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RESPIRATORY OSCILLATIONS IN DISCHARGE FREQUENCY OF CHEMORECEPTOR AFFERENTS IN SINUS NERVE OF ANAESTHETIZED CATS AT NORMAL AND LOW ARTERIAL OXYGEN TENSIONS

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SUMMARY

1. The discharge of chemoreceptor afferents in preparations of the sinus nerve in spontaneously breathing anaesthetized cats has been subjected to an averaging procedure in records obtained when the animals breathed (*a*) air and (*b*) a hypoxic gas mixture.

2. The mean discharge frequency was higher in hypoxia than at normal oxygen tension.

3. Oscillations in chemoreceptor discharge frequency with the same period as respiration were obtained by the averaging procedure both at normal arterial oxygen tensions and in hypoxia, but there was no significant increase in oscillation amplitude with hypoxia.

4. The carotid body response to arterial P_{CO_2} oscillations does not therefore appear to be amplified by hypoxia. This finding is discussed in relation to the reported dependence upon hypoxia of the ventilatory effects of tube breathing in man.

INTRODUCTION

This paper considers whether, in the cat, the response of the carotid body chemoreceptor to arterial P_{CO_2} (P_{a,CO_2}) oscillations, which result from the cyclic nature of alveolar ventilation is amplified by hypoxia. Cunningham, Howson & Pearson (1973) found that normal human subjects, under hypoxic conditions, ventilated significantly more during simulated tube breathing (where a CO_2 rich gas mixture was given early in inspiration) than was predicted from the subject's own CO_2 response curve (where a CO_2-rich gas mixture was given throughout inspiration). The extra ventilation of the 'tube breathing' occurred only under hypoxic conditions, and was attributed to a difference in the time course of alveolar P_{CO_2}, P_{A,CO_2}, with amplification by the hypoxia, of the response of the carotid body to P_{a,CO_2} oscillations.

Band, Cameron & Semple (1969) were able to show differences in the time course of arterial pH oscillations of respiratory frequency recorded during (*a*) 'CO_2 breathing' (a CO_2-rich mixture throughout inspiration) and (*b*) tube breathing (CO_2 rich alveolar

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gas reinspired early in inspiration). The pH oscillations almost certainly reflected P_{a,CO_2} oscillations, which presumably also had a different time course during 'tube breathing' and 'CO_2 breathing'.

In cats breathing air, oscillations in the frequency of discharge of the afferent chemoreceptor fibres from the carotid body can be shown to result from respiratory oscillations in P_{a,CO_2} (Hornbein, 1965; Band, Saunders & Wolff, 1971; Goodman, Nail & Torrance, 1974; Band, McClelland, Phillips, Saunders and Wolff, 1978). It seems likely that respiratory oscillations in afferent chemoreceptor discharge occur both in subjects breathing CO_2 rich gas mixtures and also in 'tube breathing' subjects, and that their time courses are different. The oscillations in discharge frequency recorded by Band *et al.* (1971) and Goodman *et al.* (1974) were large in comparison to their mean; far too large to be accounted for simply by the steady-state relationship between mean afferent sinus nerve chemoreceptor discharge and mean P_{a,CO_2} (Fitzgerald & Parks, 1971; Lahiri & Delaney, 1975). Band *et al.* (1971) suggested that the oscillations in chemoreceptor discharge might be so large because of sensitivity of the carotid body to the rate of change of P_{a,CO_2} (dynamic sensitivity) as well as to the changing level of P_{a,CO_2} (static sensitivity). Rate of change sensitivity of this kind is found with other tonic receptors such as baroreceptors and muscle stretch receptors (Patton, 1965).

In view of the much larger dynamic than static sensitivity of the carotid body to P_{a,CO_2} (Torrance, 1975) it is not possible to predict the effect of hypoxia on oscillations in afferent chemoreceptor discharge frequency from the known interaction of hypercapnia and hypoxia on the mean firing rate (Fitzgerald & Parks, 1971; Lahiri & Delaney, 1975). Indeed, Torrance (1976) has suggested that the carotid bodies may work by a mechanism which would cause them to respond to transient changes in P_{a,CO_2} to the same extent irrespective of the arterial P_{O_2} (P_{a,O_2}). We have, therefore, examined the effect of varying degrees of hypoxia on the oscillations in discharge frequency of the carotid body afferent chemoreceptor fibres (in spontaneously breathing cats). The present study has already been reported briefly (Band & Wolff, 1973).

METHODS

Eight cats were studied. They weighed 2·1–3·2 kg and were anaesthetized with I.P. sodium pentobarbitone (veterinary Nembutal) 40 mg/kg. Respiration was monitored by means of a pneumotachograph attached to a tracheostomy tube. Differential pressure across the pneumotachograph was recorded as flow (Mercury Electronics, Scotland) and this signal was integrated continuously to give a tidal volume record.

The right sinus nerve was identified in all cats, the left in six of the eight cats. In the six cats where the left sinus nerve was identified it was divided, to reduce the ventilatory response to hypoxia. Carotid chemoreceptor discharge was monitored from single or few fibre filaments of the cut right sinus nerve. The recordings were made under paraffin with bipolar silver electrodes. The signal was amplified at a Tectronix RM122 low level preamplifier, displayed on a Tectronix 502A oscilloscope and recorded photographically on a galvanometric recorder (S.E. Labs, Model 2005). The galvanometer frequency response was flat to 3000 Hz (A.5000 galvanometer).

The animals ventilated spontaneously, breathing either air, or an approximately 10% O_2 (balance N_2) mixture, passed from a Douglas bag across a T-piece attached to the outer end of the tracheostomy tube.

Arterial blood was taken for *in vitro* measurement of blood gases and pH, firstly during nerve recording in the animal breathing air and secondly, during nerve recording at least 2 min after the animal started to breathe the hypoxic gas mixture. Where further runs were recorded blood

was again taken, and the same precaution observed. This was to ensure recording when blood gases and pH were steady. Arterial pH was measured by means of a capillary electrode (Radiometer, Model BMS 1) calibrated with standard buffers (National Bureau of Standards, U.S.A.). Arterial P_{O_2} (P_{a,O_2}) and P_{CO_2} (P_{a,CO_2}) were measured using separate electrodes (Radiometer) calibrated with known gases measured on the Lloyd–Haldane apparatus. All values were corrected to the cat's body temperature (Severinghaus, 1966).

Method of analysis of the time course of sinus nerve discharge frequency

Each respiratory cycle was divided into a number of equal time 'bins' – typically eight to ten. The number of impulses present in the first bin of each cycle were added together over a number of breaths (typically forty to sixty respiratory cycles). This procedure was repeated for the rest of the bins. The number of impulses in each bin was corrected for the time and number of cycles to give a mean discharge frequency for each part of the respiratory cycle. The values obtained were smoothed by taking a running average (of the values in three adjoining bins), and the original values were subjected to Fourier analysis to give the first fundamental sine wave. The amplitude of the oscillation in sinus nerve discharge frequency was taken as either peak to trough of the smoothed oscillation, or the full wave amplitude of the first fundamental from the Fourier analysis, since there was very close agreement between them.

RESULTS

Arterial P_{O_2} values in cats breathing air were normal (11·2–13·3 kPa; 84–100 mmHg) except for one animal in which P_{a,O_2} was low (8·4 kPa; 63 mmHg). P_{a,O_2} was lowered in all animals when breathing a low O_2 mixture, though the degree of hypoxia covered a wide range (3·6–10·1 kPa; 27–76 mmHg).

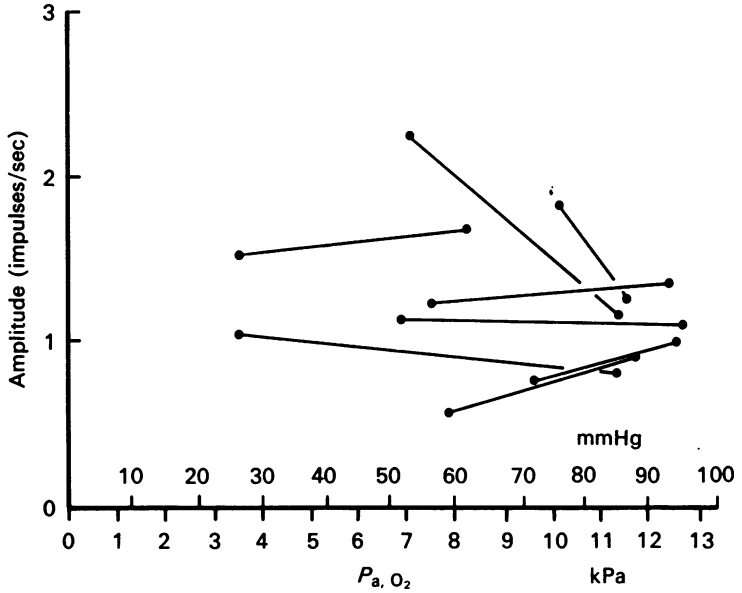

Fig. 1. Absolute amplitude of oscillation in single or few fibre afferent carotid chemoreceptor discharge plotted against P_{a,O_2}. Points from a given animal joined by a straight line. Only points for first run on air and second run on low O_2 mixture have been plotted.

The P_{a,CO_2} did not fall under the influence of hypoxia (P = 0·4–0·5) even in the two cats in which the left sinus nerve was intact. The P_{a,CO_2} over-all range was between 3·1 and 6·0 kPa (23–45 mmHg). The difference in P_{a,CO_2} between the first run

on air and the second run on a low O_2 mixture was always less than 2 mmHg (0·27 kPa). The animals therefore did not hyperventilate under the influence of the hypoxia. The arterial pH range was 7·16–7·41 (median 7·30) with no significant association with P_{a,O_2}.

The respiratory period was unchanged, between runs breathing air and the hypoxic gas mixture, in five of the cats. In one cat, with an intact left sinus nerve, the respiratory period on the low O_2 mixture was only two thirds as long as on air

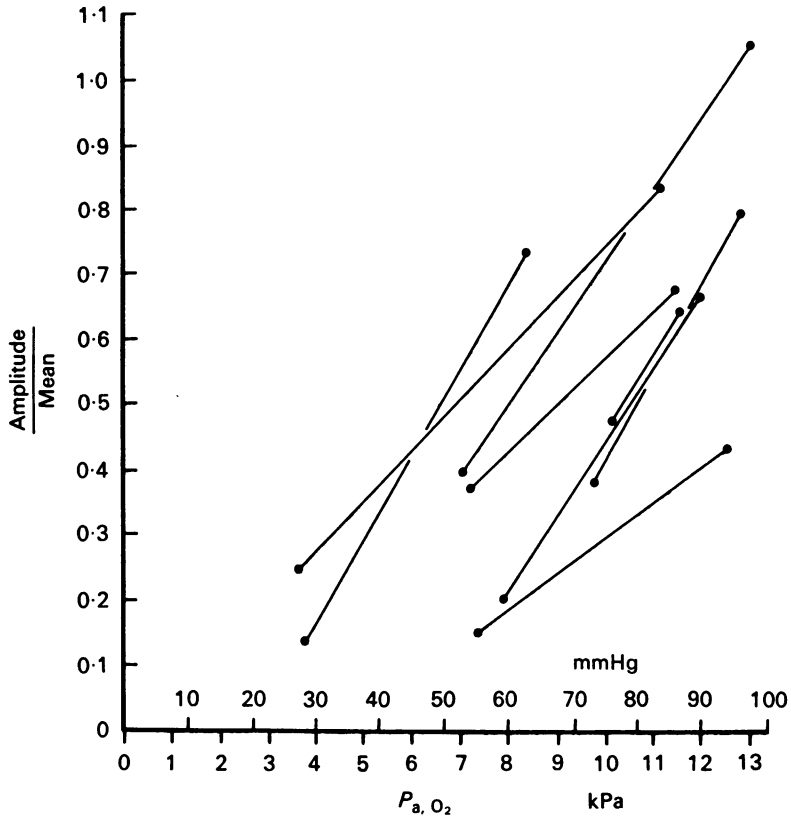

Fig. 2. Ratio of amplitude of oscillation in afferent carotid chemoreceptor discharge to the mean firing rate (relative amplitude) plotted against P_{a,O_2}. Points from a given animal joined by a straight line. Same experimental runs as in Fig. 1.

though the P_{a,CO_2} was unchanged. In the remaining two animals the respiratory period differed by one eighth of a cycle (one bin), in one case being longer in hypoxia than on air and in the other case being shorter (over-all range of respiratory rates 12–30/min).

The mean firing rate was always higher in hypoxia (range 2·00–10·14 impulses/sec) than it was at normal O_2 tension (range 0·70–3·04 impulses/sec), rising to five to six times the value found at normal O_2 tension in the two cats in which the most extreme hypoxia occurred (below 4 kPa, 30 mmHg).

The amplitude (peak to trough) of the calculated oscillation in discharge frequency, expressed in absolute terms, increased with hypoxia in two animals,

decreased in two animals, and was little changed in four animals. Fig. 1 shows the absolute amplitudes plotted against the P_{a,O_2} values for the first two runs in each animal. There is clearly no definite trend with hypoxia.

Comparison of all the absolute amplitudes (impulses/sec) of the oscillations in neuronal frequency in the low P_{a,O_2} range (under 8 kPa; 60 mmHg) with those recorded at normal O_2 tensions (above 10·7 kPa; 80 mmHg) showed no significant difference (analysis of variance; $F < 1{\cdot}0$; mean value in the normal P_{a,O_2} range 1·13 impulses/sec, mean value in the low P_{a,O_2} range 1·28 impulses/sec).

The amplitude of each oscillation in chemoreceptor discharge frequency has also been expressed in terms of the mean rate of discharge (amplitude to mean ratio). Fig. 2 shows the amplitude to mean ratio for each experiment during the initial two runs (on air and on the hypoxic gas mixture). The mean discharge always rose with hypoxia whereas the absolute amplitude tended to alter less. Therefore, the amplitude to mean ratio was markedly reduced by hypoxia.

DISCUSSION

In the present experiments the amplitudes of calculated average oscillations in the carotid chemoreceptor afferent firing rate (which have a respiratory period) have been expressed in two different ways; the absolute value of the amplitude in terms of impulses per second, and the amplitude related to the mean frequency of discharge (amplitude to mean ratio).

The amplitudes expressed in absolute terms have shown no clear tendency to change with hypoxia, whereas the mean discharge frequency was always increased. The increase in mean discharge was of the order expected from the study by Lahiri & Delaney (1975) of single unit chemoreceptor fibres and the study by Fitzgerald & Parks (1971) of whole nerve preparations. If hypoxia had, in each case, increased both mean discharge (static sensitivity of the carotid body to P_{a,CO_2}) and the oscillation amplitude (dynamic sensitivity) in equal proportion then the amplitude to mean ratio would have been unchanged by hypoxia. The fall in amplitude to mean ratio as O_2 tension was lowered (Fig. 2), shows that despite augmentation of the static sensitivity of the carotid body to P_{a,CO_2} by hypoxia there was far less, if any, augmentation of dynamic sensitivity (sensitivity to P_{a,CO_2} oscillations) by hypoxia.

The question remains as to whether hypoxia had any effect on the oscillations in chemoreceptor discharge frequency which would account for the potentiation (by hypoxia) of ventilatory responses in man to the alterations in the time course of P_{a,CO_2} oscillations brought about by 'tube breathing' (Cunningham *et al.* 1973). In the present experiments there was a small trend toward an increase in chemoreceptor discharge oscillation amplitude with hypoxia, but the trend was slight in comparison with the effect of hypoxia on mean discharge frequency. Goodman *et al.* (1974) also clearly demonstrated only a modest increase in absolute neuronal oscillation amplitude in some cats with hypoxia. Like us, they found a marked decrease in 'relative amplitudes of oscillation' (our 'amplitude to mean ratio') on changing from euoxia to hypoxia. We therefore conclude that, if the human carotid body behaves in a similar way to the cat carotid body then the enhancement due to hypoxia of the extra ventilatory effect of 'tube breathing' is likely to be a result of a mean level

or threshold phenomenon (possibly at brainstem level), rather than a direct effect of hypoxia on the amplitude of the oscillation in arterial chemoreceptor discharge frequency. It would be well worth repetition of the tube breathing experiments in the anaesthetized cat to determine whether the mechanisms in cat and man respond similarly to this kind of stimulus.

It appears, in general terms, that the way the carotid body in the anaesthetized cat responds to dynamic (or rapid) changes in P_{a,CO_2} differs from the way in which it responds to static (or mean level) changes. The oscillations in discharge frequency, in animals breathing air, are too large to represent a simple proportional response to P_{a,CO_2} oscillations (Goodman *et al.* 1974; Torrance, 1975), and the present study suggests that there is very little, if any, augmentation by hypoxia of the dynamic sensitivity of the carotid body to P_{a,CO_2} (i.e. to P_{a,CO_2} oscillations of respiratory frequency).

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REFERENCES

BAND, D. M., CAMERON, I. R. & SEMPLE, S. J. G. (1969). Effect of different methods of CO_2 administration on oscillations of arterial pH in the cat. *J. appl. Physiol.* **26**, 268–273.

BAND, D. M., MCCLELLAND, M., PHILLIPS, D. L., SAUNDERS, K. B. & WOLFF, C. B. (1978). Sensitivity of the carotid body to within-breath changes in arterial P_{CO_2}. *J. appl. Physiol.* (In the Press).

BAND, D. M., SAUNDERS, K. B. & WOLFF, C. B. (1971). The relation between chemoreceptor discharge and respiratory fluctuation of arterial pH in the anaesthetized cat. *J. Physiol.* **218**, 73–74*P*.

BAND, D. M. & WOLFF, C. B. (1973). The effect of hypoxia on the respiratory fluctuations in chemoreceptor discharge in the cat. *J. Physiol.* **234**, 71–72*P*.

CUNNINGHAM, D. J. C., HOWSON, M. G. & PEARSON, S. B. (1973). The respiratory effects in man of altering the time profile of alveolar carbon dioxide within each respiratory cycle. *J. Physiol.* **234**, 1–28.

FITZGERALD, R. S. & PARKS, DOROTHY, C. (1971). Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. *Resp. Physiol.* **12**, 218–229.

GOODMAN, N. W., NAIL, B. S. & TORRANCE, R. W. (1974). Oscillations in the discharge of single carotid chemoreceptor fibres of the cat. *Resp. Physiol.* **20**, 251–269.

HORNBEIN, T. F. (1965). Effect of respiratory oscillation of arterial P_{O_2} and P_{CO_2} on carotid chemoreceptor activity and phrenic nerve activity. *Physiologist, Wash.* **8**, 197.

LAHIRI, S. & DELANEY, R. G. (1975). Stimulus interaction in the responses of carotid body chemoreceptor single afferent fibres. *Resp. Physiol.* **24**, 249–266.

PATTON, H. D. (1965). Receptor Mechanism. In *Physiology and Biophysics*, 19th edn., pp. 105/6, ed. RUCH, T. C. and PATTON, H. D. Philadelphia and London: W. B. Saunders.

SEVERINGHAUS, J. W. (1966). Blood gas calculator. *J. appl. Physiol.* **21**, 1108–1116.

TORRANCE, R. W. (1975). Discussion of PONTE, J. C. M. R. and PURVES, M. J. On the speed of response of chemoreceptors. In *The Peripheral Arterial Chemoreceptors*, ed. PURVES, M. J., p. 369. Cambridge: Cambridge University Press.

TORRANCE, R. W. (1976). A new version of the acid receptor hypothesis of carotid chemoreceptors. In *Morphology and Mechanisms of Chemoreceptors*, ed. PAINTAL, A. S. Delhi: Vallabhbhai, Patel Chest Institute, University of Delhi.