell (Jaeger O_2 test, FRG). All signals were recorded on a polygraph, and also digitized and processed by a PDP 11/23 computer. The tidal volume, inspiratory time, expiratory time, respiratory frequency, $\dot{V}_E$, end-tidal CO_2 and end-tidal O_2 tensions were stored, on a breath-to-breath basis, on disc.

The pneumotachograph was connected to a T-piece. One arm of the T-piece was receiving a gas mixture with a flow of 80 l min^{-1} from a gas mixture system, consisting of three mass flow controllers (Bronkhorst High Tech BV-F202, The Netherlands) by which the flow of O_2, N_2 and CO_2 could be set individually at any desired level. The computer provided control signals to the mass flow controllers, so that the composition of the inspiratory gas mixtures could be adjusted to force the P_{ET,CO_2} to follow a specific dynamic pattern in time and keep the end-tidal P_{O_2} (P_{ET,O_2}) constant. The method of the 'dynamic end-tidal forcing technique' has been described in detail by Swanson & Bellville (1975). The instrumental dead space was 250 ml. In this study we generally assumed that end-tidal P_{CO_2} is equal to arterial P_{CO_2}.

Experimental design

Experiments were performed on three morning sessions, separated by periods of three weeks. The subjects were comfortably seated and encouraged to read and listen to music through headphones. There was a rest period of at least 30 min before the experiments started. The steady-state experiments preceded the pseudo-rebreathing experiments. The subjects rested 15 min between individual runs. Each session lasted approximately 3.5 h. Nine subjects performed all experiments during hyperoxia (P_{ET,O_2} 70 kPa). Experiments were performed in two subjects (A.D.A. and A.B.E.) against a background of normoxia (P_{ET,O_2}, 14·5 kPa).

To obtain data points for the steady-state response four to five stepwise P_{ET,CO_2} elevations were applied. The steps varied from 1 to 2·5 kPa. The order of the steps was randomly determined. Each steady-state experiment started with a period of steady-state ventilation of approximately 5 min during which the P_{ET,CO_2} was held slightly above resting P_{ET,CO_2} (varying among subjects from 5·4 to 5·8 kPa) and the P_{ET,O_2} at the desired level. The P_{ET,CO_2} was then elevated within one or two breaths, maintained constant for about 8 min and then returned to the original value for another 8 min.

The pseudo-rebreathing experiments started with 5–6 min of steady-state ventilation at a constant P_{ET,O_2} followed by a stepwise increase and a subsequent constant rate of rise of P_{ET,CO_2} for 3 min (see Fig. 5). Two to eight pseudo-rebreathing experiments were performed in each session.

Data analysis

In the steady-state experiments the $\dot{V}_E$ and P_{ET,CO_2} were averaged over ten breaths. Data points were collected in the minute before a P_{ET,CO_2} increase, in the minute before a P_{ET,CO_2} decrease and in the last minute of the experiment. In the non-steady-state experiments breath-to-breath data points were used. Time $t = 0$ was set at the moment of the step in P_{ET,CO_2}. Data points obtained from the first 30 s after the step in P_{ET,CO_2} were excluded from the data analysis as were sighs and swallows. The CO_2 sensitivity for both methods was estimated by linear regression of $\dot{V}_E$ on P_{ET,CO_2}. For non-steady-state experiments the rate of rise of the P_{ET,CO_2} (R) was estimated for each run from linear regression of P_{ET,CO_2} on time. The differences between the intercept of this line with the P_{ET,CO_2} axis and the resting P_{ET,CO_2}, measured during the minute preceeding the start of rebreathing was taken as an estimate of the step increase in P_{ET,CO_2} (A).

RESULTS

In the nine subjects, participating in the experiments against a background of hyperoxia, eighty-two observations of the non-steady state, together with twenty-nine steady-state responses, were obtained on twenty-nine different morning sessions.

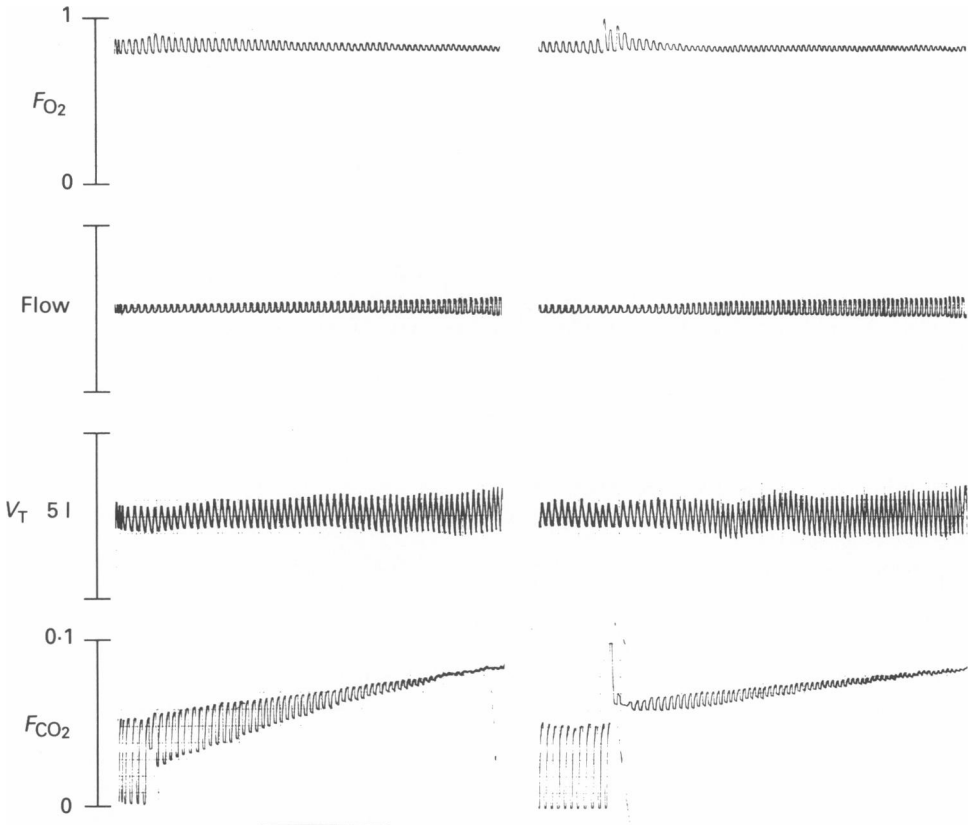

Fig. 5. Oscillographic recording of two non-steady-state responses of the same subject with different values of A and R. The flow signal is not calibrated. F_{O_2} and F_{CO_2} are the fractional concentrations of O_2 and CO_2, respectively. V_T is a tidal volume. A is 0·34 kPa and R 0·88 kPa min^{-1} for the response on the left and 1·33 kPa and 0·63 kPa min^{-1} for the response on the right. Bar represents 1 min.

Fifty-three experiments were performed with a value of R of $0{\cdot}8 \pm 0{\cdot}1$ kPa min^{-1} (range 0·7–1·0 kPa min^{-1}).

In Fig. 5 a live recording of two non-steady-state experiments of one subject during hyperoxia is given. The P_{ET,CO_2} increases linearly in time after the step increase. A is 0·34 kPa and R 0·88 kPa min^{-1} for the experiment on the left. This yields a ratio S_n over S_s of 1·08. In the experiment on the right A is 1·33 kPa and R 0·63 kPa min^{-1}. The resulting S_n/S_s is 1·5.

The magnitude of A determines the magnitude of the slope of the non-steady-state response. This is clearly seen in Fig. 6 where the ratio of non-steady-state slope to steady-state slope (S_n/S_s) is plotted against A of the hyperoxic experiments with a value of R of 0·8 kPa min^{-1} (△). There was a good correlation between S_n/S_s and A ($r = 0{\cdot}71$; $n = 53$). Linear regression analysis yielded a slope of $0{\cdot}54 \pm 0{\cdot}07$ kPa^{-1} and an intercept of $0{\cdot}75 \pm 0{\cdot}07$. It follows that a magnitude of A of 0·46 kPa results in a non-steady-state ventilatory CO_2 sensitivity which is close to the steady-state CO_2 sensitivity.

In Fig. 6 data obtained with a value of R of $0{\cdot}6 \pm 0{\cdot}05$ kPa min^{-1} (range

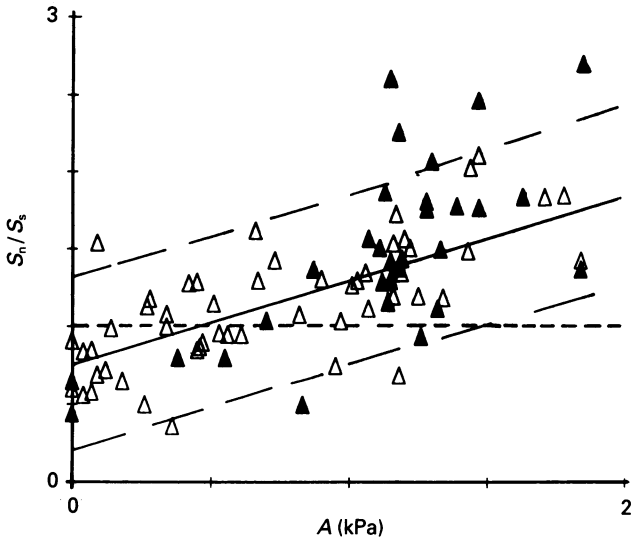

Fig. 6. The ratio response slope obtained with the non-steady-state method over the response slope obtained with the steady-state method (S_n/S_s) as a function of A of the hyperoxic data. The drawn line is the linear regression line for the data with an R of 0·8 kPa min^{-1} (△; $n = 53$); the dashed line is the 95% one-at-the-time prediction interval. ▲, data points obtained with an R of 0·6 kPa min^{-1} ($n = 29$).

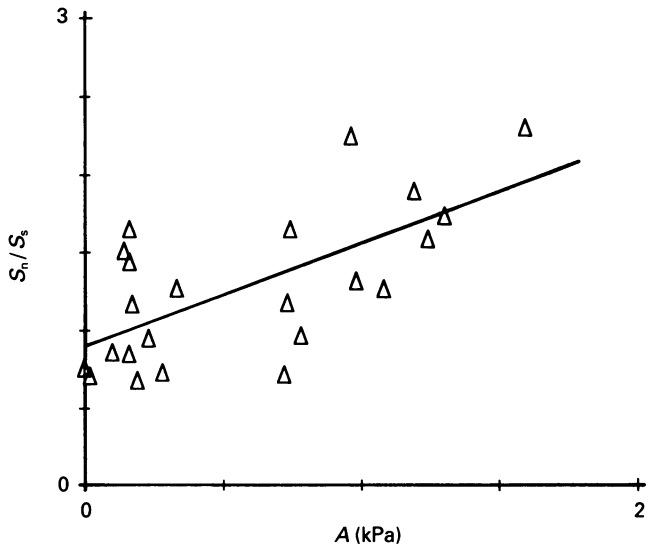

Fig. 7. The ratio response slope obtained with the non-steady-state method over the response slope obtained with the steady-state method (S_n/S_s) as a function of A of the normoxic data of two subjects. The continuous line is the linear regression line of all data points.

0·5–0·7 kPa min^{-1}) are included (▲). Most points lie within the 95% one-at-the-time prediction interval of the data with an R of 0·8 kPa min^{-1}. Only at high values of A there is some deviation of the data obtained with a low R value. This indicates that at values of $A < 1$ kPa the magnitude of R only plays a minor role in determining the slope of the non-steady-state response.

In two subjects twenty-three non-steady-state experiments together with six steady-state experiments were performed against a background of normoxia. The ratio S_n/S_s is plotted against A in Fig. 7 for all experiments. The mean value for R was $0·7 \pm 0·2$ kPa min^{-1}. Linear regression yielded a slope of $0·67 \pm 0·16$ kPa^{-1} and an intercept of $0·90 \pm 0·12$.

DISCUSSION

In 1967 Read introduced a rebreathing method to measure the ventilatory response to carbon dioxide. With this method the CO_2 sensitivity can be obtained within a short period of time with minimal discomfort to the subject. Due to these properties Read's rebreathing method became popular in drug studies and fundamental research. The experiment is performed with a small rebreathing bag (4–6 l) filled with 7% CO_2 in oxygen. Several features are observed when rebreathing is carried out in this fashion: (i) rebreathing is initiated at a CO_2 tension close to that of mixed venous blood ($P_{\bar{v},CO_2}$) and a rapid equilibrium is established between the CO_2 tension in mixed venous blood, arterial blood and gas in the lung and the rebreathing bag (Rebuck & Slutsky, 1981); (ii) ventilation increases linearly with time and the $\dot{V}_E$–P_{ET,CO_2} relation is linear (Read, 1967); (iii) as the CO_2 exchange between blood and gas is negligible during rebreathing the rate of change of P_{ET,CO_2} is independent of the ventilatory response (Fowle & Campbell, 1964). The results obtained with this type of rebreathing experiment are generally interpreted as follows. Initiating rebreathing close to the mixed venous P_{CO_2} leads to a rapid establishment of equality between arterial P_{CO_2}, mixed venous P_{CO_2} and presumably brain tissue P_{CO_2}, all of which increase at the same rate (Rebuck & Slutsky, 1981). If this interpretation holds good it follows that the CO_2 sensitivity obtained with Read's rebreathing method (S_r) should be equal to the CO_2 sensitivity at the site of the central chemoreceptors (S_t), which must be larger than the steady-state CO_2 sensitivity. This becomes clear by considering the effects of changes in cerebral blood flow upon increasing the P_{a,CO_2} (Berkenbosch *et al.* 1989) leading to a decreased cerebral venous–arterial P_{CO_2} gradient in the steady-state (Kety & Schmidt, 1948; Lambertsen, Hall, Wollman & Goodman, 1963; Fencl, Vale & Broch, 1969; Nishimura, Suzuki, Nishiura, Yamamoto, Miyamoto, Kishi & Kawakami, 1987). Indeed, we found that the CO_2 sensitivity from rebreathing experiments exceeded the steady-state CO_2 sensitivity by roughly a factor of 2. This was recently confirmed by others (Jacobi, Patil & Saunders, 1989; Bourke & Warley, 1989). These results are at variance with those of Read (1967) who found good agreement between the CO_2 sensitivities obtained with both methods.

Findings reported in the literature suggest that changes in the CO_2 sensitivity in the comparison of different physiological conditions obtained from rebreathing and

the steady state are substantially different. This has been demonstrated by Linton, Poole-Wilson, Davies & Cameron (1973) in a study on the effect on ventilation of chronic acid–base disturbances in man, and more recently by Bourke & Warley (1989) in a study on the effects of morphine on ventilation.

It is of considerable interest to discuss whether Read's rebreathing method gives the CO_2 sensitivity at the site of the central chemoreceptors. From a theoretical analysis using a physiological model described by Read & Leigh (1967) it follows that S_r is equal to S_t if the P_{t,CO_2}–P_{a,CO_2} gradient is reduced to zero at the onset of rebreathing and the rate of rise is such that this gradient is kept zero throughout this procedure. However, a practical problem arises here since the value of P_{t,CO_2} in man is unknown and difficult to determine. In animals the ventral medullary extra cellular fluid (ECF) P_{CO_2} can be measured with a P_{CO_2} electrode on the ventrolateral surface of the medulla oblongata. In cats the medullary surface ECF P_{CO_2}–P_{a,CO_2} gradients found are 1·3 kPa (Feustel, Vurek & Severinghaus, 1983), 2·0 kPa (Javaheri, Teppema & Evers, 1988) and 2·3 kPa (Javaheri, Evers & Teppema, 1989). Lambertsen (1960) and Bradley & Semple (1962) suggested that in man the jugular venous P_{CO_2} (P_{jv,CO_2}) is a reasonably approximation of the cerebrospinal fluid P_{CO_2} and hence P_{t,CO_2}, The P_{jv,CO_2}–P_{a,CO_2} gradient during hyperoxia in man varies between 1·4 and 2·0 kPa (Kety & Schmidt, 1948; Landmesser, Cobb, Peck & Converse, 1957; Lambertsen *et al.* 1963; Bradley, Semple & Spencer, 1965; Fencl *et al.*, 1969; Nishimura *et al.* 1987). The $P_{\bar{v},CO_2}$–P_{a,CO_2} gradient is about 0·8 kPa (Nunn, 1987) and much smaller than the P_{jv,CO_2}–P_{a,CO_2} gradient. Adrogué, Rashad, Gorin, Yacoub & Madias (1989) found a $P_{\bar{v},CO_2}$–P_{a,CO_2} gradient of 0·7 kPa in hyperoxia in healthy anaesthetized subjects before elective surgery. Abolishing the $P_{\bar{v},CO_2}$–P_{a,CO_2} gradient, one of the features of Read's rebreathing method (see above), may not be enough to reduce the P_{t,CO_2}–P_{a,CO_2} gradient sufficiently close to zero.

On the other hand it follows from the model that there is an infinite number of combinations of A and R that result in a linear relationship between $\dot{V}_E$ and P_{a,CO_2}. Only when $\lambda = 1$ will the response slope of the non-steady-state curve equal the slope at the site of the central chemoreceptors (see eqns (5) and (10)). Linearity of the non-steady-state response curve is therefore not a reliable criterion for judging whether the Read conditions are fulfilled. Furthermore, we found in our experiments that curvilinearity is hard to detect in single runs due to the noise superimposed on the ventilation data of human subjects. The simulations in which noise was added to the data are in agreement with these observations (see Fig. 1). Therefore the realization of the above mentioned conditions is hard or even impossible to verify in man and one cannot be sure that the CO_2 sensitivity obtained with Read's rebreathing method is equal to the CO_2 sensitivity at the site of the central chemoreceptors.

It is obvious that a search should be made for conditions of A and R which result in the measured CO_2 sensitivity approximating the steady-state CO_2 sensitivity. To this end we performed experiments in which A was varied between 0 and 2 kPa and R only changed minimally. It is clear from Fig. 6 that at a value of A of 0·5 kPa the non-steady-state response slope is about equal to the steady-state response slope. This figure also shows that the influence of R on S_n is much less important compared to the influence of A. Simulations are in agreement with these results (see Fig. 2). Our experimental results show that an increase of A of 1 kPa results in an increase of the

ratio S_n/S_s of about 50% (see Fig. 6). Therefore, the difference in the value of A in our previous study (Berkenbosch *et al.* 1989) and the study of Read (1967) can at best partly explain the large difference between the findings of Read and ours.

Performing experiments against a background of hyperoxia will only give information about the central chemoreceptors since the peripheral chemoreceptors are silent (Cunningham, Robbins & Wolff, 1986). The technique of pseudo-rebreathing enables us to perform step-ramp inputs in CO_2 against any level of oxygen. The CO_2 sensitivity obtained in such experiments is then the sum of the central and peripheral CO_2 sensitivity. We performed normoxic experiments in two subjects. The relationship between S_n/S_s and A during normoxia was similar compared to hyperoxia.

To gain some insight into the effect on the ratio S_n/S_s of changes in metabolism, initial cerebral blood flow and changes in cerebral blood flow to CO_2 we performed several simulations. They show that S_n/S_s is not very sensitive to moderate changes in $\dot{M}$, $\dot{Q}_0$ and changes in cerebral blood flow to CO_2. A very large $\dot{Q}_0$ resulted in a marked decreased sensitivity of S_n/S_s *vs.* A (see Fig. 3). Lowering $\dot{M}$ and $\dot{Q}_0$ so that the brain tissue P_{CO_2} remains equal to control almost doubled the value of A at which $S_n/S_s = 1$. Especially this latter condition is of importance as there are many drugs which decrease both $\dot{M}$ and $\dot{Q}_0$, e.g. benzodiazepines, morphine and barbiturates (Hoffman, Milietch & Albrecht, 1986; Santiago & Edelman, 1986). The consequence will be that an adjustment of A is necessary to keep the ratio S_n/S_s equal to 1 in studies involving those drugs. As the magnitude of the adjustment of A cannot be known beforehand the pseudo-rebreathing method is not very appropriate for the assessment of the ventilatory response to CO_2 in drug studies.

In our opinion the dynamic end-tidal forcing (DEF) technique (Swanson & Bellville, 1975), using a square-wave P_{ET,CO_2} challenge, is much better suited for drug studies. Beside giving the steady-state CO_2 sensitivity of the peripheral and central chemoreflexes is also provides valuable information on the dynamics of the receptors involved. A potential disadvantage of this method is that a mathematical model of the respiratory controller has to be used to extract the various physiological parameters from the overall ventilatory response. The validation of such a mathematical model for man is a difficult task and has not been accomplished satisfactorily as yet. Fortunately, the DEF technique gives the steady-state CO_2 sensitivity without interpretational difficulties and in less time than is needed for the classical steady-state method because it effectively opens the feedback loop of the respiratory control system.

In a previous study in anaesthetized cats (Berkenbosch *et al.* 1986) we showed that the ventilatory response obtained by applying a step-ramp P_{ET,CO_2} input could be satisfactorily described using the empirical two-compartment model introduced by Bellville, Whipp, Kaufman, Swanson, Aqleh & Wilberg (1979). From these results we calculated that in a pseudo-rebreathing experiment the steady-state CO_2 sensitivity could be obtained provided that the ratio of the step change A over the rate of rise R was equal to the central time constant. From the model of Read & Leigh (1967) a differential equation, similar to the differential equation for the central component in the empirical two-compartment model of Bellville *et al.* (1979), can be derived with a central 'time constant' proportional to the inverse of the cerebral blood flow (see

eqns (16) and (17)). For anaesthetized cats the assumption that the central time constant is insensitive to changes in cerebral blood flow induced by changes in tissue P_{CO_2} led to close agreement with experiment. The central time constant deduced from the pseudo-rebreathing experiments was close to that obtained from experiments using step changes in P_{ET,CO_2}. It may be that the insensitivity of the central time constant to changes in cerebral blood flow in cats is due to neuronal dynamics (Teppema, Vis, Evers & Folgering, 1982; Eldridge, Kiley & Paydarfar, 1987) overwhelming the effect of changes in cerebral blood flow on the central time constant. Moreover, the anaesthetics used are expected to depress the responsiveness of cerebral vessels to hypercapnia (Levasseur & Kontos, 1989).

Our results in awake humans seem to indicate that the central time constant is sensitive to changes in cerebral blood flow. This would lead to a greater slope in the relation S_n/S_s *vs.* A than in anaesthetized cats. The limiting time constant (see eqn (15)) has a value of about 47 s using our parameter values. It is tempting to conclude that this value is in fair agreement with a value of A/R of about 34 s, which we experimentally found when S_n and S_s are about equal, although one cannot be sure that the value of R is matched to A in these experiments. On the other hand results obtained in a few human subjects using square-wave CO_2 challenges during hyperoxia suggest that the central time constant lies in the range of 70–175 s (Gelfand & Lambertsen, 1973; Gardner, 1980). This raises some questions about the validity of the model of Read & Leigh (1967) in man, since the (effective) central time constant (see eqn (17)) cannot be larger than the limiting time constant.

To establish the role of the cerebral blood flow and possible neuronal dynamics with regards to the central time constant further investigations are required.

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