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RESPIRATORY RESPONSES TO MEDULLARY HYDROGEN ION CHANGES IN CATS: DIFFERENT EFFECTS OF RESPIRATORY AND METABOLIC ACIDOSES

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SUMMARY

1. The steady-state responses of respiration, measured as integrated phrenic nerve activity, to hypercapnic acidosis of the medullary extracellular fluid (e.c.f.) and to metabolically generated acidosis were compared in paralysed, vagotomized and glomectomized cats. E.c.f. hydrogen ion concentration ($[H^+]$) was measured directly by means of a small (2 mm diameter) pH electrode placed on the ventral medulla.

2. The results in ten cats show that changes of medullary e.c.f. $[H^+]$ were linearly related to changes of end-tidal P_{CO_2} both before ($r = 0{\cdot}999$) and after ($r = 0{\cdot}996$) development of metabolic acidosis.

3. There was a curvilinear relation between hypercapnic e.c.f. $[H^+]$ changes and the respiratory response that reflects progressive saturation of a central neural pathway between the chemoreceptors and the respiratory controller. This relation was similar in form both before and after development of metabolic acidosis. When acidosis of metabolic origin was present, apnea occurred with only small decreases of CO_2 despite a high $[H^+]$.

4. The respiratory responses to the same e.c.f. $[H^+]$ change were only about one-half as large when they were generated metabolically as when they were generated by raising P_{CO_2}. Both exogenously induced metabolic acidosis (HCl infusion) and endogenous acidosis yielded similar results.

5. We conclude that the e.c.f. $[H^+]$ does not represent the unique stimulus to the central chemoreceptors. We discuss several alternate mechanisms for the action of CO_2 and $[H^+]$ on central chemoreceptors but none can be considered definitive at the present time.

INTRODUCTION

The mechanism by which chemically sensitive structures in the medulla are activated to drive ventilation has never been fully resolved. Winterstein (1921, 1956) proposed that the key stimulus was the hydrogen ion concentration ($[H^+]$) in the medulla, the reaction theory. He suggested that CO_2 acted to stimulate breathing only through its ability to cause $[H^+]$ to increase in the brain. An alternative

hypothesis has been that CO_2 has an effect on the central chemoreceptors that is independent of the change of medullary $[H^+]$ that it causes (Nielsen, 1936; Borison, Hurst, McCarthy & Rosenstein, 1977; Borison, McCarthy & Lamb, 1980).

Studies that have supported these hypotheses have until recently depended upon estimations of extracellular fluid (e.c.f.) pH or $[H^+]$ made from measurements of arterial blood and cerebrospinal fluid (c.s.f.) pH during steady-state conditions or during ventriculo-cisternal perfusion. However, it is likely that arterial $[H^+]$ alone is not an adequate reflexion of that of the medullary e.c.f., the presumed site of the central chemoreceptors (Loeschcke, 1982). Furthermore, the assumptions for estimation of e.c.f. $[H^+]$ from arterial and c.s.f. measurements have been recently questioned (Bledsoe, Berger & Hornbein, 1983).

In the past few years there has been increasing interest in measuring medullary e.c.f. pH directly 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. Recent work by Teppema, Vis, Evers & Folgering (1982), Ahmad & Loeschcke (1982), Teppema, Barts, Folgering & Evers (1983), Millhorn, Eldridge & Kiley (1984), Kiley, Eldridge & Millhorn (1984) and Eldridge, Kiley & Millhorn (1984) supports the idea that the isolated thin layer of fluid between the flat electrode and the surface of the medulla reaches rapid equilibrium with e.c.f. and that measurements of it are a valid indicator of the $[H^+]$ in the milieu of the chemoreceptors.

In the present study we have used this technique to examine the relations between e.c.f. $[H^+]$ and respiratory responses during acidoses induced by elevated P_{CO_2} or by infusion of HCl. In order to avoid effects of peripheral feed-back, the experiments were performed in paralysed, ventilated animals whose vagi and carotid sinus nerves had been cut and phrenic nerve activity was used as the index of respiratory output.

METHODS

Studies were performed in ten healthy adult cats weighing between 2·6 and 4·5 kg. They were anaesthetized first with ether and then given chloralose (40 mg/kg) and urethane (250 mg/kg) via a catheter placed in a 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 a rigid head mounting. Both vago-sympathetic trunks were cut in the neck. The carotid sinus nerves were exposed and cut under direct vision. One phrenic nerve root (C5) 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 this electrode were built in to a small piece of acrylic plastic, which was placed in the tissue well adjacent to the nerve. Because the electrode had no fixed external attachment (the electrical connexions were flexible and moved freely) it moved with the cat. With this technique 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.

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 in 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 very narrow range around the desired level (Smith, Mercer & Eldridge, 1978). In order to change the P_{ET,CO_2} to a different level in an animal, the ventilator's tidal volume was changed and the set-point of the servocontroller adjusted to the new value.

Measurement of ventral medullary e.c.f. pH. In preparations for this measurement, 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 carefully controlled by 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 the spaces around it filled with agar, resulting in a well which subsequently filled with clear c.s.f. 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 MI404, time constant response = 1·5 s) and a reference micro-electrode (Microelectrodes, Inc., Model MI401). Both were mounted in a balanced holder which moved freely in the vertical direction. Both electrodes were lowered gently on to the surface of the medulla so that the tips were located lateral to the mid line near the upper rootlets of the 12th cranial nerve in areas that were free of visible blood vessels. Under visual inspection using a binocular microscope firm contact of the pH and reference electrode tips on the medulla was verified but indentation of the medullary surface was avoided.

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 a period of time (1 or 10 s as appropriate for a given experiment). 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_{CO_2} was an acid shift of e.c.f. pH that began within 4–7 s, or approximately the lung-to-medulla circulation time, and then an alkaline response occurring with the same lag after the first effective post-occlusion breath (Eldridge *et al.* 1984). All animals showed this response.

Experimental protocols

The experiments included three protocols performed in the order that follows.

CO_2 response. After preparations and preliminary experiments had been completed and all variables had become stable, the P_{ET,CO_2} was lowered to determine the threshold of rhythmic phrenic activity. The values of P_{CO_2} and e.c.f. pH were recorded for 1 min. The P_{ET,CO_2} was then raised slightly (usually about 1 Torr) by changing the setting on the ventilator's servocontroller. After 5–10 min when e.c.f. pH, phrenic activity and other variables had again become stable, they were recorded for 1 min. Six to seven additional step increases of P_{ET,CO_2} to as high as 40 Torr above threshold were then performed and the responses at each step determined in a similar manner.

Induction of metabolic acidosis. Following the measurement of the CO_2 response, P_{ET,CO_2} was returned to the lowest level at which rhythmic phrenic activity had been obtained (usually about 1 Torr above threshold). It was maintained at that level ($\pm$0·1 Torr) throughout the subsequent protocol. All variables were recorded after they had become stable. At this time, an I.V. infusion of HCl (0·5 mol/l) was given in nine cats over a period of 5 min. The quantity varied somewhat from cat to cat but averaged 1·3$\pm$0·5 ml/kg. All variables were measured 5 min after the end of the infusion and then every 5 min for at least 20 min. In some cats they were followed to up to 55 min after infusion. A second infusion of HCl (0·5 mol/l) was then given in seven of the cats (1·6$\pm$0·7 ml/kg) and measurements made in a similar manner.

In one cat, a metabolic e.c.f. acidosis of almost 0·2 pH units occurred spontaneously after the first CO_2 response measurement, despite the breathing of 100% O_2 and the absence of hypotension (mean arterial pressure was >100 mmHg and had not changed). This cat was included in the study but was not given HCl.

CO_2 response after metabolic acidosis. The CO_2 threshold for rhythmic phrenic activity was again determined and the respiratory response to CO_2 was measured in the same way as in the initial protocol.

Data handling

Data obtained included arterial pressure, airway P_{CO_2}, phrenic nerve impulses and e.c.f. pH, all of which were recorded on magnetic tape and on a hard copy of an oscilloscope screen. pH values were also recorded by means of a digital printer. Breath-by-breath analysis of phrenic activity was performed by 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). The inspiratory output for each breath was determined from the peak 0·1 s activity. It has been shown that this value is the neural equivalent of tidal volume of breathing (Eldridge, 1971). Neural minute activity was calculated as the product of this value and respiratory frequency. In the steady-state studies, recordings were made for a 1 min period. Since breath-to-breath variation of the indices of phrenic activity was consistently small (± 2–3 % S.E. of mean), the averaged value for each period was treated as a single measurement for purposes of analysis.

In order to compare the findings among different cats, the data were normalized by assigning a value of 85 units to the level of tidal phrenic activity found in an individual cat when P_{CO_2} was 40 Torr above the threshold for rhythmic activity. All other levels were scaled accordingly. The experimental basis for this normalization lies in the finding that the response of tidal phrenic activity to increasing CO_2 saturates at approximately this level (Eldridge, Gill-Kumar & Millhorn, 1981).

For presentation of mean results of the steady-state relations between $[H^+]$, P_{CO_2} and phrenic responses, data were grouped into bins of increasing $[H^+]$ from the original apneic threshold (0–3, 3·1–6, 6·1–10·5, 10·6–18, 18·1–30 and 30·1–55 nmol/l for the responses to CO_2 administration, and 0–5, 6–25 and 26–55 nmol/l for the responses to metabolic acidosis.

RESULTS

The original mean P_{ET,CO_2} at apneic threshold in the ten cats before induction of metabolic acidosis was $27·3 \pm 1·4$ (S.E. of mean) Torr, and the $[H^+]$ was $54·4 \pm 2·8$ nmol/l (pH = 7·265 units). The mean arterial pressure was 114 ± 5 mmHg.

Responses to hypercapnic acidosis

All ten animals studied were responsive to the hypercapnic stimulus acting only on the central chemoreceptors. The responses in one cat of minute phrenic activity are plotted against P_{ET,CO_2} (right panel, open circles) and against $[H^+]$ (left panel, open circles) in Fig. 1. The mean data for all ten animals are shown in Fig. 2 (open circles) for minute phrenic activity *vs.* P_{ET,CO_2} and in Fig. 3 (open circles) for phrenic activity *vs.* $[H^+]$. Tidal (peak phrenic) neural activity, which is not shown, exhibited similar relations. Both indices of respiratory activity showed a clear tendency for the response lines to curve to the right, indicating that there were progressively smaller increments of activity for equal increments of P_{ET,CO_2} (Figs. 1 and 2) or $[H^+]$ (Figs. 1 and 3) as these stimuli became higher. The mean data for frequency *vs.* $[H^+]$ are shown in Fig. 4 (open circles). All findings are similar to those reported for CO_2 (Eldridge *et al.* 1981) or CO_2 and $[H^+]$ (Eldridge *et al.* 1984) in earlier studies of hypercapnic acidosis.

The relation between P_{ET,CO_2} and medullary e.c.f. $[H^+]$ in the ten cats is shown in Fig. 5 (open circles). The mean slope was 0·687 Torr/nmol.l, a value similar to that (0·714 Torr/nmol.l) reported earlier in a group of twenty-six cats (Eldridge *et al.* 1984). The linearity of the relation is apparent ($r = 0·999$).

Mean arterial pressures fell slightly from their values at apneic threshold (114 ± 5 mmHg) to those (105 ± 6 mmHg) at the highest P_{CO_2} level.

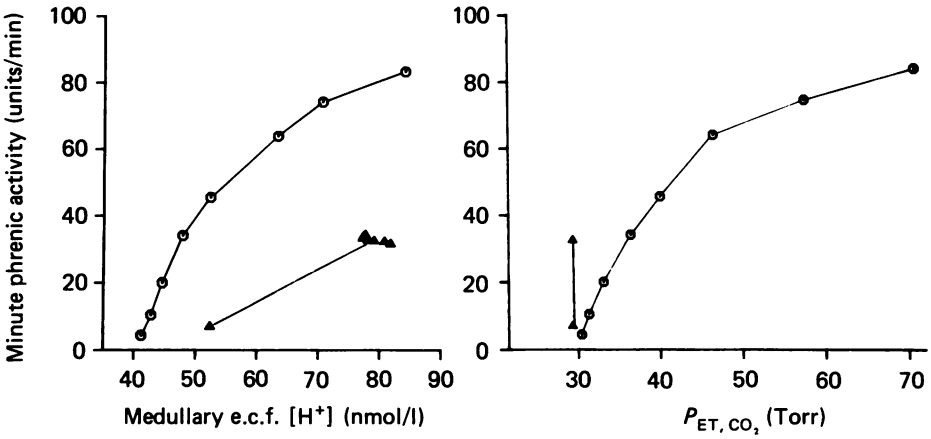

Fig. 1. Relations in one cat between medullary e.c.f. [H^+] and neural (phrenic) respiratory activity (left panel) and P_{ET,CO_2} and respiratory activity (right panel) during steady-state hypercapnia (open circles) and during metabolic acidosis (triangles) induced by infusion of HCl and followed for 40 min.

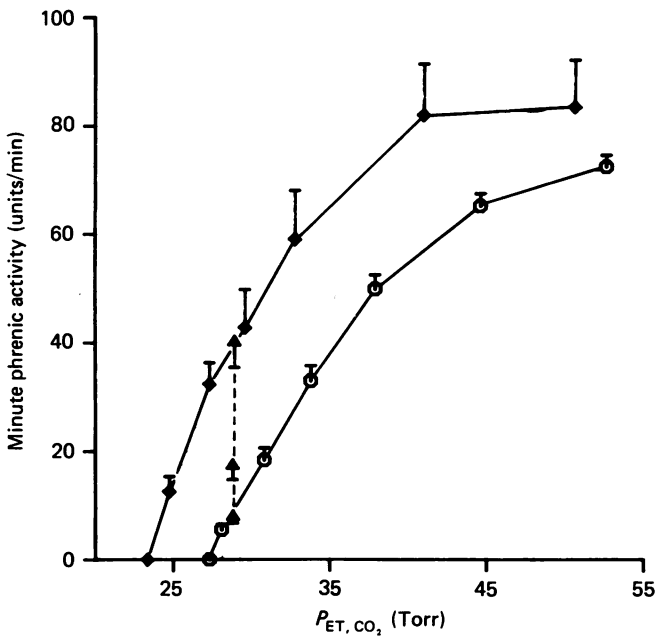

Fig. 2. Average values in ten cats of steady-state relations between P_{ET,CO_2} and phrenic activity with hypercapnia before induction of metabolic acidosis (open circles), during isocapnic metabolic acidosis (triangles) and with hypercapnia after development of metabolic acidosis (diamonds).

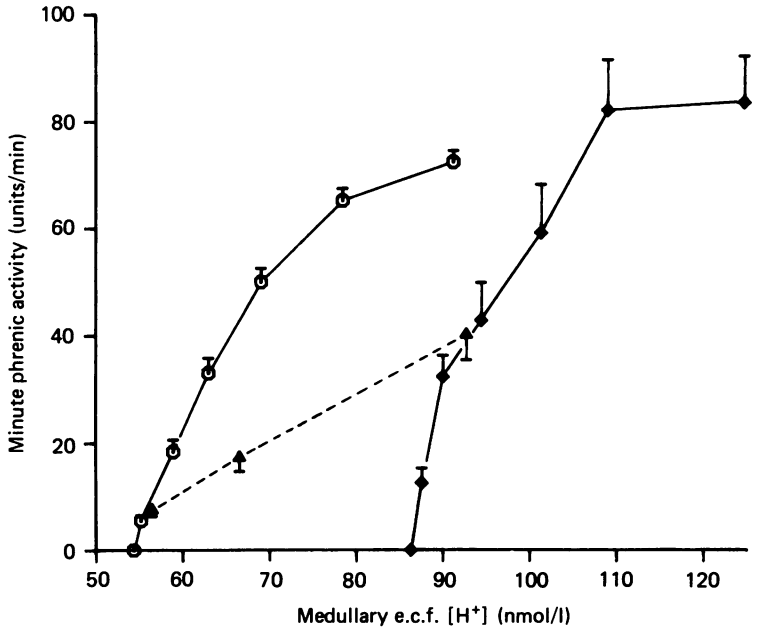

Fig. 3. Average values in ten cats of steady-state relations between medullary e.c.f. [H^+] and phrenic activity with hypercapnia before induction of metabolic acidosis (open circles), during isocapnic metabolic acidosis (triangles) and with hypercapnia after development of metabolic acidosis (diamonds).

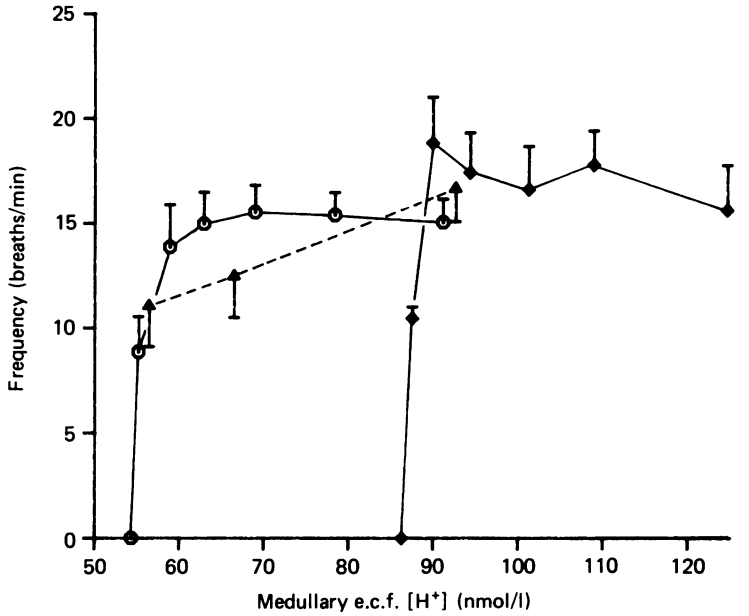

Fig. 4. Average values in ten cats of steady-state relations between medullary e.c.f. [H^+] and respiratory frequency with hypercapnia before induction of metabolic acidosis (open circles), during isocapnic metabolic acidosis (triangles) and with hypercapnia after development of metabolic acidosis (diamonds).

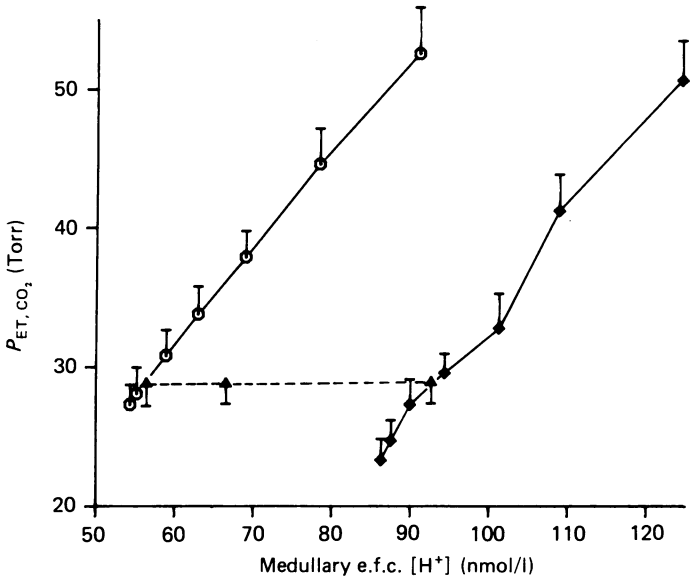

Fig. 5. Average values in ten cats of steady-state relations between medullary e.c.f. [H^+] and P_{ET,CO_2} with hypercapnia before induction of metabolic acidosis (open circles), during isocapnic metabolic acidosis (triangles) and with hypercapnia after development of metabolic acidosis (diamonds).

Responses to metabolic acidosis

The I.V. administration of HCl led to the fairly rapid (minutes) development of an acidosis of the medullary e.c.f. However, it became apparent that during the infusion which lasted 5 min, and for a variable period thereafter, the developing acidosis displaced CO_2 from the blood causing P_{ET,CO_2} to increase despite the use of the servocontroller, and also presumably displaced CO_2 and raised P_{CO_2} in brain tissue.

Since our steady-state findings show that the metabolic acidosis caused less stimulation of respiration than hypercapnic acidosis, we have interpreted larger respiratory responses during and immediately after the infusion of acid as being due to these elevations of P_{CO_2}. These effects lasted up to 15 min after start of the infusion (Fig. 6). We have therefore accepted as valid indicators of the true metabolic e.c.f. acidoses only those values obtained, in the nine cats receiving HCl, at least 20 min after induction. After this period the e.c.f. [H^+] remained relatively stable in six of the seven cats in which it was followed ($-1{\cdot}1 \pm 1{\cdot}4$ nmol/l at 30 min; $+0{\cdot}3 \pm 3{\cdot}5$ nmol/l at 40 min). In one cat it became significantly less acid with time. When this occurred, phrenic activity decreased linearly with the change of [H^+] (Fig. 6).

An example of an e.c.f. metabolic acidosis, followed for 40 min in one cat to show the stability of the relation between [H^+] and phrenic activity, is given in Fig. 1. The phrenic response to the same [H^+] (left panel, triangles) is less than half that found with hypercapnic acidosis (open circles). The constancy of the servocontrolled P_{CO_2} during the metabolic acidosis is shown in the right panel (triangles).

The mean findings are shown in Fig. 2 (triangles) for minute phrenic activity *vs.*

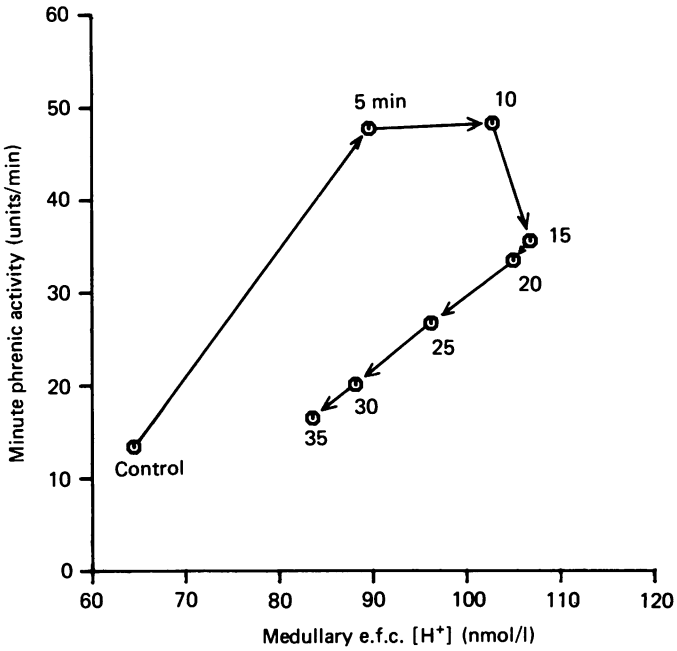

Fig. 6. Relation in one cat between medullary e.c.f. [H^+] and phrenic activity during induction of isocapnic metabolic acidosis by infusion of HCl (0·5 mol/l). During the infusion (5 min) and until 15 min, respiratory activity exceeds that caused solely by metabolic acidosis because of release of CO_2 due to the unsteady state of developing acidosis.

P_{ET,CO_2}, which did not change. Fig. 3 demonstrates the relation between minute phrenic activity and [H^+] (triangles) and shows that the respiratory response to metabolic acidosis was only about half that of a similar degree of acidosis due to hypercapnia (open circles). Fig. 4 gives the relation between frequency of breathing and e.c.f. [H^+] (triangles).

Fig. 5 shows the mean relations between P_{ET,CO_2} and medullary e.c.f. [H^+] during metabolic acidosis (triangles). Since P_{ET,CO_2} did not change, there is only a shift to the right from the original pre-metabolic acidosis relation.

Mean arterial pressure in the ten cats was 111 ± 5 mmHg before induction of metabolic acidosis and did not change significantly after development of the acidosis.

The one cat that developed spontaneously a metabolic acidosis of the e.c.f. had findings that were essentially the same as the larger group that had received HCl to induce the acidosis. The metabolic acidosis, depicted by the dashed line and arrow in Fig. 7, again led to a respiratory response only half as large as that produced by hypercapnic acidosis of the same degree.

Responses to hypercapnic acidosis after metabolic acidosis

Despite the presence of metabolic acidosis, all cats responded to hypercapnia in virtually the same manner as before the development of the acidosis. The apneic threshold for CO_2 ($23{\cdot}3 \pm 1{\cdot}5$ Torr) had decreased in all animals and had shifted $-4{\cdot}3 \pm 0{\cdot}6$ Torr on average (Fig. 2, diamonds). The curvilinear shape of the post-

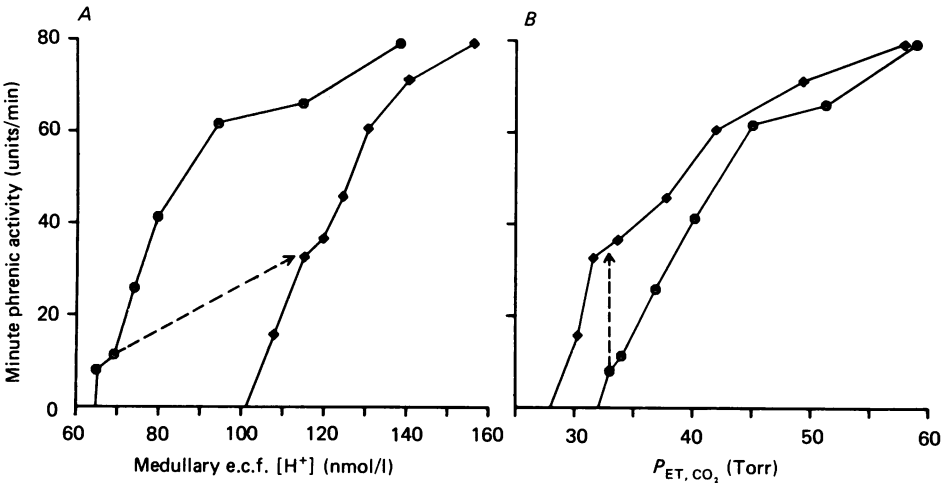

Fig. 7. Relations in one cat of medullary e.c.f. [H^+] and phrenic activity (*A*), and P_{ET,CO_2} and phrenic activity (*B*) with hypercapnia before (open circles) and after (diamonds) spontaneous development of metabolic acidosis (depicted by dashed lines).

metabolic acidosis CO_2–phrenic activity relation was similar to that of the original curve (open circles) except that it had shifted moderately to the left.

The mean post-metabolic acidosis relations between minute phrenic activity and medullary e.c.f. [H^+] are shown in Fig. 3 (diamonds). The curve is shifted in an acidotic direction from the original curve (open circles). Despite the marked metabolic acidosis which had developed at the end of the preceding experiment (rightmost triangle, [H^+] = 93·8 nmol/l), only a small decrease of [H^+] (7·5 nmol/l) led to cessation of rhythmic breathing. Thus, only a small decrease of P_{CO_2} of 4·3 Torr produced apnea despite the continuing severe e.c.f. acidosis (86·3 nmol/l). The relation between breathing frequency and changes of e.c.f. [H^+] during the post-metabolic acidosis hypercapnia was similar to that before metabolic acidosis, although shifted to a markedly higher absolute [H^+] (Fig. 4, diamonds).

After development of metabolic acidosis, the mean relation between P_{ET,CO_2} and medullary e.c.f. [H^+] remained linear (r = 0·996) (Fig. 5, diamonds) but was shifted markedly to the right of the original relation (open circles). The mean slope (0·703 Torr/nmol.l) remained approximately the same as the original slope.

Mean arterial pressures fell slightly from their values at apneic threshold (116 ± 6 mmHg) to that (103 ± 7 mmHg) at the highest P_{CO_2} level. These changes were not significantly different than those of the pre-metabolic acidosis study.

DISCUSSION

The hypothesis that [H^+] or pH in medullary e.c.f. represents the unique stimulus at the central chemoreceptor has developed a rather wide degree of acceptance (Loeschcke, 1982), but direct evidence supporting it has been meagre.

The problem has been approached by measurements of ventilation, arterial blood and c.s.f. pH and P_{CO_2} in animals (Fencl, Miller & Pappenheimer, 1966) and humans

(Fencl, Vale & Broch, 1969) during stable states of metabolic acidosis or alkalosis. It was assumed that c.s.f. values were the same as those in the medullary e.c.f. A second approach has involved measurements of ventilation during ventriculo-cisternal perfusion (Pappenheimer, Fencl, Heisey & Held, 1965) or medullary superfusion (Berndt, Berger, Berger & Schmidt, 1972) with mock c.s.f. of varying acid–base characteristics. In these studies the e.c.f. $[H^+]$ at the site of the chemoreceptors has been estimated, with the assumption that they lie at some depth from the medullary surface along the gradient from c.s.f. to deep tissues. All of these studies have shown that alveolar ventilation (Pappenheimer *et al.* 1965; Fencl *et al.* 1966; Fencl *et al.* 1969) or minute ventilation (Fencl *et al.* 1969; Berndt *et al.* 1972) can be expressed as a single function of c.s.f. $[H^+]$ in the steady-state studies or of calculated e.c.f. $[H^+]$ in the perfusion studies. The findings have been interpreted to support the concept that e.c.f. $[H^+]$ is the main if not sole central chemical stimulus to breathing.

There are interpretative difficulties with most of these studies that include the presence of carotid bodies and vagal receptors in some and the assumptions required for estimation of e.c.f. $[H^+]$ (see Bledsoe *et al.* 1983, for critical review). It is of interest that one study involving ventriculo-cisternal perfusion of artificial c.s.f. in anaesthetized cats was unable to confirm that ventilation is a unique function of calculated e.c.f. $[H^+]$ at the presumed site of the chemoreceptors (Berkenbosch, deGoede, Olievier & Quanjer, 1978).

The alternate hypothesis has been that CO_2 has an effect on the central chemoreceptors that is independent of the change of e.c.f. $[H^+]$ that it causes. Nielsen (1936) supported the latter concept because he found a much greater increase of breathing in humans from the acidosis of CO_2 inhalation than from a similar degree of metabolic acidosis. More recently Borison *et al.* (1977) reported that even in unanaesthetized, decerebrate and peripherally denervated cats the tidal ventilatory response to a given increase of arterial $[H^+]$ was far greater with CO_2-induced than with metabolically induced acidosis. An analysis of similar experiments from the same group (Borison *et al.* 1980) led to the conclusions that CO_2 and $[H^+]$ act centrally as independent and additive stimuli. This type of study has generally not been accepted as definitive because of the possibility that arterial $[H^+]$ is not an adequate reflexion of that in the medullary e.c.f.

By making direct measurements of medullary e.c.f. $[H^+]$ we avoided these problems. Our main findings show that the increase of e.c.f. $[H^+]$ produced metabolically does cause respiratory output to increase. However, it is clear that there is a significantly smaller respiratory response to the same medullary $[H^+]$ when the acidosis is produced by metabolic mechanisms than when it is produced by hypercapnia. We found, fortuitously, that the difference occurred with an endogenously developing metabolic acidosis as well as that induced by exogenously administered HCl.

We have shown in this and a previous study (Eldridge *et al.* 1984) that the respiratory response to increasing $[H^+]$ due to CO_2 has a curvilinear shape, which indicates progressive saturation of neuronal pathways between the chemoreceptors and the respiratory controller. Even if the logarithmic function of $[H^+]$, pH, is used, the respiratory response is still curvilinear although with a lesser degree of curvature. The finding is also illustrated in some of the figures of Teppema *et al.* (1983). It should

be noted that the response to CO_2-induced $[H^+]$ change retains the same curvilinear shape after induction of metabolic acidosis. We also attribute considerable significance to the finding that a small decrease of P_{CO_2} still leads to apnea, despite the high $[H^+]$ due to the metabolic acidosis. Although we have plotted our results as a function of medullary e.c.f. $[H^+]$, no significant difference in our basic findings or conclusions would have resulted if we had used pH instead.

Several other workers have used direct measurements of medullary e.c.f. pH and have reported equivalent results. Our findings are in general agreement with those of Teppema *et al.* (1983) who studied responses to hypercapnic and metabolic pH changes induced by HCl and $NaHCO_3$ in both paralysed and spontaneously breathing animals. Shams, Ahmad & Loeschcke (1981) showed that infusion of H_2SO_4, once the P_{CO_2} effects of acidification (see above) had passed, caused less respiratory stimulation than hypercapnic acidosis. Kiwull-Schone & Kiwull (1983) reported that the respiratory responses to endogenously generated (hypoxic) metabolic e.c.f. acidosis were much smaller than those due to hypercapnic acidosis.

One possible problem could be that we, and others using the technique of surface measurement of medullary e.c.f. pH, are not measuring a value that represents the pH in the interstitial fluid to which the central chemoreceptors are actually exposed. However, it has been shown that the ventilatory responses correlate very closely with the surface-measured pH changes in dynamic situations (Teppema *et al.* 1982; Ahmad & Loeschcke, 1982; Eldridge *et al.* 1984). Teppema *et al.* (1982) have suggested that the pH changes are perfusion limited and macroscopically homogeneous in the brain stem. The surface-measured $[H^+]$ also correlates well with ventilatory responses in the steady-state (Eldridge *et al.* 1984). Teppema *et al.* (1983) have considered the possibility that there are gradients of pH between the surface and the chemoreceptors and present arguments that gradients are unlikely to exist in the steady state, the conditions of their and our experiments. Even if gradients were to exist, they would, in order to affect interpretation of the results, have to be different in the two types of acidosis, an unlikely event. Finally, Cragg, Patterson & Purves (1977), using micro-electrodes, were unable to demonstrate a steady-state pH gradient in the medulla to a depth of 5 mm.

We see no alternative, therefore, to a conclusion that e.c.f. $[H^+]$ does not represent the unique stimulus to the central chemoreceptors. We have reported elsewhere that they do not respond at all to changes of c.s.f. pH produced either by CO_2 or by metabolic means (Kiley *et al.* 1984).

Several possibilities exist as explanations for the results of these studies.

(1) One is that $[H^+]$ receptor sites on the chemosensory cells are in a e.c.f. compartment that is accessible to freely diffusible CO_2 which generates $[H^+]$ locally, but not as accessible to strong ions that generate $[H^+]$.

(2) Another is that e.c.f. $[H^+]$ is not the stimulus at all, that intracellular $[H^+]$ in chemosensory cells represents the important site and mechanism of receptor stimulation. Because of the permeability of membranes to CO_2, intracellular $[H^+]$ would change more during hypercapnia than it would with extracellular metabolic changes induced exogenously.

(3) The third is that the stimulus site is extracellular, but that there is an action of molecular CO_2 that is independent of its effects on e.c.f. $[H^+]$. This would have

to mean that there are two types of receptor sites on the chemosensory cells, or that $[H^+]$ effects come from locations in the medulla that are separate from the cells that respond to CO_2. Both of these possibilities would imply that during hypercapnia some of the respiratory response is due to the CO_2 effect and some to the simultaneously generated $[H^+]$, whereas during metabolic acidosis only the $[H^+]$ mechanism is affected.

At present there is insufficient evidence to make a clear choice among these alternatives. However, the impressive effect on respiration of a small change of CO_2 after development of severe metabolic acidosis seems to us to favour the third mechanism.

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