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DYNAMICS OF MEDULLARY HYDROGEN ION AND RESPIRATORY RESPONSES TO SQUARE-WAVE CHANGE OF ARTERIAL CARBON DIOXIDE IN CATS

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SUMMARY

1. The dynamics of changes of medullary extracellular fluid (ECF) hydrogen ion concentration ($[H^+]$) and respiration, measured as integrated phrenic nerve activity, were determined in anaesthetized, paralysed, vagotomized and glomectomized cats. ECF $[H^+]$ was measured directly by means of a small (2 mm diameter) glass pH electrode placed on the ventral surface of the medulla. The variables were measured continuously after a step change of arterial P_{CO_2} produced by abruptly starting or stopping an infusion of hypercapnic fluid into the aortic arch.

2. Alteration of pH in the descending thoracic aorta at the onset or offset of infusion was complete within 1·5 s after the change began, indicating that it was nearly square wave in form.

3. In sixteen experiments, ECF $[H^+]$ began to fall within 2 s of offset of infusion, reflecting aortic–medullary circulation time. Thereafter, ECF $[H^+]$ decreased to a stable level over the next 5 min; the curve describing the decrease consisted of two exponential functions, one with a time constant (τ) of $9{\cdot}5 \pm 0{\cdot}6$ s and a second with a τ of 53 ± 3 s.

4. We interpret the findings at the offset of CO_2 infusion in terms of CO_2 wash-out from the medullary ECF. The slow function is associated with wash-out during stable medullary blood flow that develops after 1 min. The early fast function is associated with the decreasing medullary blood flow that occurs during the first minute after change from arterial hypercapnia to normocapnia.

5. We have estimated medullary blood flow using a mathematical model incorporating the two functions. The values obtained are consistent with those in the literature where other methods have been used. Changes of blood flow following the step change of CO_2 are fairly rapid, half of the response occurring in 13 s.

6. The change of respiratory activity lags the change of stimulus expressed by $[H^+]$, throughout the recovery period and respiration requires up to 8 min to reach a stable level. We attribute this slow response to slow central neural respiratory dynamics, the respiratory after-discharge.

INTRODUCTION

The dynamics of change of ventilation after changes of inhaled CO_2 have been used to make inferences about the changes of the chemoreceptor stimuli (P_{CO_2} and $[H^+]$) in medullary tissue (Gelfand & Lambertson, 1973; Severinghaus & Crawford, 1978) and of the perfusion of chemoreceptor tissue (Severinghaus & Crawford, 1978; Adams, Glasheen & Severns, 1984). However, ventilatory dynamics have been shown to be influenced by slowly changing central neural mechanisms that are independent of the specific input to the respiratory controller (Eldridge & Gill-Kumar, 1980). Thus, changes of ventilation may not directly reflect changes of stimuli at the chemoreceptor level.

The recent technique of making direct measurements of ventral medullary extracellular fluid (ECF) pH by means of a small flat-tipped H^+-sensitive electrode has allowed better evaluation of the time courses of changes of ECF pH or hydrogen ion concentration ($[H^+]$) after changes of P_{CO_2} at the mouth (Ahmad & Loeschcke, 1982*a*; Teppema, Vis, Evers, & Folgering, 1982; Eldridge, Kiley & Millhorn, 1984). These studies have shown that, after such a change of CO_2, ECF pH requires up to 5 min to reach a new steady state (Eldridge *et al.* 1984) with time constants of the order of 30–50 s (Ahmad & Loeschcke, 1982*a*; Teppema *et al.* 1982). Teppema *et al.* (1982) proposed from their findings that the dynamic e.c.f. pH changes were perfusion limited.

Because the changes of arterial P_{CO_2} in these experiments were not truly square wave in form, we felt that some information in the early part of the dynamic process may have been missed and that it would be desirable to determine $[H^+]$ responses to nearly square-wave changes of arterial P_{CO_2}. In the present experiments we measured the changes of ECF $[H^+]$ and respiratory activity following the sudden cessation of infusion of hypercapnic fluid into the aortic arch.

METHODS

Studies were performed in nine cats weighing between 2·6 and 3·8 kg. They were anaesthetized first with ether and then given chloralose (30 mg/kg) and urethane (180 mg/kg) via a catheter placed in the femoral vein. Femoral arterial pressure was measured by means of a catheter and strain gauge. Body temperature was monitored with a rectal thermistor and servocontrolled at 37·5 °C by means of an electronic circuit and d.c. heating pad. The trachea was cannulated through a neck incision and continuous sampling of airway CO_2 was accomplished by means of a catheter placed in the airway; analysis was made by an infra-red CO_2 analyser (Beckman LB-2).

Following these preparations, the animal was placed supine on a table with rigid head mounting. Both vagosympathetic trunks were cut in the neck and the carotid sinus nerves were exposed and cut under direct vision. One phrenic nerve root (C_5) was also exposed in the neck, cut, desheathed and placed on a bipolar recording electrode. The nerve and electrode were immersed in a pool of mineral oil. The platinum wires of the electrode were built into a small piece of acrylic plastic, which was placed in the tissue well adjacent to the nerve. Because the electrode had no external attachment except the electrical connexions, which were flexible, it was possible to leave the nerve and electrode untouched even during a long experiment and to maintain a relatively constant electrical coupling between nerve and electrode.

Another catheter (size 5F) was then inserted into an artery (femoral in four cats, carotid in four and subclavian in one) and its tip advanced to the aortic arch. The appropriateness of its location for these experiments was confirmed by the development of medullary ECF pH changes and a respiratory response to small injections of Ringer solution equilibrated with CO_2 (see below). All approaches yielded similar results.

All animals were ventilated with 100 % O_2 using a volume-cycled ventilator and were paralysed with gallamine triethiodide, 3 mg/kg I.V. initially, followed by a continuous infusion at the rate of 3 mg/(kg h) to maintain paralysis. In order to prevent significant changes in end-tidal and arterial P_{CO_2} secondary to changes of cardiac output and venous CO_2 return to the lungs, the d.c. voltage on the ventilator's motor could be controlled by the animal's end-tidal P_{CO_2} (P_{ET,CO_2}) through an electronic circuit. The ventilator's rate was thereby servocontrolled to maintain P_{ET,CO_2} within a narrow range around the desired level (Smith, Mercer & Eldridge, 1978). Because the profile of tidal ventilation produced by the ventilator was constant and P_{ET,CO_2} changes were minimal due to the servocontroller, changes in rate of the ventilator showed changes of CO_2 cleared by the lungs (Smith *et al.* 1978).

Measurement of pH. In order to make measurements of ventral medullary ECF pH (eight cats), we retracted the larynx and oesophagus rostrally, removed the muscle covering the basal portion of the occipital bone and chipped away the bone between the tympanic bullae. Bleeding was controlled with Gelfoam. The dura overlying the medulla was opened in the mid line by microcautery, retracted laterally and tacked to the bone by means of cautery. A 1 cm deep Teflon ring was placed over the exposed medulla and spaces around it filled with agar, resulting in a well that subsequently filled with clear cerebrospinal fluid (CSF) to a depth of approximately 1 cm.

The pH was measured by means of a flat-surfaced, pH-sensitive glass electrode with a tip diameter of 2 mm (Microelectrodes, Inc., Londonderry, NH, U.S.A., Model MI 404, time constant of response approx. 1·5 s) and a reference electrode (Microelectrodes, Model MI 401). Both electrodes were mounted in a balanced holder which moved freely in the vertical direction. They were lowered gently onto the surface of the medulla so that the tips were located lateral to the mid line near the upper rootlets of the twelfth cranial nerve in areas that were free of visible blood vessels.

A custom-built pH meter with isolated input was used to amplify the signal. The output of the meter was processed by means of an integrating digital voltmeter (Hewlett–Packard Model 5326B) and expressed as average values for each second. Calibration of the pH electrode was carried out *in vitro* with standard phosphate buffers (pH 7·0 and 7·382).

In order to show that the pH electrode was located and functioning properly, the system was tested in all cats by occluding the airway for 10–15 s. The appropriate response to the rise of P_{ET,CO_2} was an acidic shift of ECF pH that began within 4–7 s, or approximately the lung-to-medulla circulation time, and then an alkaline response occurring with the same time lag after the first effective post-occlusion breath (Eldridge *et al.* 1984). All animals showed this response.

In one additional animal, a catheter-type pH electrode (Microelectrodes, Inc., Model MI 508, time constant approximately 1·5 s) was located in the descending thoracic aorta. Calibration and processing of the signal were the same as noted above.

Experimental protocol

After preparations and preliminary experiments had been completed, the P_{ET,CO_2} was raised to approximately 3 torr above threshold to produce rhythmic breathing. After all variables had become stable, the values were recorded for 1 min. At this time, a continuous infusion into the aortic arch of warmed (37 °C) Ringer solution equilibrated with 100 % CO_2 was made by means of a roller pump (Holter, RD045). Rates of infusion varied from experiment to experiment (2–8 ml/min). The infusion caused arterial and medullary ECF pH to become acidic and respiration to increase. The infusion was continued until pH and respiratory variables had become relatively stable (usually 4–5 min). The values were recorded. The infusion pump was then suddenly turned off. Subsequent values of ECF pH and other variables were followed continuously during the recovery until they had become stable (at least 5 min) and again at 8 min. In the one cat with measurement of arterial pH, the changes were followed for only 1 min.

Data analysis

Data obtained included arterial pressure, airway P_{CO_2}, frequency of the servocontrolled ventilator, phrenic nerve impulses and measurements of pH, all of which were recorded on magnetic tape and on hard copies of the tracings on a storage oscilloscope. All data were processed by means of a computer. Phrenic activity was half-wave rectified and integrated for each 0·1 s period by means of an integrating digital voltmeter as previously described (Eldridge, 1975). It has been shown that this value is the neural equivalent of tidal volume of breathing (Eldridge, 1971). Breath-by-breath neural minute activity was calculated as the product of this value and respiratory frequency.

P_{ET,CO_2} and frequency of the ventilator were measured for each breath and were averaged for each 5 s period. Arterial pressure was averaged in 5 s periods. pH values were recorded each second during the experiment and were expressed as both absolute pH and as hydrogen ion concentration ($[H^+]$) after processing by the computer.

In addition to the direct measurements, we estimated the medullary ECF P_{CO_2} during control and at the end of recovery to be 3 torr higher than end-tidal/arterial P_{CO_2}, i.e. P_{ET,CO_2} + (0·5 times estimated arterial–venous P_{CO_2} difference of 6 torr) (Ponten & Siesjo, 1966). Since it has been shown that the relation between P_{CO_2} and medullary ECF $[H^+]$ is linear and has a slope of approximately 0·7 torr/(nmol l) (Eldridge, Kiley & Millhorn, 1985), we calculated the ECF P_{CO_2} during the CO_2 infusion from the base-line ECF P_{CO_2} + 0·7 times the change of ECF $[H^+]$.

Analysis of medullary e.c.f. $[H^+]$ changes

When the changes of ECF $[H^+]$ were plotted semilogarithmically against time, it became apparent that there were two components, one that was essentially complete by 1 min and a second (slow) linear function. For each experiment the data of the slow function between 1 and 2·5 min were analysed by linear regression of the log values of $[H^+]$ against time using the method of least squares approximation. An iterative computer program was employed to perform a systematic search for the asymptote that minimized the residual sum of squares of y, i.e. deviations of $[H^+]$ from the exponential curve. This 'best' asymptote was used in the final analysis of the slow function from which a time constant (τ_s) was calculated.

To evaluate the fast function, we extrapolated the slow function to zero time and subtracted the values of the slow function from the observed $[H^+]$ values obtained over the first 90 s. The log values of these differences were analysed by linear regression and the time constant (τ_f) calculated.

Other statistical methods included analysis of group mean differences with the unpaired t test and of mean changes from infusion base line with the paired t test. Differences were considered significant if the P value was less than 0·05. It should be pointed out that when more than one experiment was performed in the same cat, the physiological state often varied considerably from one run to the next, i.e. initial conditions were different due to the prior infusion, there were markedly different rates of infusion of CO_2–Ringer solution and therefore different maximal ECF $[H^+]$ changes (e.g. 19·9 *vs.* 81 nmol/l) and calculated medullary blood flow rates. In the presentation of mean data, we therefore treated each of the sixteen experiments as independent ($n = 16$) rather than averaging the findings from a single cat and using an n of 8.

RESULTS

The changes of arterial pH measured in the descending aorta after the sudden onset or offset of infusion of Ringer solution equilibrated with 100 % CO_2 for one cat are shown in Fig. 1. With both there are response delays of approximately 1 s representing circulation times from catheter tip to pH electrode. Thereafter, both the on- and off-transients are virtually complete in another 1·5 s. Since the time constant of the pH electrode is about 1·5 s, we interpret these results to show that there is almost a square-wave change of P_{CO_2} and acidity in the arterial blood following either the onset or offset of infusion.

Actual recordings in one cat of the changes of medullary ECF $[H^+]$ and phrenic activity at onset (Fig. 2*A*) and offset (Fig. 2*B*) of CO_2–Ringer solution infusion are shown in Fig. 2. The delays from change of infusion to onset or offset of the ECF $[H^+]$ response were about 2 s in this cat. The mean offset delay in all cats (sixteen experiments) was $1·75 \pm 0·2$ s (S.E. of mean), and presumably represented the circulation time from catheter to brain. In the analyses that follow, zero time was considered to be the time at which the change of ECF $[H^+]$ began, i.e. time of offset of CO_2 infusion plus the circulatory delay.

It proved to be impossible to maintain a perfectly stable infusion rate with the roller pump used in these experiments, so ECF $[H^+]$ showed variations during the

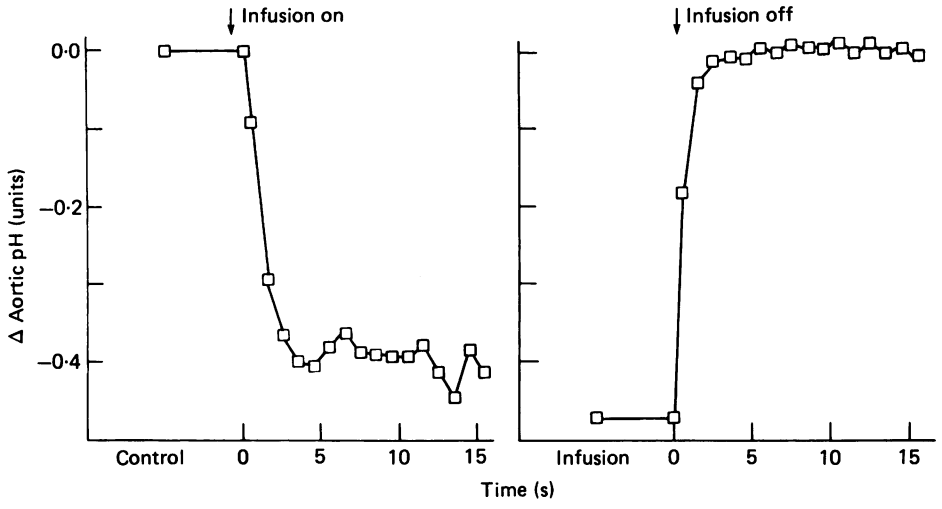

Fig 1. Examples in one cat of rapid change of arterial pH in descending aorta after onset (left) or offset (right) (shown by arrows) of continuous infusion of hypercapnic Ringer solution into aortic arch. Time zero is that at which actual change of pH began.

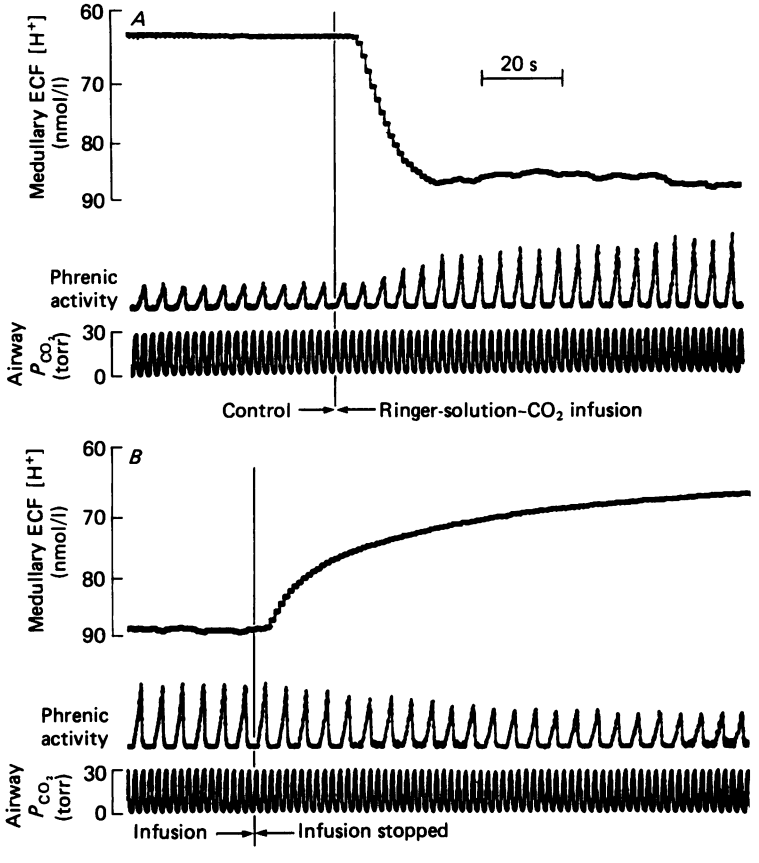

Fig. 2. Example in one cat of changes of medullary ECF [H^+] and phrenic activity after onset (*A*) and offset (*B*) of continuous infusion of hypercapnic Ringer solution into aortic arch. In *A* the infusion rate was changed 22 s after the start, so a full curve was not obtained.

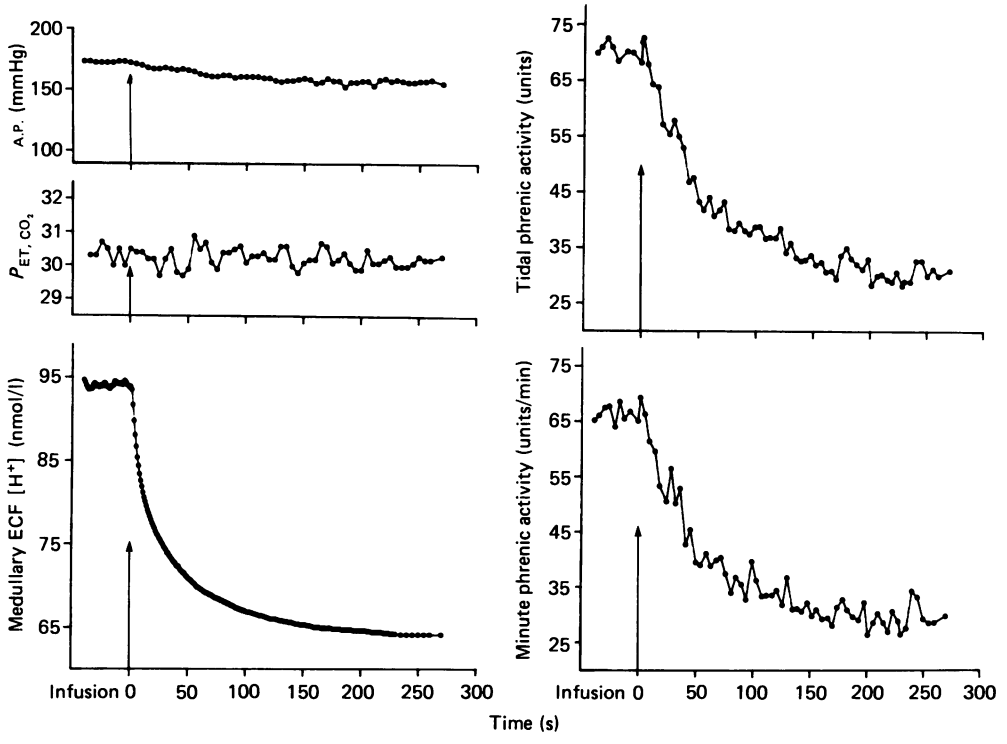

Fig. 3. Example in one cat of changing variables followed for 4·5 min after cessation of infusion of hypercapnic Ringer solution into aortic arch. Time zero in this and subsequent graphs is that at which the change of ECF [H^+] begins. A.P., arterial pressure.

TABLE 1

	During Infusion	End of Recovery	Change
P_{ET,CO_2} (torr)	29·4 ± 1·0	29·4 ± 1·0	0·0
ECF P_{CO_2} (torr)	57·7 ± 3·9†	32·4 ± 1·0*	−25·3 ± 3·5
ECF [H^+] (nmol/l)	99·4 ± 5·6	63·4 ± 1·8	−36·0 ± 5·1
Arterial pressure (mmHg)	129·4 ± 8·4	123·6 ± 8·1	−5·8 ± 2·5
Ventilator rate (per min)	33·9 ± 1·8	32·1 ± 0·8	−1·8 ± 0·7

Values are means ± S.E. of mean.

* Calculated from P_{ET,CO_2} + (0·5 × estimated arterial–venous P_{CO_2} difference [6 torr]) (Ponten & Siesjo, 1966).

† Calculated from the relation, recovery ECF P_{CO_2} + (Δ ECF [H^+] × 0·7) (Eldridge, Kiley & Millhorn, 1985).

infusion (see Fig. 2A) that precluded precise analysis of the time course of changes after onset of CO_2 infusion. On the other hand, the square-wave offset led, after the circulatory delay, to a smooth curve of decreasing [H^+] (see Figs. 2*B* and 3), provided P_{ET,CO_2} remained stable throughout the recovery period. This was the case in sixteen experiments in the eight cats. Three additional experiments were rejected for analysis because of unacceptable oscillations of both P_{ET,CO_2} and ECF [H^+] during the recovery period.

Fig. 3 is a graph of the changes of ECF [H^+], phrenic activities, P_{ET,CO_2} and arterial

pressure followed in another experiment for 270 s after offset of the CO_2 infusion. The smoothly declining curve of ECF $[H^+]$ reaches a stable level only after 4·5 min. The decreasing ECF $[H^+]$ is associated, with some lag (see below), with decreasing phrenic activities. The servocontrolled ventilator maintained end-tidal, and presumably

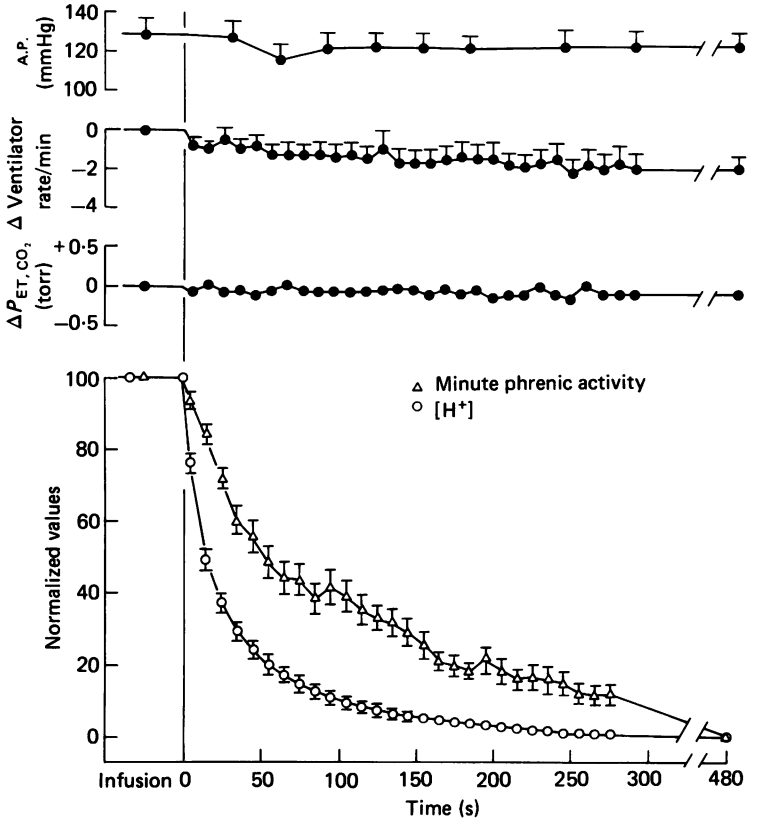

Fig. 4. Mean data from sixteen experiments of changes of medullary ECF $[H^+]$, minute phrenic activity, P_{ET,CO_2}, ventilator rate and arterial pressure (A.P.) following cessation of infusion of hypercapnic Ringer solution into aortic arch. Note that phrenic activity decreases more slowly than ECF $[H^+]$.

arterial, P_{CO_2} relatively stable (±0·5 torr) after the step change, which indicates that variations of arterial P_{CO_2} throughout the recovery period, did not contribute significantly to the changes of ECF $[H^+]$ in this cat.

Mean values in the sixteen experiments of measured variables, P_{ET,CO_2}, ECF $[H^+]$, arterial pressure and ventilator rate, and the calculated variable, ECF P_{CO_2}, during CO_2 infusion and at the end of recovery (5 min after stopping the infusion) are shown in Table 1. The time courses of arterial pressure, the changes of ECF $[H^+]$ and minute phrenic activity, both scaled from 100 to 0, P_{ET,CO_2} and ventilator rate following the offset of infusion are shown in Fig. 4. The decrease of the servocontrolled ventilator rate is significant and reflects a 6 % decrease of presentation of CO_2 to the lungs following offset of the infusion, but stability of P_{ET,CO_2} due to the servocontroller is apparent.

Medullary ECF [H^+] changes

Fig. 5 gives in four cats semilogarithmic plots of the changes of medullary ECF [H^+] after cessation of CO_2 infusion. They clearly show the two components of the recovery process. The early fast components were virtually complete by 1 min. The

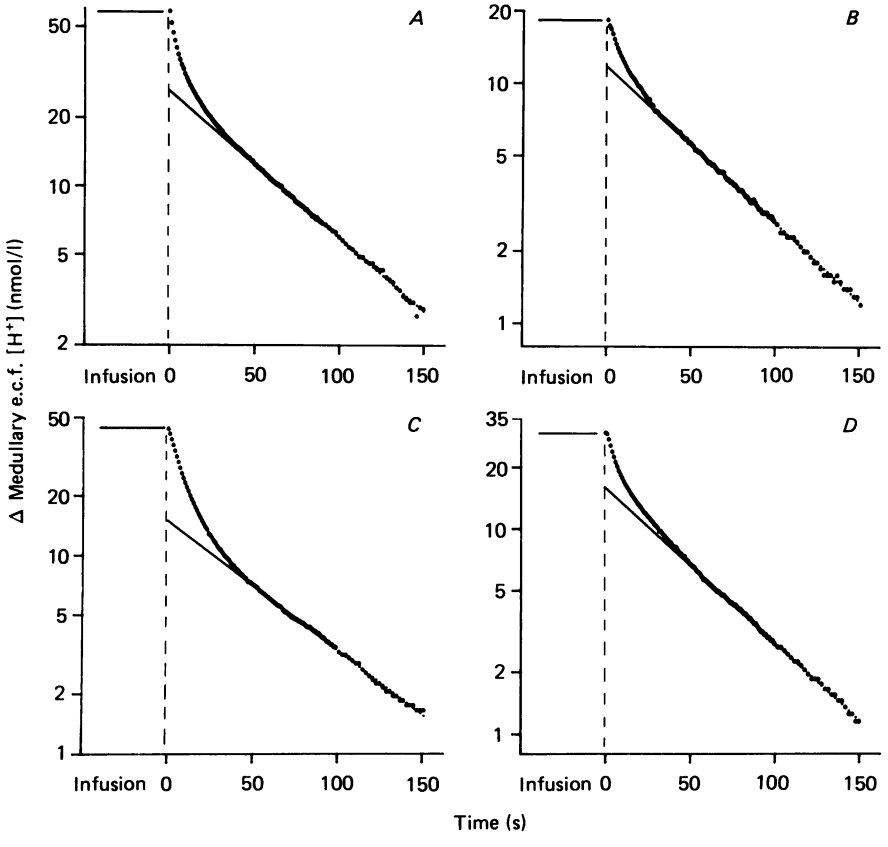

Fig. 5. Semilogarithmic plot in four experiments in different cats of change of ECF [H^+] with time after offset of hypercapnic infusion. The diagonal continuous line represents the best fit regression (extrapolated to time zero) of the points after 1 min. There is a separate early function during which ECF [H^+] has a more rapid decrease.

time constants (τ_s) of the slow function in these four cats ranged from 57·5 to 67·5 s. Correlation coefficients (r) of the four regressions ranged from −0·994 to −0·999.

Semilogarithmic plots of the data of all other cats were similar. The mean value of τ of the slow function in the sixteen experiments was 53·2 ± 3·1 s (S.E. of mean). Average r of the regressions was −0·993 ± 0·002. There was no significant correlation between τ_s and the magnitude of changes of ECF [H^+] ($r = -0{\cdot}174$, $P > 0{\cdot}5$) (Fig. 6), or between τ_s and the peak ECF [H^+] or calculated ECF P_{CO_2}.

The fast component of the change of [H^+] was present in every experiment and accounted for an average of 50·1 ± 3·1 % of the total change. Fig. 7 shows the mean values (scaled to 100) *vs.* time. The curve has the form of an exponential function

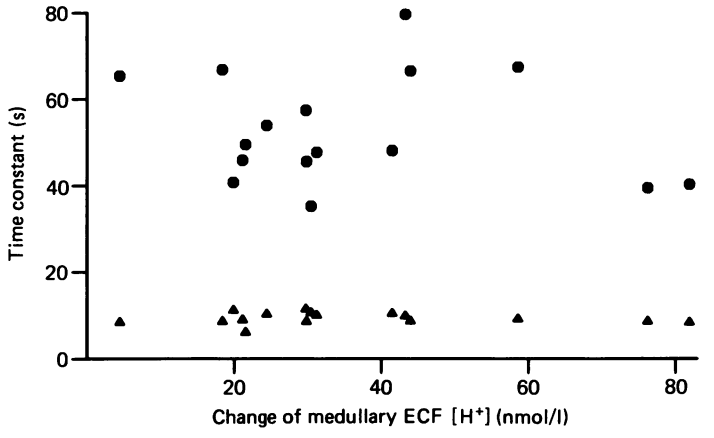

Fig. 6. Plot of time constants in sixteen experiments of the slow function (τ_s, circles) and those of the fast function (τ_f, triangles). There is no significant correlation of either with magnitude of ECF $[H^+]$ change or with each other.

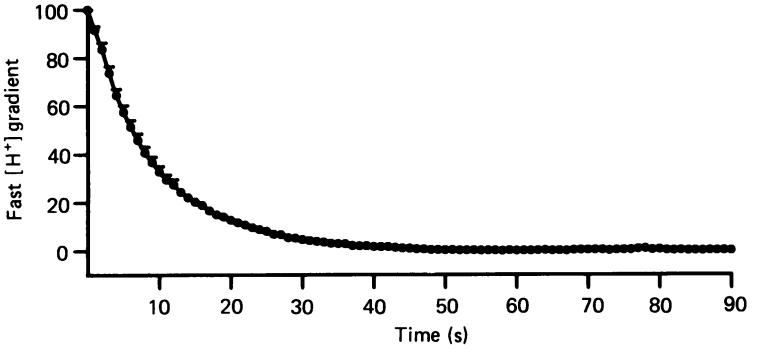

Fig. 7. Plot of mean changes (sixteen experiments) of fast ECF $[H^+]$ gradient that is present during the first minute after cessation of hypercapnic Ringer solution infusion into aortic arch.

which approaches an asymptote by approximately 1 min. The mean time constant (τ_f) is 9·5 s and the r of the regression is −0·995. The τ_f of individual experiments showed a narrow range of values (mean $9{\cdot}5 \pm 0{\cdot}6$ s) and there was no correlation with change of ECF $[H^+]$ ($r = -0{\cdot}27$, $P > 0{\cdot}2$) (Fig. 6), or with peak ECF $[H^+]$ or calculated ECF P_{CO_2}. τ_s and τ_f exhibited no significant correlation with each other ($r = 0{\cdot}43$, $P > 0{\cdot}05$).

Relation between phrenic activity and medullary ECF $[H^+]$

Both tidal and minute phrenic activity began to fall in all cats soon after the decrease of ECF $[H^+]$ following offset of CO_2 infusion (Fig. 3). The mean data show that minute phrenic activity had decreased significantly ($P < 0{\cdot}002$) from infusion base line by 5 s (Fig. 4). However, Fig. 4 also shows that the mean decrease of minute phrenic activity lagged significantly ($P < 0{\cdot}001$) that of medullary ECF $[H^+]$ at all times during recovery. Even though ECF $[H^+]$ had stabilized at 275 s, phrenic

activity was still decreasing and reached a stable level only after 8 min. The magnitude of the lag is more clearly seen in Fig. 8 where the changes of minute phrenic activity and ECF [H^+], normalized from 100 to 0, are plotted against each other.

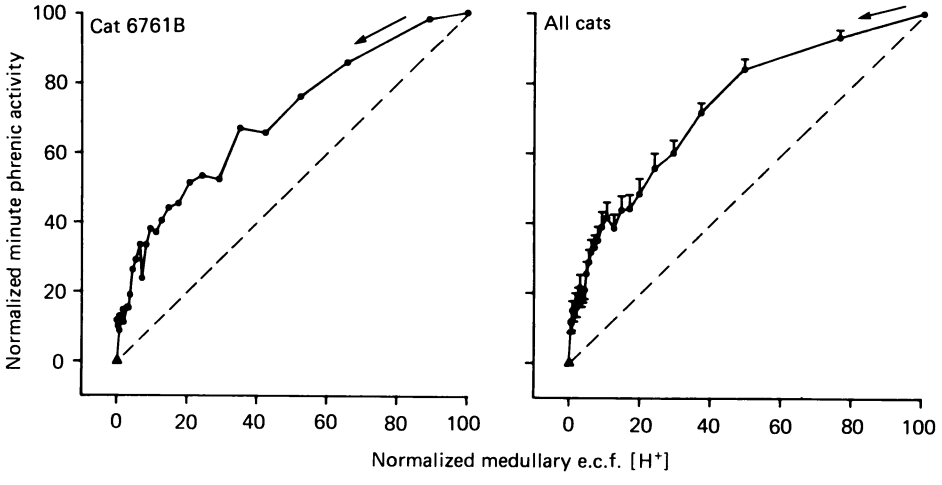

Fig. 8. Plot of changes of minute respiratory (phrenic) activity *vs.* changes of medullary ECF [H^+] after offset of hypercapnic infusion. Example in one cat (left panel) and mean of sixteen experiments (right panel). Note that respiratory activity falls more slowly than ECF [H^+]. Successive circular points are 10 s apart. Triangles represent value at 8 min.

DISCUSSION

Measurement of medullary extracellular fluid pH (or [H^+]) by means of a small flat-tipped H^+-sensitive electrode placed squarely on the ventral surface of the medulla in the region thought to be related to chemosensitivity has been reported by a number of investigators in the past few years. The basis of the technique is the observation that ECF has free access to fluid on the surface of the brain (Dermietzal, 1976) and that there is a continuous outward movement of fluid from ECF through the surface channels (Cserr, Cooper & Milhorat, 1977). It is believed that the isolated thin layer of fluid between the surface of the medulla and the flat electrode reaches rapid equilibrium with ECF. Evidence that the fluid being measured represents ECF comes from the studies of Kiley, Eldridge & Millhorn (1985) who showed that the measurement is not affected by changes of surrounding c.s.f. pH and that there are marked differences in response times of ECF and CSF [H^+] to change of blood P_{CO_2}. It has been shown that respiratory responses correlate closely with surface-measured pH changes in dynamic conditions (Teppema *et al.* 1982; Ahmad & Loeschcke, 1982*a*; Eldridge *et al.* 1984; Kiley *et al.* 1985; Shams, 1985) and in the steady state (Eldridge *et al.* 1984, 1985). The possibility of gradients of pH between the surface and the actual chemoreceptor sites has been examined by Teppema *et al.* (1982) and felt to be unlikely. Furthermore, Cragg, Patterson & Purves (1977) using micro-electrodes, were unable to demonstrate gradients in the medulla to a depth of 5 mm.

Our study measured the responses of medullary e.c.f. [H^+] and respiration to

sudden changes of arterial P_{CO_2} accomplished by starting or stopping infusions of CO_2-laden Ringer solution into the aortic arch. We showed that the resulting arterial pH changes were, after a brief circulatory delay (see also Eldridge *et al.* 1984), close to being square wave in form.

With this technique we found that onsets and offsets of medullary ECF $[H^+]$ responses were quite rapid i.e. mean of 1·75 s after change of P_{CO_2} at the aortic arch. This lag reflects the circulation time from injection site to the medulla. Lags of onset of response of carotid body discharge to injections of CO_2 in the aortic arch are about 0·5 s (Eldridge, 1972) and the inhibitory ventilatory response to Tris injected into a vertebral artery lags 0·4 s (Nye, Hanson & Torrance, 1983). The lags in our study are consistent with the slightly longer circulatory pathway from the aortic arch to the medulla and are also consistent with the moderately longer lags (4–8 s) of onset of medullary ECF $[H^+]$ changes, due to delays both in the lungs and in the longer circulatory pathway, when CO_2 is changed at the mouth (Cragg *et al.* 1977; Teppema *et al.* 1982; Ahmad & Loeschcke, 1982*a*; Eldridge *et al.* 1984; Millhorn, Eldridge & Kiley, 1984; Kiley *et al.* 1985).

These findings indicate that ECF $[H^+]$ begins to change almost as soon as the altered arterial P_{CO_2} reaches the medulla. On the other hand the full response requires a much longer time, up to 5 min in our experiments and similar to that of other investigators (Ahmad & Loeschcke, 1982*a*; Teppema *et al.* 1982).

Two separate functional components of the over-all change of $[H^+]$ after square-wave offset of CO_2 infusion become readily apparent in the semilogarithmic plots of Fig. 5. The slow function, which can be separately quantified after 1 min, is an exponential with a mean time constant of 53 s in the sixteen experiments. Other investigators who have measured changes of medullary ECF pH following step changes of CO_2 at the mouth have reported mean time constants of approximately 50 s (Ahmad & Loeschcke, 1982*a*) and 40 s (Teppema *et al.* 1982), which are similar to those found in the present study.

The finding of an early fast function is new. Neither of the previous groups interpreted their data as showing the existence of this function. Reasons for this may include: (1) that changing P_{CO_2} at the mouth did not provide an accurate square-wave change of the arterial blood perfusing the medulla (Ahmad & Loeschcke, 1982*a*), whereas our studies came close to producing a square-wave change; (2) although Teppema *et al.* (1982) analysed their data using a P_{CO_2} forcing function rather than assuming that it was a square wave, the model used for analysis included the assumption that there was a single first-order system (as did Ahmad & Loeschcke, 1982*a*). Our data suggest this to be incorrect. In addition, a perusal of Fig. 4 A (graph M_2/Δ pH) of Teppema *et al.* (1982) shows that there was a larger change of pH over the first minute after a P_{ET,CO_2} down-step than predicted by their model. Thus, we believe that their data may be consistent with our findings regarding the two functions.

We believe that an interpretation can be made relating our findings to the magnitude of blood-flow in the medullary area under our electrode. The presence of carbonic anhydrase in glial cells ensures that there is rapid HCO_3^-–CL^- exchange between ECF and glial intracellular fluid (Ahmad & Loeschcke, 1982*b*); its contri-

bution to the functions found in this study should be negligible. Teppema *et al.* (1982) present arguments supporting the view (Ohta & Farhi, 1979) that, because of the density of capillaries and rapid CO_2 diffusion, the wash-out of CO_2 and changes of $[H^+]$ are perfusion limited. We have therefore assumed that clearance of CO_2 and $[H^+]$ is proportional to medullary blood flow and to the concentration gradient from tissue to blood. Since we found that log of $[H^+]$ was a linear function with time after the first minute of recovery, medullary blood flow must have been constant after this time but changing during the first minute.

Estimates of central chemoreceptor flow have previously been made from the time courses of ventilatory responses to sudden changes of P_{ET,CO_2} (Gelfand & Lambertsen, 1973; Severinghaus & Crawford, 1978; Adams *et al.* 1984). However, central neural dynamic processes that affect ventilatory responses (Eldridge & Gill-Kumar, 1980) and that are slower than the ECF CO_2 and $[H^+]$ changes (Teppema *et al.* 1982) affect the validity of these analyses.

In our study we measured ECF $[H^+]$ directly. We have shown elsewhere (Eldridge *et al.* 1985) that the relation between ECF $[H^+]$ and end-tidal/arterial P_{CO_2} is linear with a slope of approximately 0·7 torr/nmol. Thus, the changing ECF $[H^+]$ should reflect the local changes of P_{CO_2} and the clearance pattern of CO_2 from the tissue. Since the change of arterial P_{CO_2} in our experiments was close to being square wave in form and since we were able, through the use of a servocontrolled ventilator, to keep end-tidal/arterial P_{CO_2} almost constant during the period of recovery from CO_2 infusion, the changing gradient of P_{CO_2} and $[H^+]$ between tissue and blood should have been reflected by our measurements of ECF $[H^+]$.

The studies using CO_2 wash-out to estimate medullary blood flow (Gelfand & Lambertsen, 1973; Severinghaus & Crawford, 1978; Adams *et al.* 1984) have used analytical techniques similar to those commonly used in measuring brain blood-flow by inert tracer wash-out (Kety, 1951; Young, 1980). In these studies a mass balance differential equation, based on the Fick principle, is used. This equation relates the clearance of a tracer to the blood flow and the concentration gradient. We applied this equation to our ECF $[H^+]$ data to estimate medullary blood-flow. Solving for flow, F(t), expressed in ml/(min 100 g), for all times (t) greater than zero results in

$$\mathrm{F}(t) = \left\{\frac{1/\tau_s[G_s e^{-t/\tau_s}] + 1/\tau_f[G_f e^{-t/\tau_s}]}{[G_s e^{-t/\tau_s}] + [G_f e^{-t/\tau_s}]}\right\} \lambda\ 100\ \mathrm{g}, \quad (1)$$

where G_s and G_f are respectively the zero-time $[H^+]$ intercepts of the slow and fast functions, τ_s and τ_f are the time constants, expressed in minutes, of the slow and fast functions, and λ is the tissue–blood partition coefficient for CO_2, estimated at 0·7 (Severinghaus & Crawford, 1978). The model assumes that CO_2 exchange can be treated as a well-stirred system but allows for a gradient from arterial to venous P_{CO_2} owing to metabolism (Adams *et al.* 1984).

When t is much greater than τ_f (> 1 min in our experiments), the terms of eqn. (1) relating to the fast function become negligible. In this situation flow is not changing and becomes inversely proportional to the time constant of the slow function,

$$\mathrm{F} = 1/\tau_s\ \lambda\ 100\ \mathrm{g}. \quad (2)$$

In our sixteen experiments, stable medullary flow calculated from individual τ_s averaged 83·2±4·6 ml/(min 100 g) (range 53–119). Five representative examples showing the range of stable flows are graphed in Fig. 9*A*. Our findings can be compared with studies of other workers. Ahmad & Loeschcke (1982*a*) and Teppema

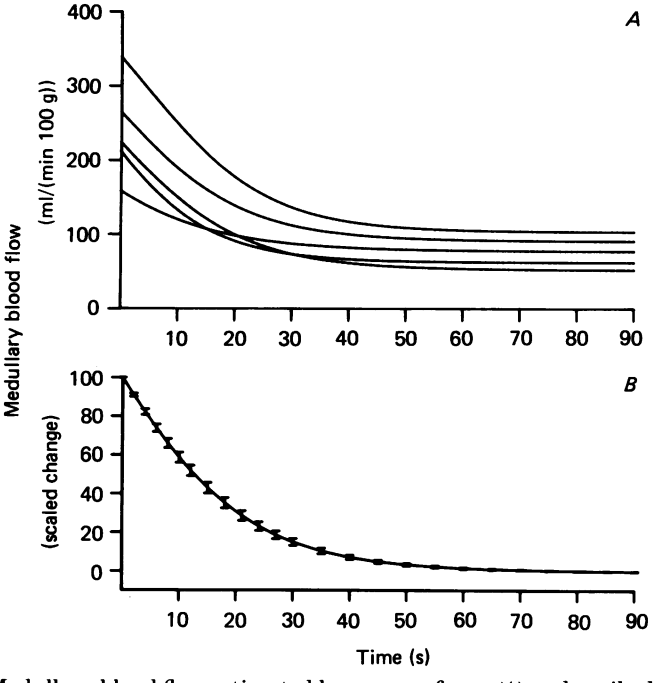

Fig. 9. Medullary blood flow estimated by means of eqn. (1) as described in text. *A*, typical examples in five cats of absolute flow levels at and following offset of infusion of hypercapnic Ringer solution into aortic arch. *B*, mean data of sixteen experiments of changes of flow following offset of infusion. Note that flow decreases with half-time of 13 s and becomes stable by approximately 1 min.

et al. (1982), using a technique similar to ours in cats anaesthetized with chloralose and urethane and whose P_{ET,CO_2} was in the same range as ours, found mean time constants of 50 and 40 s respectively, which would represent mean stable flows of 84 and 105 ml/(min 100 g). Kagstrom, Smith & Siesjo (1983), using a [^{14}C]/iodoantipyrine technique for measuring local blood flow in halothane-anaesthetized rats with arterial P_{a,CO_2} averaging 38 torr, found values of 82, 105 and 85 ml/(min 100 g) for cerebellum, hypothalamus and septal nucleus respectively, and higher values in other parts of the brain but do not report a value for the medulla. Feustal, Stafford, Allen & Severinghaus (1984), using inert gas (H_2) wash-out from the chemoreceptor areas of the ventrolateral medulla in pentobarbitone-anaesthetized cats, found a mean blood flow of 57·8±27·5 ml/(min 100 g) (S.D.) when P_{a,CO_2} was approximately 32 torr. These values may be somewhat low because of the use of barbiturate anaesthesia, which is known to reduce brain blood flow and metabolism (Nilsson & Siesjo, 1975).

It is well known that hypercapnia dilates cerebral blood vessels (Raper, Kontos & Patterson, 1971) and causes blood flow to increase (Kontos, 1981), so it would be

expected that medullary flow during the infusion of CO_2 in our experiments would be higher than the stable level at the end of recovery. Using eqn. (1), we have estimated medullary flows at time zero and at various times after stopping the infusion. Peak flow (at time zero) was elevated over stable flow in all experiments. It averaged $246 \pm 15{\cdot}8$ ml/(min 100 g) (range, 148–352), representing a 3-fold increase of flow for a mean increase of ECF P_{CO_2} of $25{\cdot}3 \pm 3{\cdot}5$ torr. The relation between peak flows and change of P_{CO_2} in individual experiments yielded a positive slope of 6 ml/(min 100 g) per torr with an r of 0·65, $P < 0{\cdot}01$. Representative examples of the peak and changing medullary flows in five cats are shown in Fig. 9*A*.

Our findings are consistent with those of Dahlgren, Ingvar & Siesjo (1981) and Kagstrom *et al.* (1983) who also found a 3-fold increase of flow in various grey matter areas of the brain when P_{a,CO_2} increased 35–40 torr. Vis & Folgering (1980) reported a 3-fold increase of vertebral flow when P_{ET,CO_2} was increased 30 torr. Feustal *et al.* (1984) found a smaller increase, 1·3-fold for an increase of P_{a,CO_2} of 12·6 torr, but this again may be low because barbiturate anaesthesia reduces the responsiveness of cerebral blood vessels to changes of CO_2 (Edvinsson & McCulloch, 1981).

The mean changes (scaled from 100 to 0) of medullary flow estimated from our experiments are plotted against time in Fig. 9*B*. It can be seen that flow reaches a stable level by approximately a minute after the step change of P_{a,CO_2}. The half-time of the change is approximately 13 s. A number of investigators have determined the response of cerebral blood flow to changes of P_{a,CO_2} in human beings, cats and dogs (Shapiro, Wasserman & Patterson, 1965, 1966; Severinghaus & Lassen, 1967; Tuteur, Reveich, Goldberg, Cooper, West, McHenry & Cherniack, 1976; Vis & Folgering, 1980; Wilson, Trystman & Rapela, 1985). A problem with most of these studies is that the change of CO_2 was made at the mouth during spontaneous breathing or during constant ventilation in paralysed animals. Thus, the arterial P_{CO_2} itself was not changed rapidly, often requiring some minutes to reach what was considered a stable value (Shapiro *et al.* 1965, 1966; Tuteur *et al.* 1976; Wilson *et al.* 1985). The relatively long response times for change of cerebral flow found by these investigators therefore probably do not reflect only the vascular response to a square change of P_{CO_2}.

Severinghaus & Lassen (1967), on the other hand, studied the change of cerebral blood flow after a relatively square-wave decrease of P_{CO_2} during controlled hyperventilation in men. They found a mean time constant of 18 s (half-response time of about 14 s) which is consistent with our results. The very long time constants for vertebral flow changes (339 s for dilatation, 41 s for constriction) reported by Vis & Folgering (1980) after a step-change of P_{CO_2} in paralysed cats are not consistent with our results or others in the literature.

We should point out that the quantitation of medullary flow requires assumptions that metabolic production of CO_2 ($\dot{V}_{CO_2}$) is not changing during the recovery after offset of CO_2 infusion and that no CO_2 diffuses to or from a poorly perfused compartment. Either of these may be incorrect. A decreasing $\dot{V}_{CO_2}$ would lead to an over-estimation of flow rates. Over- or underestimation of flow would result from the second factor depending on whether there was loss to or gain from the underperfused compartment. Although these problems may have led to quantitative errors in our estimations, they are probably not very large (Siesjo, 1980).

Finally, we have shown that respiratory activity decreases, after the square-wave

change of arterial P_{CO_2}, even more slowly than does the hypercapnic stimulus represented by ECF $[H^+]$. This finding is consistent with the existence of a central neural mechanism, termed a respiratory after-discharge, that has the property of maintaining an increased but slowly declining respiratory activity for some minutes after cessation of the primary stimulus (Eldridge & Gill-Kumar, 1980). We suggest that the lag of respiratory change in the present experiments is explained by such a central neural dynamic process. The studies of Teppema *et al.* (1982) led to the same conclusion. The existence of this mechanism precludes the use of dynamic changes of ventilation of any respiratory output signal as a direct index of ECF $[H^+]$ chemoreceptor stimulation.

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