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EFFECT ON RESPIRATION OF CHANGES IN THE FORM OF THE NATURALLY OCCURRING OSCILLATION IN ARTERIAL pH

D. M. BAND, P. EBDEN, S. J. G. SEMPLE and C. B WOLFF

Department of Medicine, St. Thomas' Hospital,
Medical School, London, England

Abstract. We have previously shown that the respiratory control system in the cat has the requisite sensitivity and speed to respond to changes in arterial pH which are equal to, or smaller than, the normal fluctuations in pH with respiration. A respiratory response was only observed when the changes in pH were produced by alterations in PCO_2 and not when they were induced by non-gaseous acids. We describe now the respiratory effect produced by various procedures which modify or abolish the naturally occurring arterial pH oscillations before they reach the peripheral chemoreceptors. The pH oscillations were abolished by a mixing chamber, whilst their phase relationship to respiration was altered by delay coils; by presenting a moving plastic surface to the arterial blood stream it was possible to distort the shape of the oscillations without increasing the transport lag between lung and peripheral chemoreceptors. All manoeuvres produced a brief period of respiratory stimulation which was greater than could occur by chance. We were particularly concerned in these experiments with the possibility of artefacts in our experimental design. We feel that we have excluded respiratory effects due to changes in arterial pressure, blood temperature and mean pH. We are less confident that we have excluded changes in ventilation resulting from the release of a substance (or substances) from the contact of blood with plastic or glass surfaces. This problem is discussed in relation to experiments on the control of breathing where blood, which is perfusing the chemoreceptors, has been in contact with artifical surfaces.

INTRODUCTION

The relationship between pulmonary ventilation and the mean levels of the chemical stimuli to breathing have been well studied. Recent interest has been concerned with additional components of these signals other than their mean levels. Yamamoto and Edwards (1960) suggested

that temporal fluctuations in the arterial partial pressure of carbon dioxide ($PaCO_2$) could be one such component. Dutton and his colleagues have provided experimental evidence that the rate of rise of $PaCO_2$ may stimulate breathing independant of the absolute partial pressure (Dutton et al. 1968). Black and Torrance (1967) demonstrated that the respiratory effects of an abrupt change in $PaCO_2$ at the carotid body depended on the timing of the change in the respiratory cycle. In part this work has been hampered by the technical difficulties of measuring rapid and transitory changes in $PaCO_2$ and/or pH in vivo. This difficulty has largely been overcome by the development of an indwelling arterial pH electrode with a rapid response time (Band and Semple 1967). With this electrode we set out to determine the sensitivity and speed of response of the respiratory control system, and also whether temporal fluctuations in arterial pH might play a part in chemical control of breathing.

The pH of arterial blood has been found to fluctuate at the same frequency as respiration in man, dog, rabbit and cat. Evidence has been presented that these oscillations in pH are due to changes in $PaCO_2$ and are secondary to fluctuations in alveolar PCO_2. Injections of saline equilibrated with 100% CO_2 timed to produce changes at the carotid body chemoreceptors of the cat during inspiration caused an increase in tidal volume of that breath. The amplitude of the pH changes so produced were comparable with those of the oscillations in pH produced by respiration itself. The respiratory responses occurred whether the animal was breathing air of 100% O_2. Injections of lactic or hydrochloric acid were without effect except when pH changes were in excess of 0.1 pH units. Finally infiltration of the carotid sinus nerve area with procaine temporarily abolished the respiratory response to injections of saline equilibrated with 100% CO_2 in the cat. These results have been published in detail (Band et al. 1969*ab*, 1970).

These preliminary reports suggested that the respiratory control system in the cat had the necessary sensitivity and speed to respond to the normal fluctuations in $PaCO_2$. Fluctuations in arterial pH within the physiological range produced by non-gaseous acids were without effect on respiration. We report here the effects of various manoeuvres designed to abolish, distort or change the time of arrival of the naturally occurring oscillations in $PaCO_2$ in the carotid arteries on respiration and carotid artery pH in the cat.

METHODS

The cats, weighing 3.0–5.0 kg, were anaesthetized with intraperitoneal sodium pentobarbitone (30 mg/kg). Blood pressure was recorded from the right femoral artery and the root of the aorta was cannulated retrograde-

ly via the left femoral artery so that injections of saline equilibrated with 100% CO_2 could be given. This enabled us to see if the animal had a respiratory response to abrupt injections of CO_2 in saline and if this sensitivity to CO_2 injections was retained throughout the experiment. The animals were tracheotomised and inspiratory tidal volume measured by integration of the flow signal from a Fleisch head. The CO_2 in the airway was measured with an infra-red CO_2 analyser (Beckman LB1), sampling from a point distal to the pneumotachograph. Both common carotid arteries of the cat were cannulated and connected to a common channel (the shunt), after which the blood flow was connected to the headward continuation of both common carotids (Fig. 1). By application

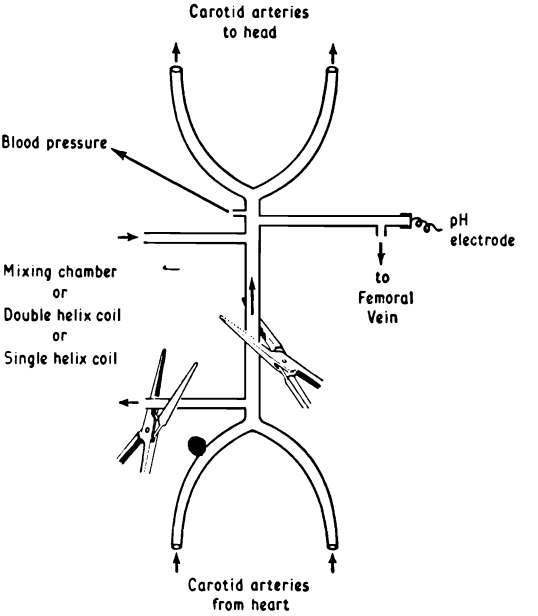

Fig. 1. Diagram of system to divert carotid blood flow through mixing chamber or delay coil.

of a clamp to the shunt blood could be diverted to a coil or mixing chamber thereby altering the pattern of the oscillations and their time of arrival at the carotid body. Arterial pH and blood pressure were recorded distal to the shunt and to the coil or chamber (Fig. 1). The glass electrode fitted into a short length of needle tubing through which blood flowed at 3–4 ml/min and returned to the animal via a femoral vein. The reference connection was a saturated calomel electrode making contact with the blood through a salt bridge and ceramic plug liquid — liquid junction. The animal's rectal temperature was monitored with a thermistor and maintained between 37 and 38°C with a heating pad.

The arterial pH oscillations were abolished by closing the shunt and passing the carotid flow through a mixing chamber (volume 15 ml.) which was surrounded by a water jacket maintained at the same temperature as that of the animal (Fig. 2).

The relationship between the respiratory cycle and the simultaneously occurring pH oscillation at the carotid body was changed by prolonging

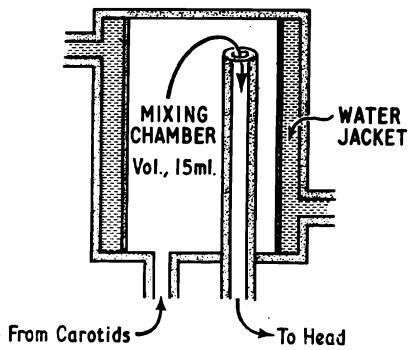

Fig. 2. Diagram of mixing chamber for the abolition of oscillations in the PCO_2 of carotid artery blood.

the circulation between lung and electrode. It was proposed, by changing the circulation time, to change a rising pH at the carotid body at the beginning of inspiration to a falling one or vice versa (Fig. 3). If, as shown by Black and Torrance (1967), the effect of a chemical stimulus

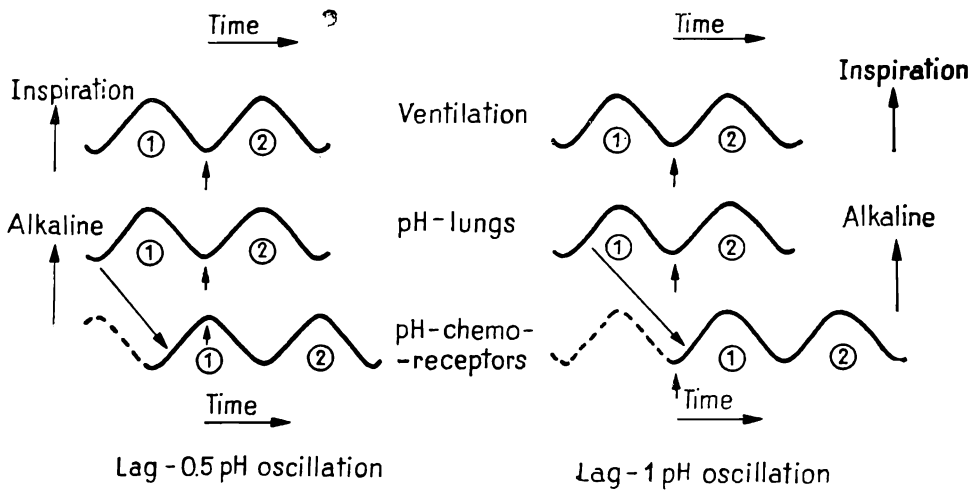

Fig. 3. Diagram to illustrate the effect of circulatory delay between lungs and chemoreceptors on the phase relationship between oscillations in $PaCO_2$ and respiration.

depends on its timing in the respiratory cycle, then conversion of a rising pH to a falling pH at the beginning of inspiration should stimulate breathing whereas respiration should be depressed by changing a falling pH to a rising one.

Alteration in the circulation time between lung and carotid body was produced by a variable teflon delay coil. This coil consisted of a semi-circular section two-start thread machined on a teflon rod. This screwed through a silicone rubber sealing nut and into a teflon sleeve. The sleeve converted the threads into two spiral channels. An inlet and an outlet pipe passed through the sleeve close to the sealing nut and picked up the two threads. The threads stopped short of the end of the rod and were joined by a short bridging channel. When the rod was screwed fully out, blood entered by the inlet pipe, passed along this bridge and left by the exit pipe. As the rod was screwed further through the nut and into the sleeve blood passed along one of the threads, across the bridge and returned by the other thread. In the experiments with the delay coil the shunt remained closed and blood flow through the coil was continuous.

The relationship between the respiratory cycle and the simultaneously occurring pH oscillations at the carotid body is referred to as the phase relationship between these two variables. To see if there had been a phase change when analysing the experimental results we determined the point on the pH oscillation with which inspiration coincided before and after changing the circulation time.

Analysis of results

Determination of whether there had been a respiratory response to diversion of the carotid flow from the shunt to the chamber or coil could readily be detected by inspection of the trace. Because this method of determination was open to observer bias we adopted the following procedure. The mean and standard deviation (SD) of the tidal volume was determined for the four breaths before the shunt was closed or the coil turned. The SD was then multiplied by 3.18 which is the value of t for a p value of 0.05 when the degrees of freedom are three. This value ($3.18 \times$ SD) was then added and substracted from the mean of the control breaths and if, after closing the shunt or turning the coil, the tidal volume of any of the subsequent five breaths exceeded or was below range, then a respiratory response was considered to have occurred. This procedure was not adopted for statistical purposes but was designed to avoid observer bias in deciding whether a change in ventilation had occurred. During each experiment this analysis was carried out on nine consecutive breaths between experimental observations when the cham-

ber or coil had not been used. We did this to determine the number of occasions a respiratory response would have been recorded due to spontaneous variation in tidal volume. In all cats the frequency of a respiratory response never exceeded 15% of all these control observations.

RESULTS

Mixing chamber: abolition of the oscillations produced a brief period of hyperventilation on 32 occasions in 66 trials in 7 cats (Fig. 4 and Table I). Two cats showed no respiratory response in all trials, two responded on every occasion and three cats responded in about half the number of trials.

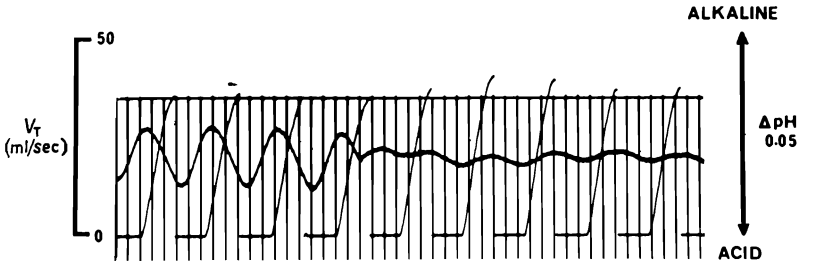

Fig. 4. Changes in tidal volume and carotid artery pH produced by mixing chamber. The point of closure of the shunt and diversion of blood through the chamber is where the oscillations in pH diminish. The vertical lines are 1 sec apart.

Delay coil: changing the phase relationship between the respiratory cycle and the pH oscillation produced a brief period of hyperventilation on 74 occasions in 129 trials in six cats (Fig. 5 and Table I). All cats responded with approximately the same frequency, namely in about 50% of these trials. The magnitude of the phase change was usually between

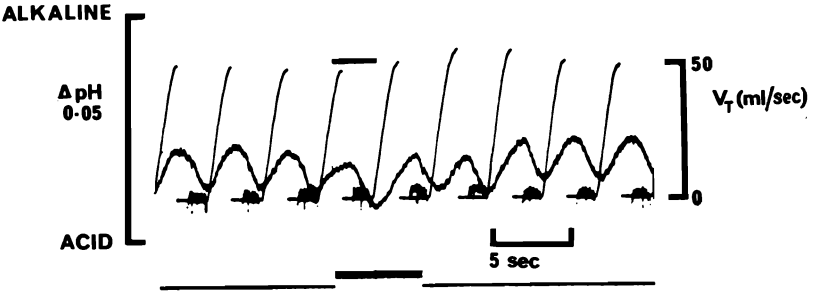

Fig. 5. Changes in tidal volume and carotid artery pH during lengthening of the delay coil. The start of the turn of the coil is indicated by the signal at the bottom of the record.

TABLE I

Frequency of the respiratory responses in the mixing chamber and delay coil experiments

	Mixing chamber	Delay coil
Number of cats	7	6
Number of trials	66	129
Respiratory response		
Number	32	74
Percentage	49	58
No respiratory response		
Number	34	55
Percentage	51	42

0.25 to 0.5 of a pH oscillation. In Fig. 5 the phase change produced was 0.25 of a pH oscillation and occurred in the fifth and sixth breath, thereafter the phase relationship returned to its original position, that is the beginning of inspiration coincided with the trough of the pH oscillation. In the majority of trials the phase relationship once changed remained that way, after a brief disturbance due to the hyperventilation, till the next turn of the coil. In some trials, as in the experiment shown in Fig. 5, the phase relationship resumed its original position after the hyperventilation. As the duration of the breath during the control period was the same as that after the hyperventilation, the resumption of the original phase relationship must be due to a change in circulation time between lung and electrode.

In addition to the change in phase relationship, there was distortion of the shape of the pH oscillation during the turn of the coil. This can be seen in Fig. 5 by comparing the fourth, fifth and sixth pH oscillation with the oscillations before the coil was turned.

The expectation that one particular phase change would stimulate breathing and another depress it was not confirmed. There was no particular phase change associated with the occurrence or absence of a respiratory response and when there was a response there was nearly always an increase in ventilation; depression only being seen on two occasions.

GENERAL POINTS ABOUT THE CHAMBER AND COIL EXPERIMENTS

There was little or no change in mean pH when carotid blood flow was directed through the mixing chamber or the delay coil turned. When there was a change it was in no consistent direction, was less than 0.02

units and the direction of the change in mean pH in no way determined whether there was a respiratory response.

When blood was directed through the mixing chamber or the delay coil lengthened there was a drop in systolic pressure of approximately 10 mm Hg and only 1–3 mm Hg change in diastolic pressure beyond the chamber or coil; there were no changes in femoral blood pressure. Systolic pressures were never below 100 mm Hg and usually above 120 mm Hg. Shortening of the coil produced rises in blood pressure of approximately the same degree. If, under the circumstances of these experiments, blood pressure can be used as an indicator of blood flow then changes in flow were small and actually increased when the coil was shortened.

The mixing chamber was surrounded by a water jacket maintained at the animal's rectal temperature, so it is unlikely that in these experiments there was any significant cooling of the blood by the chamber. In two experiments we cooled headward blood flow which always led to a rise in mean pH (0.01–0.03 units) but no change in ventilation. As there was usually no change in mean pH in the coil and chamber experiments it is unlikely that the experimental procedures had any significant effect on blood temperature.

DISCUSSION

These experiments involved dissection and cannulation of both carotid arteries and the use of a plastic chamber or coil to modify the pH oscillations. In these circumstances it is obviously possible that the stimulation of breathing was artefactual due to the release of an agent directly from the plastic surfaces or from the blood itself from contact with an artificial surface. In the coil experiments blood flow was continuous through the coil and when it was shortened no new surface was presented to the blood and the area exposed was reduced yet breathing was stimulated. However, when the coil was turned the frictional forces between two plastic surfaces might have released an agent which stimulated breathing; this would not have occurred in the chamber experiments but, when the shunt was open, blood was stationary in the chamber and the concentration of any abnormal agent in the blood affecting breathing might have increased during this time. The time that the shunt was open was kept to a minimum (15–30 sec) for this reason. If, however, stagnation of blood did produce the effects observed then this would still not be relevant to the coil experiments where there was no such stagnation. The choice of two completely different techniques for modifying the pH oscillations make it unlikely that our results are due to an artefact but this cannot be excluded on the present data.

If the changes in ventilation were not due to an artefact then certain conclusions can be drawn from the present experiments. From the chamber experiments it would appear that in some, but not all cats, oscillations in $PaCO_2$ are exerting an inhibitory effect on breathing under the circumstances of our experiments. Changes in the phase relationship between the respiratory cycle and the oscillations in $PaCO_2$ at the carotid body or changes in the shape of the oscillations or both briefly stimulates breathing. The hyperventilation is presumably not maintained because of the fall in mean $PaCO_2$ which results from the increase in respiration. Although changes in shape of the oscillations were small this does not preclude them from having an effect on respiration, for in tube rebreathing relatively trivial alteration in the shape of the $PaCO_2$ oscillation has a specific effect on ventilation (Brown et al. 1968).

The fact that the respiratory response was not consistent, that there was no particular phase change which stimulated breathing and that ventilatory depression was virtually never seen suggests that the respiratory control mechanism is more complex than was anticipated when the experiments were designed. In these experiments the phase relationship has been determined at the beginning of inspiration on the assumption that this relationship will be maintained throughout the rest of the respiratory cycle; with distortion of the shape of the oscillation this assumption is invalid. Thus in some trials of the coil the actual phase change which had produced the respiratory response was not identified because of uncertainty at what point in the respiratory cycle the phase change should be determined. Two other explanations at least could account for the failure to obtain a consistent pattern between phase change and respiratory response. First the phase relationship between the respiratory cycle and pH oscillation at the electrode may not be the same as at the carotid body. There is a significant and variable time delay from electrode to carotid body (D. M. Band, unpublished data); in addition efferent sympathetic activity might alter blood flow through the carotid body during an experiment which would change the phase relationship at the carotid body but not at the electrode. Second, efferent activity in the intact carotid sinus nerve may modify the oscillations in afferent activity observed in the cut sinus nerve. If this does occur then it might not be possible to identify the exact mechanism whereby oscillations in $PaCO_2$ affect breathing by measurement of arterial pH and respiration alone.

If the phase relationship between the respiratory cycle and the pH oscillation determines in part the chemical stimulus to respiration then abrupt changes in the duration of the respiratory cycle and circulation

time would immediately alter the chemical stimulus. This reflex chemical control of respiration would be faster than the circulation time between lung and carotid body.

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D. M. BAND, P. EBDEN, S. J. G. SEMPLE and C. B. WOLFF, Department of Medicine. St. Thomas' Hospital, Medical School, London W1N 8AA, England.