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THE EFFECT ON RESPIRATION OF ABRUPT CHANGES IN CAROTID ARTERY pH AND P_{CO_2} IN THE CAT

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SUMMARY

1. An *in vivo* pH monitoring technique was used to assess changes in pH, and by inference changes in P_{CO_2}, in the carotid artery of anaesthetized cats. The changes in carotid artery pH and respiration following abrupt injections of various acids into the carotid artery or aorta were investigated.

2. Injections of saline equilibrated with 100 % CO_2, timed to produce changes at the carotid body chemoreceptors during early inspiration caused an increase in the tidal volume of that breath. The amplitude and rate of change of the pH changes so produced were comparable with those of the oscillations in pH produced by respiration itself.

3. The respiratory responses to injection of saline equilibrated with 100 % CO_2 occurred whether the animal was breathing air or 100 % O_2.

4. Injections of lactic or hydrochloric acid were without an effect on respiration, except when pH changes larger than 0·1 pH unit were produced. A $NaHCO_3$ solution equilibrated with 30 % CO_2 stimulated respiration, even though the solution was alkaline to the cat's arterial blood and induced an alkaline change in arterial pH.

5. Infiltration of the carotid sinus nerve area with procaine temporarily abolished the respiratory response to injections of saline equilibrated with 100 % CO_2.

INTRODUCTION

Yamamoto (1960) suggested that oscillations in the arterial P_{CO_2} (P_{a,CO_2}) may provide a stimulus to respiration different from that due to a steady level of P_{a,CO_2} at the mean of the oscillations.

That such oscillations exist is indicated by the findings that the P_{O_2} of the blood in the carotid artery of the cat oscillates at the same frequency

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as respiration (Purves, 1965) and that the pH of the blood in the brachial artery of conscious man and in the carotid arteries of the anaesthetized cat shows similar oscillations (Band & Semple, 1966, 1967). The pH oscillations have been further studied in the cat (Band, Cameron & Semple, 1969*a*) and the effects of different methods of CO_2 administration on these oscillations have been described (Band, Cameron & Semple, 1969*b*). The amplitude of the oscillations, about 0·01–0·02 pH unit, is compatible with their arising from the fluctuations in alveolar P_{CO_2} that are known to occur throughout each respiratory cycle. The oscillations in pH are abolished by rebreathing a CO_2 mixture that matches the end tidal P_{CO_2}. Whilst breathing air, the amplitude of the oscillations is related directly to tidal volume and inversely to respiratory rate. They are reduced in amplitude by breathing CO_2 at a concentration less than the end tidal value but are increased when blood equilibrated with a high P_{CO_2} is infused intravenously. Tube rebreathing modifies the contour of each pH oscillation in a manner to be expected from the calculated modification of alveolar P_{CO_2} induced by this manoeuvre (Brown, Cunningham, Goode & Howson, 1968).

It seems likely, therefore, that oscillations in the arterial pH at the frequency of respiration reflect oscillations in P_{CO_2} and that these arise in the arterial blood from the cyclical nature of breathing itself. Whether the pH or P_{CO_2} oscillations are of any significance in the control of the breathing that is producing them is unknown.

Recordings from the sinus nerve have shown that the carotid body chemoreceptor discharge has a respiratory rhythm (Hornbein, Griffo & Roos, 1961; Biscoe & Purves, 1965). The chemoreceptor itself may, therefore, be able to respond to such breath by breath oscillations.

More recently Black & Torrance (1967) found in anaesthetized cats that injections of saline equilibrated with CO_2, given retrogradely down the external carotid artery so as to reach the carotid body, cause an increase in the tidal volume of the breath taking place at the time. In order to produce this response the injections had to be timed to reach the chemoreceptor during inspiration. When given during expiration the expiratory pause was prolonged. These results illustrate a new feature of the problem, that the effect of a stimulus may depend upon its timing in relation to the respiratory cycle. A continuously rising P_{CO_2} would be expected to affect both inspiration and expiration and may not be an equivalent stimulus to repeated rises coinciding with inspiration alone.

The relevance of this observation to the normal control of respiration depends upon whether the magnitudes of the changes in the pH and P_{CO_2} that are necessary to elicit the response described by Black & Torrance (1967) are comparable with the spontaneously arising respiratory oscillations.

This paper describes the respiratory effects of abrupt injections of non-gaseous and gaseous acids in solution into the carotid artery or root of the aorta in anaesthetized cats. The changes in arterial pH produced by these injections were monitored *in vivo* by the technique described by Band & Semple (1967). The effect of systemic hyperoxia and hypoxia on the respiratory response to these injections was also investigated. These findings have already been briefly communicated (Band, Cameron & Semple, 1968).

METHODS

Cats weighing 3·0–5·0 kg were anaesthetized with either intraperitoneal sodium pentobarbitone, 30 mg/kg (Veterinary Nembutal, Abbott Laboratories Ltd.) or, by induction with ethyl chloride vapour (Evans Medical Ltd.) and subsequent intravenous injection of chloralose (40 mg/kg, B.D.H.). Supplementary doses of intravenous sodium pentobarbitone were given as required.

The animal's rectal temperature was monitored with a thermistor and maintained between 37 and 38° C with a heating pad. A catheter was introduced into the right femoral artery and the arterial blood pressure recorded using a pressure transducer (Statham P 23 Db). This catheter was also used for anaerobic withdrawal of arterial blood samples, which were analysed *in vitro* for pH, P_{CO_2} and P_{O_2}. pH was measured with a micro-capillary electrode (Radiometer, Copenhagen) standardized with buffers compatible with the National Bureau of Standards (Washington, D. C.) pH scale. P_{CO_2} was measured with a Severinghaus type electrode (Radiometer, Copenhagen) and P_{O_2} with a modified Clark cell developed in this laboratory. All measurements were made at 37·5° C and were corrected to the animal's rectal temperature using the results of Rosenthal (1948) and of Bradley, Stupfel & Severinghaus (1956).

The technique for the continuous recording of arterial pH has been described elsewhere (Band & Semple, 1966, 1967; Cowell, Band & Semple, 1967; Band *et al.* 1969*a*, *b*). A glass electrode in the form of a blind-ended capillary fits closely into the lumen of a modified Cournand needle; blood flows in the narrow annular space between the two. The reference connexion is either a silver/silver chloride electrode in direct contact with the blood or a saturated calomel electrode making contact with the blood through a salt bridge and ceramic plug liquid–liquid junction. The system samples blood at 3–4 ml./min and returns it via a side arm from the needle to a catheter in the femoral vein. The electrode needle and the returning catheter are surrounded by a water jacket maintained at the animal's rectal temperature.

The response time *in vitro* of the electrode system was found to be approximately 40 msec to 90%. The sensitivity of each electrode was measured *in vitro* before and after each experiment; the span was always better than 97% of the theoretical relation between pH and voltage at the temperature at which standardization was performed. The pH changes indicated *in vivo* by the electrode were calculated from the responses to voltages equivalent to 0·05 and 0·1 pH unit at 37·5° C, applied between the inputs of the pH meter.

In all the experiments the needle was placed to sample blood flowing through the common carotid artery close to the carotid body. Provision was made to allow injections into the blood passing to the chemoreceptor and the electrode. In the majority of experiments a polyethylene loop was inserted into the right common carotid artery about 1·5 cm below the carotid sinus. The loop was interrupted by a mixing chamber designed to promote turbulence; the tip of the needle housing the pH electrode was inserted through a side arm distal to this (for details of the loop see

Fig. 1*A*). The total volume of the loop and mixing chamber was 0·35 ml. Injections were made either with a fine needle inserted through a silicone rubber sleeve on the loop proximal to the mixing chamber, or via a catheter passed from the left femoral artery and reaching beyond the origin of the right common carotid artery, so that its tip lay at the root of the aorta. The passage of this catheter was aided by observing the deflexion of the pH trace when an injection of saline equilibrated with 100% CO_2 was made. Correct positioning of the catheter was confirmed in every experiment at post-mortem examination.

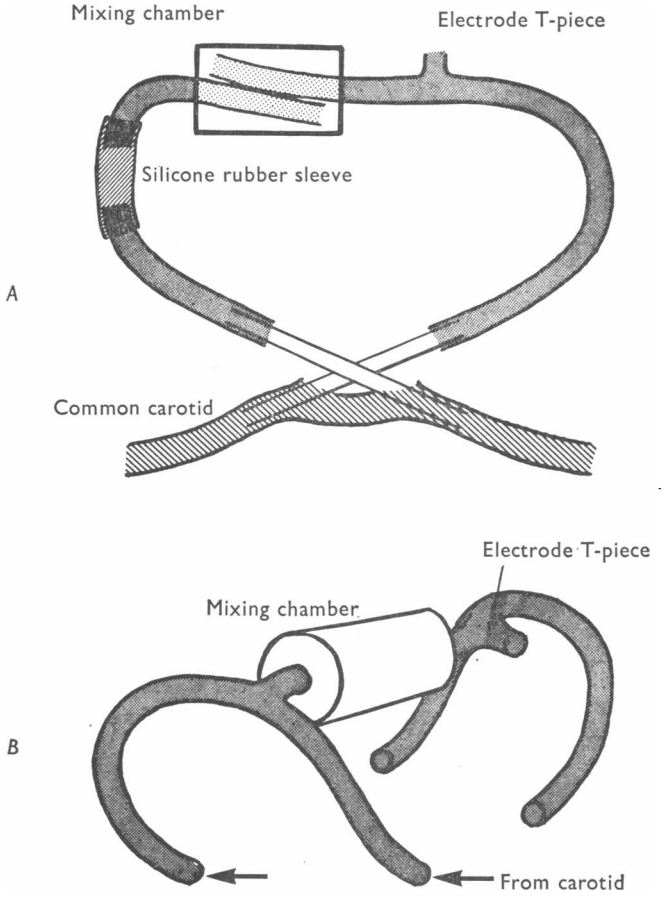

Fig. 1. *A*, unilateral loop and mixing chamber which was inserted into the right common carotid artery. *B*, bilateral loop and common mixing chamber.

In two of the experiments the electrode needle was inserted into a short nylon cannula tied either into the external carotid or lingual arteries, distal to the carotid sinus. A small gate clamp on the tube connecting the side arm of the needle to the femoral venous cannula was adjusted to vary the flow through the system. The pressures in the loop and the cannula were measured and the gate clamp adjusted to give about 5 mm Hg difference in the diastolic pressures. This corresponded to a flow of about 20 ml./min when measured directly into a syringe.

In the remainder of the experiments both common carotids were cannulated and the blood led to a common mixing chamber at the outlet of which the electrode needle tip was inserted. The flow was then conducted to the headward continuation of the common carotids (see Fig. 1*B*). Injections were made via a catheter in the root of the aorta. The total volume of the double loop and mixing chamber was about 0·5 ml.

The action of the mixing chamber in promoting turbulence and so preventing streaming of the injectate was checked *in vitro*, using dyes. The flow entering and leaving the loop was measured in one cat using an electromagnetic flow probe. No change in the headward flow signal was seen when the shunt through the electrode was opened or closed, although on opening the shunt there was a small (3–4 ml.) increase in the flow rate into the loop that corresponded to the amount drawn off by the electrode system. Heparin (3000 i.u./kg, Burroughs Wellcome Ltd.) was given intravenously before blood was allowed to flow through the electrode system.

Injections of various pre-warmed solutions were given to produce transient changes in the carotid artery blood pH and P_{CO_2}. When given directly into the carotid artery loop the volume injected was less than 0·05 ml.; when injected into the root of the aorta the volumes injected ranged from 0·1 to 0·3 ml. In some experiments the aortic catheter had a double lumen enabling two solutions to be injected alternately, so facilitating a comparison of their effects.

All the cats breathed spontaneously through a tracheostomy which was connected to a pneumotachograph (Fleisch); the inspiratory flow signal was integrated and recorded as tidal volume. The CO_2 in the airway was measured with an infra-red CO_2 analyser (Beckman LBI), sampling from a point distal to the pneumotachograph. When required, 100% O_2 or 10% O_2 was passed across a T-piece attached to the end of the airway, the flow being adjusted to exceed the peak inspiratory flow rate.

All the signals were recorded by a photographic recorder (Electronics for Medicine Inc., White Plains N.Y., model D.R.8), the multitrace monitor oscilloscope of which was used to facilitate timing of the injections.

RESULTS

The effect of control injections on carotid artery pH and respiration

Saline solutions equilibrated with room air or with nitrogen were used as controls for the injections of gaseous and non-gaseous acids. These control injections had no measurable effect on the pH of carotid artery blood or on respiration when the volume injected was 0·3 ml. or less; larger volumes did affect pH and respiration (see below).

The respiratory effect of injections of saline equilibrated with 100 % CO_2

Injections of saline equilibrated with 100% CO_2 were given to produce changes in carotid artery pH comparable with those occurring with breathing. They were timed to coincide with approximately every fifth breath. In any cat described as responding to these injections, the breath associated with the injection was of greater tidal volume than the four

preceding or succeeding breaths and four or more consecutive injections showed this effect.

Nine out of eighteen cats (50 %) responded when such injections were made into the unilateral carotid loop (Fig. 1*A*). Nineteen out of twenty-four cats (79 %) responded when injections were made via the catheter in the root of the aorta. Injections were made both into the unilateral carotid loop and into the root of the aorta in three cats. One cat responded to the injections at either site; the other two were completely unresponsive. There was no difference in the pH changes produced by the injection at the two sites, nor in the individual responses to these injections.

Initially all responsive cats showed respiratory effects to injections that produced pH changes of less than 0·04 pH unit. The majority became less sensitive as each experiment proceeded. Progressively larger injections were required to produce a response until pH changes of 0·1 pH unit were without effect. This change of sensitivity with time prevented further analysis of the results in the majority of cats; four cats (A, B, C and D), however, maintained a constant sensitivity throughout the experiment. All had bilateral carotid loops (Fig. 1*B*) and injections were made via a catheter in the aorta. In these four cats injections which produced pH changes equal to or less than the amplitude of the previous respiratory oscillation were associated with a mean increase in tidal volume of 15·5 % ± 4·2 (± S.D. of an observation) as compared to the previous tidal volume.

The changes in pH induced by the injections occurred at varying points in the train of normal respiratory oscillations. The amplitude of the change is expressed as the difference between the pH value at the peak of the produced pH change and the pH value at the corresponding instant of the previous oscillation. This was achieved by superimposing a tracing of the previous oscillation over the oscillation that had been interrupted by the change induced by the injection. The maximum rate of change of pH produced by an injection or a normal oscillation was estimated from the slope of the line drawn through the position of maximum rate of change of pH; such measurements can only be taken as an approximate guide in comparing the rate of change of pH.

The maximum rate of change of pH produced by injections of saline equilibrated with 100 % CO_2 ranged from 0·015 to 0·002 (mean 0·007) pH unit/sec; the maximum rate of change of the respiratory oscillations preceding these injections ranged from 0·012 to 0·005 (mean 0·007) pH unit/sec. Fig. 2 shows the respiratory responses produced by injections of saline equilibrated with 100 % CO_2 which induced changes in pH of various amplitudes and with varying maximum rates of change.

Timing of the injections

In order to affect the volume of a given inspiration an injection had to be timed to produce a deflexion of the pH trace soon after the start of that inspiration. The effective period was usually less than one-fifth of the period of a respiratory cycle.

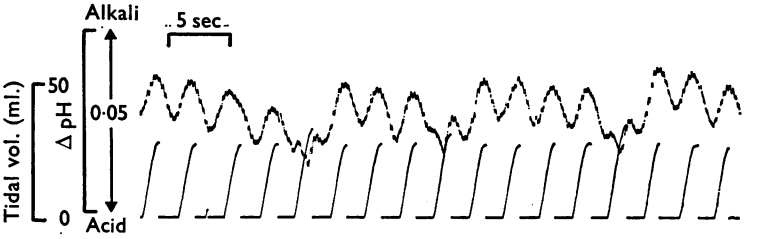

Fig. 2. The respiratory response to injections of saline equilibrated with 100% CO_2 given via a catheter in the root of the aorta (volume injected was 0·2 ml.). Upper trace, carotid artery pH. Lower trace, tidal volume. The injections were given on the fifth, ninth and fourteenth breaths on this trace. Alkaline changes are represented by an upward deflexion of the pH trace.

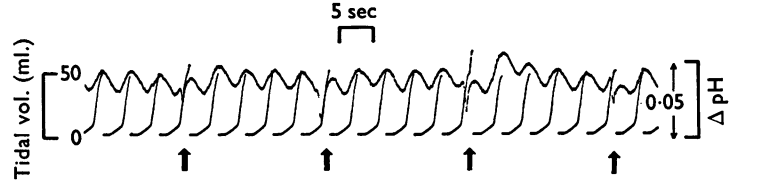

Fig. 3. The respiratory responses to injections of saline equilibrated with 100% CO_2 given via a catheter in the root of the aorta (volume injected was 0·3 ml.). Upper trace, carotid artery pH. Lower trace, tidal volume. Alkaline changes are represented by an upward deflexion of the pH trace. Times of injection are indicated by arrows. The second injection has been timed too early and the fourth injection too late in inspiration to produce an increase in tidal volume.

Fig. 3 illustrates this feature of the response. Inspiration on this trace is taking place as the pH is rising in the carotid loop. In order to affect the inspiratory tidal volume, the injections had to be timed so as to arrive in the carotid loop shortly after the pH had started to rise.

Fig. 4 shows a similar trace, but in this animal inspiration is coincident with a falling pH in the carotid loop. Here the injections had to be timed so as to produce changes as the pH was falling. Thus the response is governed by the timing of the change in pH induced by the injection in relation to the respiratory cycle, and not to some point in the pH oscillation occurring with breathing.

The phase relation between the respiratory cycle and the simultaneous

changes in arterial pH at the electrode is determined by the respiratory rate and the lung to electrode circulation time (Band *et al.* 1969*a*); this relation can vary in the same cat at different times. The response to the injections was not affected by these variations in phase; it was always governed by the timing of the injection in relation to the respiratory cycle and not to the pH oscillation.

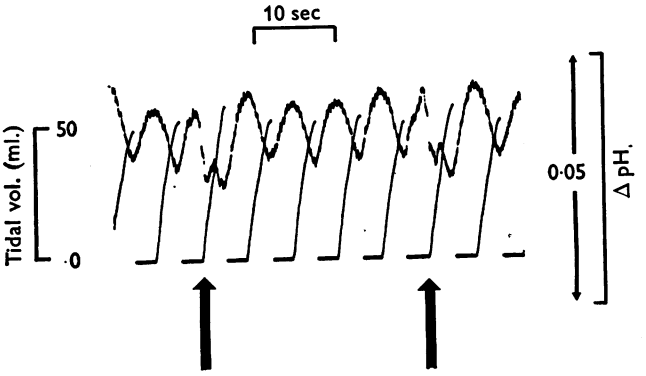

Fig. 4. The respiratory responses to injections of saline equilibrated with 100% CO_2 given via a catheter in the root of the aorta (volume injected was 0·3 ml.). Details as for Figs. 2 and 3. The injection had to arrive while the arterial pH was falling to coincide with early inspiration and produce a respiratory effect.

The effect of injections given during expiration was variable and was not fully investigated. In general these injections did not affect the next inspiration nor the expiratory volume, but on occasion the expiratory pause was prolonged.

Variation of sampling sites

In two cats the electrode needle sampled from the external carotid artery. The respiratory pH oscillations were between 0·015 and 0·02 pH unit in amplitude. Respiratory responses were seen to injections that produced pH deflexions comparable to these respiratory pH oscillations.

Both the respiratory oscillations and the pH changes following injections recorded from the external carotid artery were similar to those recorded from the common carotid artery. The external carotid sampling site was above the origin of the occipito-pharyngeal trunk and the carotid body whilst the common carotid site was below. The similarity of the observations at these two sites makes it unlikely that the changes indicated by the electrode in the common carotid artery loop are different from those occurring at the carotid body itself.

The effect of blocking the sinus nerve

In three cats the effect of blocking the sinus nerve on the respiratory response to injections of saline equilibrated with 100 % CO_2 was investigated. The response to such an injection was first established and the region of the junction of the sinus nerve with the glossopharyngeal nerve was then infiltrated with procaine. The respiratory response to the injections was abolished as was the response to lobeline injections. The respiratory response to both these injections reappeared 20 min after the block had been produced. In two of the cats a second block was performed and the responses again recovered after 20 min.

The respiratory response to injections of non-gaseous acids

HCl was diluted in isotonic saline so that volume for volume the injections produced pH changes ranging from 1 to 10 times those induced by saline equilibrated with 100 % CO_2. Lactic acid solutions were similarly prepared.

A respiratory response to saline equilibrated with 100 % CO_2 was established in twenty-three cats. Twenty of these were then tested for the respiratory response to injection of HCl solution and three for the response to lactic acid. No animal had a respiratory response to an injection of these acids when the pH changes produced were comparable with those evoked by the test solution of saline equilibrated with 100 % CO_2; three animals responded to HCl injections causing pH changes greater than 0·1 pH unit.

This trial was not entirely conclusive since fifteen of these cats were found to have lost their response to saline equilibrated with 100 % CO_2 when this was re-tested after the non-gaseous acid. The remaining eight cats, including one that did not respond to lactic acid, responded normally to the second series of injections of saline equilibrated with 100 % CO_2. The tendency for the response to disappear with time might account for the loss of the respiratory response to saline equilibrated with 100 % CO_2 in some animals. The absence of a respiratory response to non-gaseous acid injections was confirmed in a further three cats in which injections of saline equilibrated with 100 % CO_2 were alternated with injections of HCl (two cats) or lactic acid (one cat) using a double lumen catheter with its tip placed in the root of the aorta. All three cats responded to injections of saline equilibrated with 100 % CO_2 and none to the injection of HCl or lactic acids. Since alternate injections could be given at short intervals over a considerable period by this technique, the possibility that the results were caused by variation in the respiratory response with time was excluded. The results from an experiment with a double lumen catheter are shown in Fig. 5.

The respiratory response to injections of a bicarbonate solution equilibrated with 30 % CO_2

A solution of $NaHCO_3$ (150 m-equiv/l.) was equilibrated with a mixture of 70 % O_2 and 30 % CO_2 at 37° C. This solution had a pH of 7·45 and was alkaline with respect to the arterial blood of the cats studied. The solution was injected via a catheter in the root of the aorta and stimulated the breath taking place at the time of injection, even though the pH change so produced was alkaline (see Fig. 6).

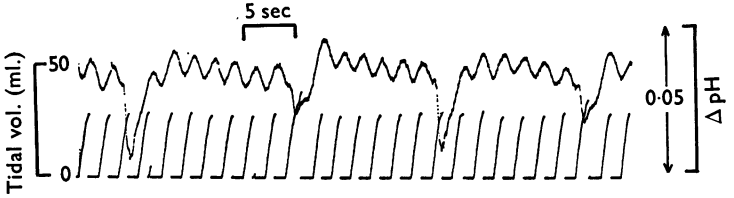

Fig. 5. Alternate injections of saline equilibrated with 100 % CO_2 and of HCl. The injections were given via a double lumen catheter in the root of the aorta. The upper trace shows the changes in carotid artery pH (acid changes downwards) and the lower trace tidal volume. The first and third acid deflexions were caused by HCl injections, and second and fourth by saline equilibrated with CO_2. Respiratory responses occur only with the injection of saline equilibrated with CO_2.

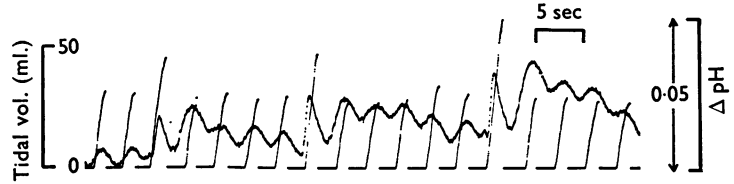

Fig. 6. The respiratory responses to injections of $NaHCO_3$ solution (150 m-equiv/l.) equilibrated with 30 % CO_2 at 37·5° C. Injections were given via a catheter in the root of the aorta. Upper trace, carotid artery pH; lower trace, inspiratory tidal volume. Alkaline changes are represented by an upward deflexion of the pH trace. The volume of solution injected was 0·3 ml.; injections were given on the third, eighth and fourteenth breaths.

Injections of a NaOH solution diluted in saline to give comparable alkaline changes in pH for a similar volume produced no respiratory response.

The effect of systemic hypoxia or hyperoxia in the response to injections of saline equilibrated with 100 % CO_2

The respiratory responses to injections of saline equilibrated with 100 % CO_2 were compared during periods of hypoxia and hyperoxia. The animals breathed the following gas mixtures in the sequence—air—100 %

O_2—air—10% O_2—air. The four cats (A, B, C and D) with the most constant respiratory response to these injections while breathing air were used for investigation. The period of 10% O_2 breathing in cat D was abandoned because of the development of cardiac arrhythmias.

The timing of such an injection in the respiratory cycle and the size of the pH change produced are important in determining the magnitude of the respiratory response (Band *et al.* 1968). Thus to compare the respiratory responses to these injections while the animal was breathing different O_2 mixtures, each injection should be given at the same point in the respiratory cycle and produce a similar change in pH. It was impracticable to produce an injection apparatus that could control precisely the change in pH induced by each injection. The injections were, however, carefully timed to give maximum respiratory responses; the correct timing and amplitude of pH change necessary to produce a maximum response were determined by trial injections at the beginning of each series of injections.

This series of experiments, in which the respiratory response to injections of saline equilibrated with 100% CO_2 was investigated in animals breathing air, 10% O_2 and 100% O_2, confirmed that the magnitude of the respiratory response varies with the amplitude of the pH change induced. This was established by a rank correlation analysis of these two variables; the correlation was significant in all four cats breathing air, in three of the four when breathing 100% O_2 and in two of the three animals in which it was examined while breathing 10% O_2 (see Table 1*a*).

The results of these experiments were distributed too abnormally to allow comparison of the dose–response relation during the inhalation of each gas by linear regression analysis. It was necessary, therefore, to use a non-parametric test. The pH deflexions were paired for equal size of amplitude in the same cat breathing each gas mixture and the respiratory responses compared by Wilcoxon's test for pair differences. In this way the respiratory responses for identical pH changes breathing air and 10% O_2 could be compared and similarly those breathing air and 100% O_2. The results of this analysis are shown in Table 1*b*. No consistent effect of hypoxia or hyperoxia was found on the respiratory response to injections of saline equilibrated with 100% CO_2. Respiratory responses to induced pH changes of less than 0·02 pH unit were seen in animals with a P_{a,O_2} greater than 500 mm Hg. Comparison of the respiratory responses during the first and last air breathing periods by Wilcoxon's test showed that there had been no significant change in these responses during the course of any of the experiments.

The pH changes and respiratory responses produced by injection of isotonic saline

It was noted that increasing the volume of the saline control injections to about 0·5 ml. produced an alkaline pH change in carotid artery blood. The respiratory response to this alkaline pH change was investigated in seven cats in a manner identical to the studies using saline equilibrated with 100 % CO_2. When these injections were timed to produce alkaline

TABLE 1. The effects of systemic hypoxia and hyperoxia on the respiratory response to injections of saline equilibrated with 100 % CO_2

(*a*) Rank correlation analysis between the amplitude of the induced pH change and the magnitude of the respiratory response. The analysis has been carried out for each cat breathing air, 100 % O_2 and 10 % O_2 (no test during 10 % O_2 was possible in cat D because of cardiac arrhythmias). The correlation was significant ($2\alpha < 0{\cdot}05$) in nine out of eleven tests.

(*b*) The effect of inhalation of air, 10 % O_2 and 100 % O_2 on the respiratory response to injections of saline equilibrated with 100 % CO_2. Respiratory responses breathing air and 10 % O_2 and air and 100 % O_2 have been compared by pairing induced pH deflexion for equal amplitude and testing the responses by Wilcoxon's test. The Table shows if the differences were significant ($2\alpha < 0{\cdot}05$) or not significant (N.S.). Air > 100 % O_2 means that the respiratory responses to injections of saline equilibrated with 100 % CO_2 were greater breathing air than 100 % O_2. Inhalation of 100 % or 10 % O_2 produced no consistent effect on these respiratory responses.

(*a*) Rank correlations

	Air	100 % O_2	10 % O_2
Cat A	$2\alpha < 0{\cdot}001$ $r = 0{\cdot}662$ $n = 32$	$2\alpha < 0{\cdot}001$ $r = 0{\cdot}819$ $n = 36$	$0{\cdot}02 < 2\alpha < 0{\cdot}05$ $r = 0{\cdot}633$ $n = 14$
Cat B	$0{\cdot}01 < 2\alpha < 0{\cdot}02$ $r = 0{\cdot}439$ $n = 33$	$2\alpha < 0{\cdot}001$ $r = 0{\cdot}811$ $n = 37$	$0{\cdot}05 < 2\alpha < 0{\cdot}1$ $r = 0{\cdot}252$ $n = 42$
Cat C	$0{\cdot}001 < 2\alpha < 0{\cdot}01$ $r = 0{\cdot}485$ $n = 38$	$0{\cdot}001 < 2\alpha < 0{\cdot}01$ $r = 0{\cdot}355$ $n = 55$	N.S. $n = 10$
Cat D	$0{\cdot}01 < 2\alpha < 0{\cdot}02$ $r = 0{\cdot}385$ $n = 44$	N.S. $n = 38$	

(*b*) Wilcoxon's pair differences

	Air: 100 % O_2	Air: 10 % O_2
Cat A	$0{\cdot}05 < 2\alpha < 0{\cdot}1$ Air > 100 % O_2	$0{\cdot}01 < 2\alpha < 0{\cdot}02$ Air < 10 % O_2
Cat B	$2\alpha < 0{\cdot}05$ 100 % O_2 > Air	N.S.
Cat C	$2\alpha < 0{\cdot}01$ Air > 100 % O_2	N.S.
Cat D	$0{\cdot}01 < 2\alpha < 0{\cdot}05$ Air > 100 % O_2	

changes in pH in early inspiration, the tidal volume of that breath was decreased. The mean decrease in tidal volume was 7·3%. The alkaline changes in pH produced by these injections varied between 0·005 and 0·02 pH unit.

DISCUSSION

The present work confirms and extends the findings of Black & Torrance (1967) that injections of saline equilibrated with 100% CO_2, which produced changes in carotid artery pH no greater than the normal respiratory oscillations, were found to stimulate the breath taking place at the time of injection. This response only occurred if the injections were timed to coincide with early inspiration. Injections of lactic acid and hydrochloric acid which produced comparable changes in carotid artery pH had no effect on respiration. Injection of a $NaHCO_3$ solution of high P_{CO_2} stimulated respiration, although the pH changes produced by this solution were alkaline. These observations indicate that this respiratory response is produced by a transient rise in P_{CO_2} rather than by a fall in pH.

Both Joels & Neil (1960) and Hornbein & Roos (1963) have investigated the relative importance of changes in pH and P_{CO_2} as chemoreceptor stimuli. Joels & Neil (1960) perfused the carotid body of anaesthetized cats with solutions of varying P_{CO_2} and $[HCO_3^-]$; it was possible to change P_{CO_2} at a constant pH, or, to change the pH of the perfusate at constant P_{CO_2}. Chemoreceptor discharge was increased in both situations. It appeared that changes in either pH or P_{CO_2} were independent stimuli to the carotid body. Hornbein & Roos (1963) investigated the effect on chemoreceptor discharge of increasing the inspired P_{CO_2} in anaesthetized cats, first at a normal $[HCO_3^-]$ and second 1 hr after the $[HCO_3^-]$ had been approximately doubled. They found that there was a change in the relation between discharge and P_{CO_2}, but that there was no change in the relation between discharge and pH. They concluded that chemoreceptor discharge could be related solely to extracellular pH, a finding opposite to that of Joels & Neil (1960). Hornbein & Roos (1963) reconciled their findings with those of Joels & Neil (1960) by postulating that pH appeared to be the dominant stimulus in their experiments since they had allowed a relatively long time (1 hr) for equilibration after a change in $[HCO_3^-]$. Such a view would seem reasonable since it might be expected that CO_2 would diffuse rapidly into a critical compartment of the chemoreceptor, whereas HCO_3^- or H^+ ions would move relatively slowly. This interpretation is supported by the observations of Jacobs (1920*a*, *b*) on the relative speeds of diffusion of CO_2, H^+ and HCO_3^- through tissues. Gray (1968) investigated further the changes in impulse traffic in the sinus nerve when the pH or P_{CO_2} of the solution perfusing the carotid chemoreceptor was

altered. An increase in P_{CO_2} or a reduction in $[HCO_3^-]$ both produced a change in impulse frequency. The chemoreceptor response produced by an increase in P_{CO_2} occurred approximately twice as fast as it did when $[HCO_3^-]$ was reduced. It would seem reasonable to postulate that in the rapid respiratory responses produced by transient changes in P_{CO_2} or pH in our experiments the effect of P_{CO_2} might be expected to dominate. This view is substantiated by the experimental findings which confirm that these respiratory responses were produced by a transient rise in carotid artery blood P_{CO_2} but not when a comparable fall in pH was otherwise induced.

This absence of a respiratory effect with injections of HCl or lactic acids was unexpected, since lowering the pH of blood in a closed system *in vitro* by the addition of either of these acids produces a corresponding rise in P_{CO_2}. Over the small range of pH of the oscillations or the induced changes, the change in P_{CO_2} giving rise to a change of approximately 0·02 pH unit should be almost identical with the P_{CO_2} rise caused by lowering the pH of blood by 0·02 pH unit with a non-gaseous acid. It is necessary to postulate, therefore, that *in vivo* the acid injections do not liberate a significant quantity of CO_2 in the time taken (1–2 sec) for blood to pass from the root of the aorta to the carotid chemoreceptor.

When saline equilibrated with 100 % CO_2 is added to the plasma most of the CO_2 is in the form of gaseous dissolved CO_2. This can diffuse rapidly to the carbonic anhydrase within the red cell. Here it is hydrated and plasma pH rapidly lowered. The work of Constantine, Craw & Forster (1965) shows that 1–2 sec is probably adequate for this hydration and buffering. Changes in plasma pH would be expected, therefore, to mirror the changes in P_{CO_2} that induced them.

When a non-gaseous acid is added to the plasma H_2CO_3 is formed immediately. Outside the red cells the dehydration of H_2CO_3 is slow. The relatively slow rate of diffusion of H_2CO_3 into the red cells may be a limiting factor in preventing a significant rise in P_{CO_2} in the time available.

The decrease in tidal volume produced by the larger saline injections (*ca.* 0·5 ml.) could be due to a sudden lowering of P_{CO_2} by dilution of the blood. The alkaline changes observed in carotid artery blood during the saline injections are, however, unexpected. Diluting blood with saline *in vitro*, in a closed system, lowers the P_{CO_2} but does not change the pH (Van Slyke, Weisinger & Van Slyke, 1949). The discrepancy between these findings recorded *in vivo* and those previously established *in vitro* could be explained in terms of an artifact due to the greater relative volume of saline injected in these experiments. Alternatively it may serve to emphasize that *in vivo* changes in $P_{a,\,CO_2}$ and arterial pH cannot be predicted from *in vitro* observations.

The explanation for the absence of the respiratory response in some animals and its disappearance with time in others is not apparent. There was no detectable change in tidal volume, in respiratory rate, in the phase relation between the respiratory cycle and the oscillation in arterial pH or in the blood pressure when the sensitivity to injections decreased or was lost.

Arterial O_2 tensions as high as 500 mm Hg failed to abolish the response to saline equilibrated with 100% CO_2; cat B was surprisingly more sensitive breathing 100% O_2 than breathing air. Hypoxia induced by inhalation of 10% O_2 did not consistently enhance the response. These findings are of importance, since it has been found that peripheral chemoreceptor activity contributes little to the respiratory response to steady-state CO_2 administration under hyperoxic conditions (Neil & Joels, 1963).

It may be concluded that the carotid chemoreceptor is extremely sensitive to small, abrupt changes in $P_{a,\,CO_2}$ and that these changes can affect respiration if they occur at an appropriate point in the respiratory cycle. The chemoreceptor response to transient changes in $P_{a,\,CO_2}$ differs from the steady-state response since the response to P_{CO_2} persists during the inhalation of 100% O_2; this contrasts with results obtained during steady-state conditions. Our results emphasize that predictions based on steady-state observations may not be applicable to rapidly changing states.

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