An assessment of central–peripheral ventilatory chemoreflex interaction using acid and bicarbonate infusions in humans

I. D. Clement, J. J. Pandit, D. A. Bascom, K. L. Dorrington, D. F. O'Connor and P. A. Robbins*

University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, UK

1. The object of this study was to investigate the effect of central chemoreceptor stimulation on the ventilatory responses to peripheral chemoreceptor stimulation.

2. The level of central chemoreceptor stimulation was varied by performing experiments at two different levels of end-tidal CO_2 pressure (P_{CO_2}). Variations in peripheral chemoreceptor stimulus were achieved by varying arterial pH (at constant end-tidal P_{CO_2}) and by varying end-tidal O_2 pressure (P_{O_2}).

3. Two protocols were each performed on six human subjects. In one protocol ventilatory measurements were made during eucapnia, when the arterial pH was lowered from 7·4 to 7·3. The variation in pH was achieved by the progressive infusion of acid (0·1 M HCl). In the other protocol ventilatory measurements were made during hypercapnia, when the arterial pH was increased from 7·3 to 7·4. The variation in pH was achieved by the progressive infusion of 1·26% $NaHCO_3$. In each protocol ventilatory responses were measured during euoxia (end-tidal P_{O_2}, 100 Torr), hypoxia (end-tidal P_{O_2}, 50 Torr) and hyperoxia (end-tidal P_{O_2}, 300 Torr), with end-tidal P_{CO_2} held constant.

4. The increase in ventilatory sensitivity to arterial pH induced by hypoxia (50 Torr) was no~t significantly different between protocols (acid protocol, -104 ± 31 l min^{-1} (pH unit)$^{-1}$ *vs.* bicarbonate protocol, -60 ± 44 l min^{-1} (pH unit)$^{-1}$; mean ± S.E.M.; not significant (n.s.)). The ventilatory sensitivity to hypoxia at an arterial pH of 7·35 was not significantly different between protocols (acid protocol, $14{\cdot}7 \pm 3{\cdot}3$ l min^{-1} *vs.* bicarbonate protocol, $15{\cdot}6 \pm 2{\cdot}4$ l min^{-1}; mean ± S.E.M.; n.s.). The results provide no evidence to suggest that peripheral chemoreflex ventilatory responses are modulated by central chemoreceptor stimulation.

The question of whether the ventilatory responses to central and peripheral chemoreceptor stimulation may be treated as independent and additive or whether there is some degree of interaction between the two reflexes remains unsettled. Some workers find no evidence of interaction in humans (Dahan, DeGoede, Berkenbosch & Olievier, 1990; Clement *et al.* 1992) or in experimental animals (van Beek, Berkenbosch, DeGoede & Olievier, 1983). Other experimental work has suggested a degree of interaction between the central and peripheral chemoreflexes both in conscious humans (Robbins, 1988) and in anaesthetized cats (Majcherczyk & Willshaw, 1973).

In order to study central–peripheral chemoreflex interaction it is necessary to be able to manipulate the inputs to the central and peripheral chemoreceptors separately. It is possible to do this in human subjects by taking advantage of the different responses of the central and peripheral chemoreceptors to CO_2 and H^+ in the arterial blood. The central chemoreceptors respond primarily to changes in arterial CO_2 pressure (P_{a,CO_2}) and are relatively insensitive to acute, metabolically induced changes in arterial pH (pH_a) (Mitchell & Singer, 1965; Knill & Clement, 1985). The peripheral chemoreceptors respond to hypoxia and to changes in pH_a, but are thought to respond to changes in P_{a,CO_2} primarily indirectly through the effects of P_{a,CO_2} on pH_a (Hornbein & Roos, 1963; Donnelly, Smith & Dutton, 1982). Thus interaction in human volunteers may be studied by manipulating stimuli to the peripheral chemoreceptors (pH_a and hypoxia) and stimuli to the central chemoreceptors (P_{a,CO_2}) independently of each other.

The particular plan behind the present experiments was to compare the ventilatory sensitivity to pH_a during eucapnia (conditions of normal central chemoreceptor stimulation) with the ventilatory sensitivity to pH_a during hypercapnia (conditions of higher central chemoreceptor stimulation).

* To whom correspondence should be addressed.

Table 1. Experimental variables from acid infusion and bicarbonate infusion protocols

		Acid				Bicarbonate			
Subject no.	Wt (kg)	P_{CO_2} (Torr)	Initial pH	Final pH	Dose (mmol)	P_{CO_2} (Torr)	Initial pH	Final pH	Dose (mmol)
796	85	42	7·365	7·299	149	50	7·327	7·380	150
850	77	40	7·401	7·306	105	54	7·319	7·371	285
751	70	40	7·420	7·326	123	54	7·311	7·390	300
834	89	42	7·409	7·305	156	54	7·326	7·375	300
833	76	39	7·393	7·288	133	54	7·311	7·384	305
802	72	41	7·389	7·294	126	54	7·303	7·373	270

These ventilatory sensitivities were to be assessed in hypoxia, euoxia and hyperoxia. In the eucapnic condition, variation in pH_a was to be achieved by acid infusion, and in the hypercapnic condition the variation in pH_a was to be achieved by infusion of bicarbonate. The results should enable the ventilatory responses to stimuli acting mainly at the peripheral chemoreceptors (hypoxia and pH_a) to be compared at different levels of central chemoreceptor stimulation. If there is no central–peripheral chemoreflex interaction, then the ventilatory responses mediated by the peripheral chemoreflexes will be independent of the level of central chemoreflex stimulation. Alternatively, if there is significant central–peripheral chemoreflex interaction, then ventilatory responses mediated by the peripheral chemoreflex will vary with the level of central chemoreflex stimulation.

METHODS

Subjects

Experiments were performed on six healthy young men aged between 18 and 23 of height 1·81 ± 0·06 m (mean ± S.D.) and weight 78·2 ± 7·41 kg. Subjects gave informed consent but were unaware of the specific aims of the study. Ethical permission for this study was obtained from the Central Oxford Research Ethics Committee.

General methods

All subjects undertook two experimental protocols, an acid infusion protocol and a bicarbonate infusion protocol. In all experiments, ventilatory measurements were performed with the subject seated comfortably in a chair while breathing through a mouthpiece with the nose occluded. Ventilatory flows and volumes were recorded using a pneumotachograph and a turbine device (Howson, Khamnei, O'Connor & Robbins, 1986), respectively. Gas composition at the mouth was sampled continuously using a mass spectrometer and changes in end-tidal gas composition were imposed using a computer-controlled dynamic end-tidal forcing apparatus (Robbins, Swanson & Howson, 1982; Howson, Khamnei, McIntyre, O'Connor & Robbins, 1987). In all experiments blood samples were drawn from a venous catheter (14G Venflon, Helsingborg, Sweden) placed aseptically in a dorsal left-hand vein under local anaesthetic. During experiments the hand was kept warm with hot, wet towels in order to obtain arterialized venous blood samples.

Acid infusion protocol

The intention in the acid infusion protocol was to generate a change in pH_a of approximately 0·1 pH units at constant end-tidal P_{CO_2} (P_{ET,CO_2}). A predicted dose for HCl of 1·75 mmol kg^{-1} was calculated to achieve an isocapnic pH change of 0·1 pH units during eucapnia. The calculation was based on a required base deficit of 5 mM and an effective bicarbonate space of 0·3 × body wt (Martin & Matzki, 1982). The infusion solution contained 100 mM H^+, 100 mM Na^+ and 200 mM Cl^-, and was prepared commercially by a specialist pharmaceutical company (Manmed Ltd, Aylesbury, UK). Immediately following infusion into the blood the H^+ would be almost completely buffered, leaving an infusion solution close to isotonic, with a total osmolality of approximately 300 mequiv (100 mM Na^+ and 200 mM Cl^-). Infusion of HCl into peripheral arm veins in humans has been reported to be very painful (Knill & Clement, 1985). To avoid this difficulty acid was infused into a large blood flow using a central venous catheter. A drum catheter (13G; Abbott Ltd., Sligo, Ireland) was introduced aseptically under local anaesthesia into the right antecubital vein. The position of the catheter tip in the superior vena cava or upper right atrium was verified by chest X-ray. The total doses of acid administered to each subject are shown in Table 1 and were given in three or four separate infusion periods, each lasting approximately 15 min with an infusion rate of approximately 20 ml min^{-1}.

Respiratory measurements were made before the first infusion and then after each infusion. Each respiratory measurement protocol consisted of an initial 8 min euoxic period followed by 2 min measurement periods of euoxia (P_{ET,O_2} = 100 Torr), hypoxia (P_{ET,O_2} = 50 Torr) and hyperoxia (P_{ET,O_2} = 300 Torr). Isocapnia was maintained throughout each respiratory measurement period with P_{ET,CO_2} held at 1–2 Torr above resting levels (Table 1). The average ventilation from the second minute of each of these periods was used in the subsequent analysis. Blood samples were drawn to coincide with the middle of the second minute of each period. A breath-by-breath record of a typical respiratory measurement protocol is shown in Fig. 1. The total experimental duration was approximately 3 h.

Bicarbonate infusion protocol

Bicarbonate infusions were delivered via a peripheral venous catheter placed in the right arm aseptically under local anaesthesia. The infusion preparation was 1·26% $NaHCO_3$ (150 mM). The predicted bicarbonate doses were the same as the acid dose in the acid infusion protocol (1·75 mmol kg^{-1}) with the intention of generating a metabolic acid–base disturbance equivalent to a change in pH_a of 0·1 pH units. In practice these

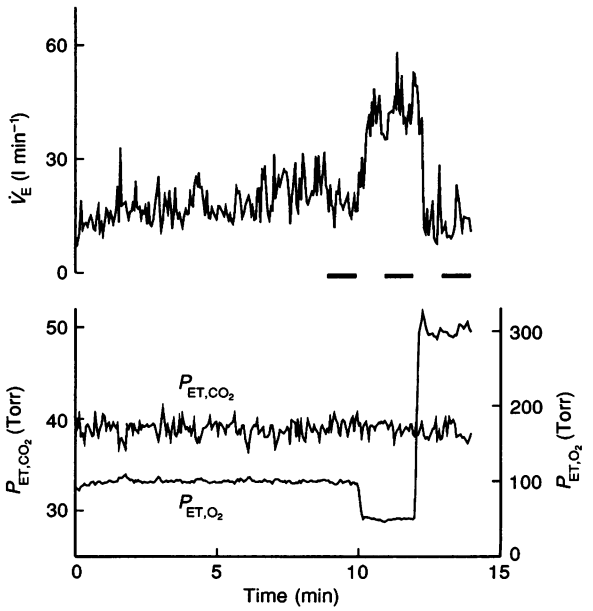

Figure 1. End-tidal stimuli and ventilatory responses from a typical respiratory measurement protocol

Breath-by-breath data from the acid infusion study for Subject 833. Upper panel shows ventilation. Lower panel shows end-tidal P_{CO_2} and P_{O_2}. The dynamic end-tidal forcing system adjusts the inspired gas composition so as to maintain the end-tidal gas values as close to the ideal values as possible. In this example, P_{ET,CO_2} was maintained close to 39 Torr throughout the study. The filled bars show the 1 min periods over which the data were averaged. Blood samples were drawn in the middle of these periods.

doses were too low, and the doses actually administered are shown in Table 1. Respiratory measurements were made at high P_{ET,CO_2} in order to induce respiratory acidosis. The protocol was designed to give respiratory measurements over a range of pH_a from 7·3 (uncorrected respiratory acidosis) to 7·4 (respiratory acidosis corrected by full bicarbonate dose). Respiratory measurements were made using the protocol described above, with P_{ET,CO_2} held at 54 Torr in five of the subjects. The sixth subject (subject 796) was unable to tolerate a P_{ET,CO_2} of 54 Torr comfortably, so a value of 50 Torr was used (Table 1).

Data analysis

Since the two protocols were performed on different days and repeated arterial catheterization was considered undesirable in healthy volunteers, pH_a could not be measured directly. Instead blood was collected from a dorsal hand vein with the hand kept warm to keep the blood composition as close as possible to arterial. An arterial pH value was then calculated from the values for pH, bicarbonate, haemoglobin oxygen saturation and haemoglobin content obtained from the arterialized venous blood samples. Values obtained for P_{ET,CO_2} were used as estimates of arterial P_{CO_2}. The calculations used to derive pH_a from these measured values are described in the Appendix. The magnitude of these calculated corrections was generally small.

RESULTS

Changes in pH_a achieved with protocols

The changes in pH_a achieved with the acid infusion protocol are shown in Table 1 and can be seen in Fig. 2. The desired change of approximately 0·1 pH units was achieved in all subjects. The calculated dose was administered in five subjects and three-quarters of the calculated dose in the sixth subject (Subject 850). In the bicarbonate infusion protocol, doses up to 2·5 times those calculated were given to achieve smaller changes in pH_a (Table 1 and Fig. 3). The bicarbonate infusions induced a mild diuresis in some

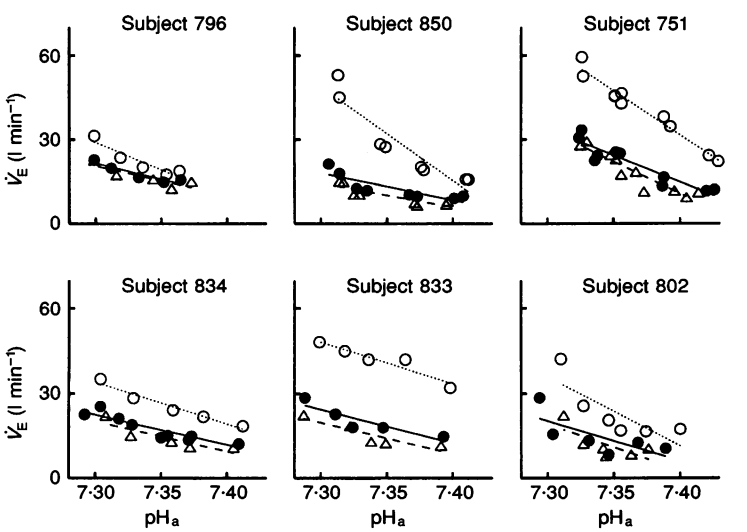

Figure 2. Acid infusion experiments

Ventilation–pH_a plots for each subject. △, hyperoxia (P_{ET,O_2} = 300 Torr); ●, euoxia (P_{ET,O_2} = 100 Torr); ○, hypoxia (P_{ET,O_2} = 50 Torr). Best fit straight lines are shown for each oxygen level: dashed line, hyperoxia; continuous line, euoxia; dotted line, hypoxia.

Table 2. Slopes of ventilation–pH_a relationships for acid infusion and bicarbonate infusion protocols (l min^{-1} (pH unit)$^{-1}$)

	Acid			Bicarbonate		
Subject no.	Hyperoxia	Euoxia	Hypoxia	Hyperoxia	Euoxia	Hypoxia
796	−107	−115	−203	59	58	−42
850	−85	−94	−336	92	109	217
751	−228	−191	−316	−59	−72	−92
834	−109	−107	−149	−19	19	−32
833	−112	−120	−146	76	16	−62
802	−165	−145	−249	−225	73	−146
Mean	−134	−129	−233	−13	34	−26
S.E.M.	22	14	33	49	26	52

subjects but sufficient time was available between respiratory measurement periods for the subject to empty his bladder.

Quality of end-tidal gas control during hypoxic steps

In comparing the responses to hypoxia between the protocols we assume that the size of the hypoxic stimulus was the same in all cases and that P_{ET,CO_2} was held constant at the euoxic level throughout the period of hypoxia. The mean hypoxic value for P_{ET,O_2} in the acid protocol was 49·9 ± 0·1 Torr (mean ± S.E.M.) and for the bicarbonate protocol it was 49·8 ± 0·1 Torr (mean ± S.E.M.), the intended P_{ET,O_2} being 50·0 Torr. The mean difference in P_{ET,CO_2} between euoxia and hypoxia was less than 0·1 Torr in both protocols. Thus the control of P_{ET,O_2} and P_{ET,CO_2} during the experiments was good.

General appearance of ventilatory data

The ventilatory results from the acid infusion experiments are shown for each subject in Fig. 2, and the results from the bicarbonate infusion experiments are shown in Fig. 3. The best fit straight lines shown on each plot were determined by linear regression. The results for the acid protocol (Fig. 2) demonstrate a reasonably linear relationship between the rate of expiratory ventilation ($\dot{V}_E$) and pH_a. In most subjects the steepness of this relationship shows an interaction with hypoxia, but the slopes are generally not zero even in hyperoxia. The same pattern of results is not consistently apparent in the bicarbonate infusion experiments (Fig. 3) and this will be covered further in the Discussion (see also Table 2). The general level of ventilation observed in the bicarbonate experiments is much higher than that in the acid experiments, consistent with the greater stimulation of the central chemoreceptors by CO_2.

Tests for the presence of interaction

In order to address the issue of central–peripheral chemoreflex interaction, ventilatory data from the two experimental protocols were compared. Three tests for central–peripheral chemoreflex interaction were employed: (1) a direct comparison of $\dot{V}_E$–pH_a slopes between protocols; (2) a comparison of the hypoxic sensitivity of $\dot{V}_E$–pH_a

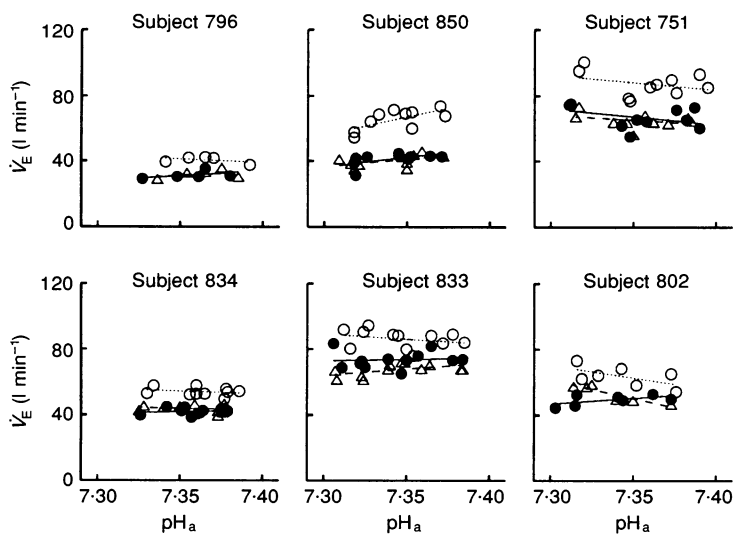

Figure 3. Bicarbonate infusion experiments

Ventilation–pH_a plots for each subject. Symbols and lines as for Fig. 1.

Table 3. Ventilatory hypoxic responses calculated as the difference between hypoxic and euoxic ventilation at a pH_a of 7·35 for each protocol

	Hypoxic responses (l min^{-1})		
Subject no.	Acid	Bicarbonate	Difference
796	3·4	10·3	6·9
850	19·0	24·9	5·9
751	23·1	21·2	−1·9
834	9·5	12·6	3·0
833	22·5	12·4	−10·1
802	10·5	12·2	1·7
Mean	14·7	15·6	0·9
S.E.M.	3·3	2·4	2·6

slopes between protocols; and (3) a comparison of ventilatory hypoxic sensitivities at fixed pH_a between protocols. The results of each of these comparisons will now be described in turn.

Comparison of $\dot{V}_E$–pH_a slopes between protocols

In the case of the null hypothesis, where the peripheral and central chemoreflexes are independent of each other, the slopes of the $\dot{V}_E$–pH_a data should not differ between the acid and bicarbonate protocols, provided the other experimental assumptions are valid. Linear regression slopes have been used to estimate the slopes of the $\dot{V}_E$–pH_a relationship in each of the different experimental conditions and these values are shown in Table 2. Comparison of the slopes between protocols using Student's paired t tests show that the acid and bicarbonate protocol slopes differ significantly by 122 ± 39 l min^{-1} (pH unit)$^{-1}$ during hyperoxia ($P < 0·05$), by 163 ± 17 l min^{-1} (pH unit)$^{-1}$ during euoxia ($P < 0·001$) and by 207 ± 72 l min^{-1} (pH unit)$^{-1}$ during hypoxia ($P < 0·05$) (mean ± S.E.M.). At each level of P_{ET,O_2} the slopes are less steep in the bicarbonate experiments than in the acid experiments. This result may appear to suggest the presence of some form of central–peripheral interaction. This is considered further in the Discussion.

Comparison of hypoxic sensitivity of $\dot{V}_E$–pH_a slopes between protocols

As an alternative to looking at the absolute values of the $\dot{V}_E$–pH_a slopes it is possible to look at the change in slope between hypoxia and either euoxia or hyperoxia and then to compare this between protocols. In the case of the null hypothesis of no interaction, the variation of the $\dot{V}_E$–pH_a slope between hypoxia, euoxia and hyperoxia should not differ between the two protocols. For the hypoxia–euoxia comparison the change in slope is -104 ± 31 l min^{-1} (pH unit)$^{-1}$ (mean ± S.E.M.) for the acid protocol and -60 ± 44 l min^{-1} (pH unit)$^{-1}$ for the bicarbonate protocol (difference n.s.; Student's paired t test). For the hypoxia–hyperoxia comparison the change in slope is -99 ± 32 l min^{-1} (pH unit)$^{-1}$ for the acid protocol and -14 ± 41 l min^{-1} (pH unit)$^{-1}$ for the bicarbonate protocol (difference n.s.; Student's paired t test). The results of this comparison thus provide no support for interaction between the central and peripheral chemoreflexes in this experiment.

Comparison of hypoxic sensitivity at fixed pH_a between protocols

As an alternative method for testing for the presence of interaction, the ventilatory effect of hypoxia at a fixed pH may be compared between protocols. In the absence of central–peripheral interaction the hypoxic response at any given pH_a will be independent of P_{CO_2} and therefore will not be different between the two protocols, provided that the assumption that the peripheral chemoreceptors do not respond directly to P_{a,CO_2} (i.e. independently of pH) is valid. The hypoxic response at the same pH_a in each experimental condition has been assessed using the $\dot{V}_E$–pH_a regression lines. The hypoxic response was calculated as the difference between the ventilation predicted by the hypoxic and euoxic regression lines for a pH_a of 7·35. This value was chosen since it falls approximately at the mid-point of the range of pH_a values seen for all experiments and subjects. The values from each protocol are shown in Table 3. The mean values for the hypoxic response from each protocol were very similar and not significantly different on a Student's paired t test. This result provides no support for central–peripheral interaction.

DISCUSSION

Interaction of central and peripheral chemoreflexes

The data in the current study allowed the question of interaction (non-independence) between the central and peripheral chemoreflexes to be studied in three ways. Firstly the $\dot{V}_E$–pH_a slopes were compared between the hypercapnic and the eucapnic protocols. Secondly, the increase in $\dot{V}_E$–pH_a slope induced by hypoxia was compared

between the two protocols. Thirdly, the hypoxic response measured at a pH_a of 7·35 was compared between protocols. The first of these comparisons may have suggested the presence of interaction, whilst the other two comparisons provided no evidence of interaction.

Considering the data used in the first comparison, it is apparent that the mean slopes derived from the bicarbonate infusion data (hypercapnia) were less steep than those derived from the acid infusion data (eucapnia) (Table 2). If it is assumed that acute changes in H^+ do not stimulate the central chemoreceptors at all, and that the central chemoreflex contribution to ventilation is constant within each of the protocols, then the $\dot{V}_E$–pH_a slopes should be the same in each protocol under the null hypothesis of no central–peripheral chemoreflex interaction. The results of this analysis therefore provide no evidence in favour of *positive* central–peripheral chemoreflex interaction and may be taken to suggest either that there is a *negative* central–peripheral chemoreflex interaction or that one or more of the assumptions used in interpreting the analysis is incorrect. Exposure to hypercapnia is often associated with a progressively rising baseline ventilation (Reynolds, Milhorn & Holloman, 1972; Khamnei & Robbins, 1990). In the bicarbonate infusion experiments this would tend to flatten the $\dot{V}_E$–pH_a slopes since measurements at more alkaline pH_a levels were made later in the experiment. This may explain the differences in slopes when compared with those from the acid infusion protocol, in which all measurements are made during eucapnia. In this respect the second method of comparison may be better than the first method, because any progressive effect of CO_2 will be controlled for in the second method of comparison but not the first.

The third way of addressing the question of central–peripheral chemoreflex interaction is to compare the hypoxic responses at matched pH_a values between protocols. At a pH_a of 7·35 the mean hypoxic responses were very similar between the two protocols (Table 3). This would suggest that *at a pH_a of 7·35* the peripheral chemoreflex hypoxic response is independent of central chemoreceptor activity, although this may not necessarily be so at other pH_a values. The pH_a value of 7·35 was chosen for the comparison since this was the mid-point of the pH_a range over which measurements were made. It is also possible to use the data to determine how well interaction has been excluded. The mean difference in hypoxic responses (at a pH_a of 7·35) between the protocols is $0·9 \pm 2·6$ l min^{-1}, which is equivalent to 5·9% of the average hypoxic response. With a S.E.M. of 2·6 l min^{-1}, any interactive component of the hypoxic response would need to be at least 33% of the average hypoxic response to reach statistical significance.

To summarize the findings of this study with regard to central–peripheral chemoreflex interaction, we find that: (1) a direct comparison of $\dot{V}_E$–pH_a slopes was not helpful because of other confounding factors; (2) a comparison of the degree to which hypoxia increased the $\dot{V}_E$–pH_a slope showed no significant difference between protocols although the results were variable; and (3) no evidence was found to indicate interaction from the hypoxic sensitivities at a pH_a of 7·35. These results provide no evidence to suggest that ventilatory responses mediated by the peripheral chemoreflex are modulated by the level of central chemoreceptor stimulation.

Basis of the experiments

Effect of arterial pH changes on the central chemoreflex

The experimental design of the current study rests on the concept of the central chemoreceptors being stimulated primarily by changes in arterial P_{CO_2} and being relatively insensitive to changes in arterial pH, at least acutely. Studies in anaesthetized, chemodenervated animals showed a partial permeability of the blood–brain barrier for H^+ when more severe variations in arterial pH were employed than was the case in the present study. Teppema, Barts, Folgering & Evers (1983) showed that about 30% of the change in arterial pH was measurable at the surface of the medulla. However Eldridge, Kiley & Millhorn (1985) showed that when these changes in medullary surface pH were induced by a metabolic acidosis the ventilatory effect was only 50% as great as that generated by an equal medullary acidosis induced by raising P_{CO_2}. It would therefore seem that, even in this experimental preparation, the ventilatory effects of changes in arterial pH mediated by the central chemoreceptors are small.

In humans, it is generally accepted that some breakdown in the impermeability of the blood–brain barrier to H^+ occurs when changes in the acid–base status are severe (such as in diabetic keto-acidosis) or are prolonged. However, for metabolic acid–base changes over the pH_a range and time scale employed in the current study, there is evidence to suggest that there is little or no central chemoreflex response to metabolic acidosis (Mitchell & Singer, 1965; Knill & Clement, 1985). Finally, the assumption in the current study that metabolic acidosis does not give rise to stimulation of the central ventilatory chemoreflex is required only for the first two methods of comparing the results between the protocols. In the third method, in which hypoxic responses are compared between the two protocols, the only requirement is that the level of central chemoreflex stimulation is different between the two protocols. This is clearly the case, as is evident by comparing the general level of ventilation seen in the two protocols.

Effect of P_{CO_2} changes on the peripheral chemoreflex

A further assumption of the current study is that the peripheral chemoreceptors respond primarily to changes in arterial pH and not directly to changes in P_{a,CO_2} (Hornbein

& Roos, 1963). Donnelly *et al.* (1982) observed in anaesthetized cats that the peripheral chemoreceptor response to a metabolic acidosis became equal to that of an equivalent respiratory acidosis in 20 min or less in most cats. These results suggest that the peripheral chemoreceptors respond uniquely to H^+ provided sufficient time is allowed for equilibration of H^+ between the arterial blood and the transducer site. In the current study, the interval between the infusion and the subsequent respiratory measurements should be sufficient to allow this equilibration to occur, provided that the dynamics of this process in humans are similar to those seen by Donnelly *et al.* (1982).

Effect of acid infusion

Orr, Shams, Fedde & Scheid (1987) reported that infusion of HCl in anaesthetized cats caused pulmonary vasoconstriction and respiratory changes which might affect the interpretation of our experiments. Shams, Peskar & Scheid (1988) showed this effect to result from a platelet-mediated thromboxane cascade. In their study, Orr *et al.* infused 0·2 mmol min^{-1} H^+ into an extracorporeal circuit with a blood flow of 20 ml min^{-1}. In the current study, H^+ was infused at approximately 2 mmol min^{-1} (see Methods) into the superior vena cava. Assuming a blood flow of 1–2 l min^{-1} (one-quarter to one-third cardiac output), the acid concentration in the blood just downstream of the infusion catheter would be 5–10 times less in our experiments. In addition Orr *et al.* reported that the effect was evident only on the first infusion and that the respiratory effects were largely on respiratory pattern with little effect on the overall ventilation.

Estimation of pH_a

Repeated arterial catheterization may be considered undesirable in healthy volunteers and arterial catheters can be a source of discomfort to the subject. Consequently it was decided to determine pH_a indirectly. Blood samples were drawn from a venous catheter placed in a dorsal hand vein and two procedures used in combination to estimate pH_a. Firstly, hot wet towels were used on the hand to obtain arterialized venous blood samples. Knill & Clement (1985) used this technique together with arterial lines in some subjects and showed that arterialized venous blood P_{CO_2} overestimated arterial P_{CO_2} by about 1 Torr. In the current study, a difference in P_{CO_2} of $1·9 \pm 0·4$ Torr (mean ± S.E.M.) was detected between the venous blood sample and the end-tidal measurement. Secondly, an estimate of the true pH_a was calculated from the measured arterialized venous pH, P_{CO_2}, oxygen saturation and haemoglobin content using P_{ET,CO_2} as an estimate of arterial P_{CO_2} in the way described in the Appendix. Used on their own, arterialized venous blood measurements may not provide an entirely accurate estimate of true pH_a. The calculated corrections, although generally small, should improve the accuracy of the estimation of pH_a.

Use of linear regression

A linear regression was used to characterize the relationship between pH and $\dot{V}_E$ in the current study. A linear relationship has been described by Gray (1950) between $[H^+]$ and $\dot{V}_E$, and over the pH range of 7·3–7·4, pH is approximately linearly related to $[H^+]$. This finding was generally supported by our observations, although the response to acid in subjects 820 and 850 did appear somewhat curvilinear. This curvilinearity appeared to be present in both euoxic and hypoxic conditions in such a way as to limit its effect on our assessments of interaction, and it did not appear to be sufficiently extreme to justify fitting a more complicated response curve to the data.

Comparison with previous studies

The authors are unaware of any other study to have used acid and bicarbonate infusions in human subjects to address the question of central–peripheral chemoreflex interaction. Infusion of alkali during respiratory acidosis and infusion of acid during eucapnia have previously been reported separately, on different groups of human subjects. Lambertsen, Semple, Smyth & Gelfand (1961) infused bicarbonate during hypercapnia. Knill & Clement (1985) infused L-arginine hydrochloride to induce acidosis during eucapnia over a similar range of pH_a to that of Lambertsen *et al.* Comparison of the mean ventilatory sensitivities to pH_a during euoxia obtained from the data reported in each study suggests a greater ventilatory sensitivity to pH_a during hypercapnia. This is the reverse of the trend observed in the current study. This discrepancy may be related to differences in subjects between those two earlier studies, whereas in the current study the comparison has been made on the same set of subjects.

Clement *et al.* (1992) studied the hypoxic responses at matched pH_a values similar to the value used in the current study. The responses were compared between a CO_2 inhalation protocol and a hypocapnic post-exercise protocol. No significant differences were observed, suggesting the absence of central–peripheral interaction. A potential criticism of that study relates to the difficulty in comparing ventilatory results between resting and post-exercise protocols. Factors other than the metabolic acidosis may have influenced the results in the post-exercise protocol. However the findings of that study are consistent with those of the current study, which did not involve exercise.

Other studies have approached the issue of central–peripheral interaction in different ways. In humans, it is possible to dissociate the responses mediated by the central and peripheral chemoreflexes on the basis of the different speeds of response of the two reflexes. Bellville, Whipp, Kaufman, Swanson, Aqleh & Wiberg (1979) fitted a two-compartment model to the ventilatory responses to steps in P_{ET,CO_2} and in six out of seven subjects the slow component (central chemoreflex) gain was greater in hypoxia than

euoxia, suggesting the possibility of a positive interaction. However, Dahan *et al.* (1990), using similar methods, observed no such effect.

Robbins (1988) used a step reduction in P_{ET,CO_2} to generate a period of time when the central P_{CO_2} was still relatively high but the pH_a was near normal. In two out of three human subjects the hypoxic response was significantly greater during this period compared with control, suggesting the presence of interaction. One possible explanation for the apparent difference in the results of the current study from those of Robbins (1988) may concern the dynamics of the buffering of CO_2 in the blood (Michel, Lloyd & Cunningham, 1966). Following a sudden change in arterial P_{CO_2}, the arterial pH is relatively well buffered. However, some equilibration of bicarbonate between the intravascular and the extravascular fluid compartments then takes place, giving the blood (but not the whole animal) a relatively lower buffering capacity. Following a step reduction in arterial P_{CO_2}, this has the effect of causing a transient relative acidosis of the arterial blood until the equilibration of bicarbonate is complete. Hypoxic sensitivity during this period may therefore appear elevated compared with a control hypoxic sensitivity determined against a background of constant P_{CO_2}.

In animals, a more direct approach is possible. van Beek *et al.* (1983) perfused central and peripheral chemoreceptors independently in anaesthetized cats. The ventilatory responses mediated by the two sets of chemoreceptors were independent and additive.

In conclusion, evidence relating to the issue of central–peripheral chemoreflex interaction is contradictory and results may be dependent upon the experimental approach. The current study addresses this issue in humans by manipulating P_{CO_2} and pH independently without the added complication of exercise. The results provide no support for central–peripheral chemoreflex interaction. However studies concerning this issue in humans are necessarily difficult to design and execute, and some caution is required in interpreting the results given the absence of stimuli which are entirely specific for each set of chemoreceptors.

APPENDIX

This Appendix describes the calculation of pH_a from the measured values of venous pH, bicarbonate, haemoglobin oxygen saturation, haemoglobin concentration and P_{ET,CO_2}. The calculation is based upon the observation that there is a linear relationship between pH and bicarbonate concentration (the Davenport diagram) in the blood in response to changes in P_{CO_2} (Michel *et al.* 1966):

$$pH = A + B[HCO_3^-]. \quad (A1)$$

The term 'buffer line' is used to describe this relationship. The strategy is first to quantify the equation for the venous blood buffer line, which passes through the experimentally measured pH–$[HCO_3^-]$ point. From this an arterial blood buffer line can be derived (the values of A and B are dependent upon changes in oxygen saturation). The equation for this line together with the Henderson–Hasselbalch equation can then be used to calculate pH_a from arterial P_{CO_2}, which is assumed to be the same as the measured value for P_{ET,CO_2}.

Buffer line for venous blood. Equation (A1) may be differentiated to yield:

$$B = \frac{d(pH)}{d([HCO_3^-])}. \quad (A2)$$

Lloyd & Michel (1966) give an equation for the *in vitro* buffer line as follows:

$$\left(\frac{d(pH)}{d(HCO_3^-)}\right)_{\text{in vitro}} = -0{\cdot}005 - (0{\cdot}273 - 0{\cdot}01D)/[Hb], \quad (A3)$$

where D is the fractional haemoglobin desaturation and [Hb] is the concentration of haemoglobin in the blood. The intercept of the venous blood buffer line A_v can be calculated from eqn (A1) using the experimentally measured values for venous pH and bicarbonate concentration, $[HCO_3^-]$:

$$A_v = pH - B_v[HCO_3^-]. \quad (A4)$$

Calculation of arterial blood buffer line. Lloyd & Michel (1966) describe A for the *in vitro* buffer line as being linearly dependent upon D as follows:

$$\frac{dA}{dD} = 0{\cdot}034 + 0{\cdot}004[Hb]. \quad (A5)$$

After integration,

$$A_a = A_v + (0{\cdot}034 + 0{\cdot}004[Hb])\Delta D, \quad (A6)$$

where

$$\Delta D = D_a - D_v, \quad (A7)$$

the difference between arterial and venous blood desaturation. Arterial blood desaturation was measured by pulse oximetry. The slope of the arterial blood buffer line, B_a, was calculated from eqn (A3),

$$B_a = -0{\cdot}005 - (0{\cdot}273 - 0{\cdot}01D_a)/[Hb]. \quad (A8)$$

Calculation of arterial pH. We now have an equation for the arterial blood buffer line:

$$pH = A_a + B_a[HCO_3^-]. \quad (A9)$$

The Henderson–Hasselbalch equation relates pH, P_{CO_2} and $[HCO_3^-]$ as follows:

$$pH = pK_1 + \log\left(\frac{[HCO_3^-]}{0{\cdot}03\,P_{CO_2}}\right), \quad (A10)$$

where the dissociation coefficient, $pK_1 = 6{\cdot}1$. The $[HCO_3^-]$

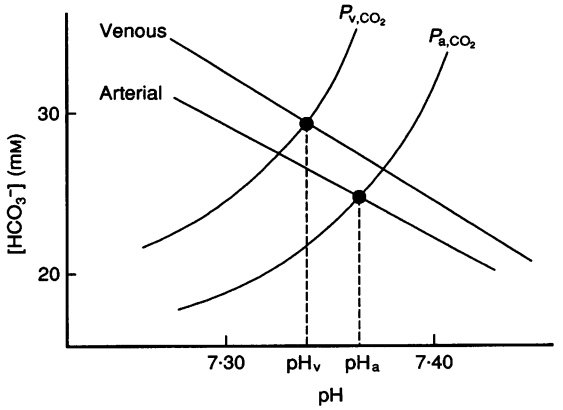

Figure 4. Schematic Davenport diagram illustrating the calculation of arterial pH

The diagram shows the venous blood buffer line (Venous) and the arterial blood buffer line (Arterial). The slopes and intercepts of these lines are calculated as described in the text. P_{v,CO_2} (venous P_{CO_2}) is measured directly. P_{a,CO_2} (arterial P_{CO_2}) is assumed to be equal to P_{ET,CO_2}.

term can be eliminated from eqns (A9) and (A10) to give an equation only in terms of pH (unknown) and arterial P_{CO_2} (assumed to be the same as P_{ET,CO_2}) as follows:

$$pH = pK_1 + \log\left(\frac{pH - A}{B\,0{\cdot}03\,P_{CO_2}}\right). \quad (A11)$$

This equation was solved for pH iteratively by Newton–Raphson approximation using a FORTRAN computer program.

The slope of the *in vivo* buffer line differs from the slope of the *in vitro* line since the *in vivo* blood buffering capacity is diluted to some extent by the extracellular fluid (Michel *et al.* 1966). However, in circulating from the venous to the arterial side of the systemic circulation the plasma will not be in exchange with the bulk of the extracellular fluid so the *in vitro* equations of Lloyd & Michel are the most appropriate for the correction described above. The process of calculating pH_a is shown schematically in Fig. 4.

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