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THE FREQUENCY OF NERVE IMPULSES IN SINGLE CAROTID BODY CHEMORECEPTOR AFFERENT FIBRES RECORDED *IN VIVO* WITH INTACT CIRCULATION

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SUMMARY

1. The responses of single afferent fibres of carotid body chemoreceptors to independent changes in arterial O_2 and CO_2 tensions and pH were studied in the cat *in vivo*.

2. The response curve obtained relating chemoreceptor activity to changes in arterial P_{O_2} was similar to an hyperbola; the frequency of nerve impulses at first decreased rapidly as the P_{a,O_2} was raised and then more slowly. The arterial P_{O_2} at which the slow decrease was reached varied among the different fibres; the mean level was 190 mm Hg (S.D. $\pm$ 40 mm Hg).

3. Single chemoreceptor afferent fibres continued to discharge even when the arterial P_{O_2} was more than 600 mm Hg.

4. The discharges of single chemoreceptor afferent fibres increased both with increasing P_{a,CO_2} at constant pH and P_{a,O_2}, and with increasing arterial H^+ at constant P_{a,CO_2} and P_{a,O_2}.

5. It is concluded that single carotid body chemoreceptor afferent fibres of the cat can be activated *in vivo* by an increase in either arterial H^+ or arterial P_{CO_2} as well as by a decrease in arterial P_{O_2}.

INTRODUCTION

The responses of carotid body chemoreceptors to changes in O_2 and CO_2 tensions and hydrogen ion concentration of arterial blood have been described in terms either of the overall reflex response of ventilation (Heymans, Bouckaert & Dautrebande, 1931) or the number of nerve impulses occurring in the sinus nerve per unit of time (Euler, Liljestrand & Zotterman, 1939; Eyzaguirre & Lewin, 1961; Hornbein, Griffo &

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Roos, 1961; Witzleb, 1963). The techniques have involved the measurement of nervous activity in the whole sinus nerve (Hornbein *et al.* 1961; Hornbein & Roos, 1963) or in a multifibre strand of it (Eyzaguirre & Lewin, 1961).

The present series of experiments was carried out to study the effects of independent changes in either arterial O_2 or CO_2 tension or hydrogen ion concentration in arterial blood $[H^+]_a$ on the discharge of single chemoreceptor afferent fibres in the sinus nerve of the cat. The carotid body was perfused naturally, that is, no attempt was made to control blood pressure in the carotid sinus segment or to measure the volume of carotid body blood flow. The results therefore are to be considered along with those described in the preceding paper (Biscoe, Bradley & Purves, 1970) in which the carotid body was artificially perfused and which involved considerable cannulation of vessels near the carotid body. A preliminary account of some of the results of the present series of experiments has been published (Biscoe, Sampson & Purves, 1967).

METHODS

Twenty-five cats, ranging in weight from 2·0 to 4·0 kg, were used in this study. They were anaesthetized with a mixture containing allobarbitone (100 mg/ml.) and urethane (400 mg/ml.) (Dial with urethane, CIBA: 0·6 ml./kg), administered intraperitoneally and supplemented when necessary with intravenous pentobarbitone (5 mg/kg). The cats were paralysed with gallamine triethiodide (Flaxedil: American Cyanamid Co.), 10 mg/kg, intravenously and artificially ventilated.

The methods used to expose the carotid body and sinus nerve, and to identify and record from afferent chemoreceptor fibres from the carotid body, were those described in the previous paper (Biscoe *et al.* 1970) with certain modifications. When a steady state with respect to P_{a,O_2}, P_{a,CO_2} or pH_a had been obtained, nerve potentials, displayed on the oscilloscope and subjected to Z-axis modulation to improve their brightness, were photographed on moving film for 20–60 sec. The number of nerve impulses occurring in 20 sec periods was read from the film. In some experiments, a discriminator was used to select action potentials and convert them to standard size pulses which were then led to a rate meter or to a pulse counter. When the counter was used, the time for 100 nerve impulses to occur was measured.

In the majority of experiments in which changes in P_{a,O_2} were measured, the cat was given heparin (Pularin–Evans), 15 mg/kg, intravenously and a Beckman macro-oxygen electrode and cuvette were inserted in a femoral arteriovenous loop. The cuvette was maintained at 37·5° C. The electrode could be calibrated *in vivo* by introducing saline equilibrated with room air and at 37·5° C through stopcocks which were placed on either side of the cuvette. The output of the electrode was led to a Beckman physiological gas analyser and P_{a,O_2} was monitored continuously. Changes in P_{a,CO_2} and pH_a were made and recorded in the same way as described in Biscoe *et al.* (1970).

In many experiments, a continuous intravenous infusion of heparinized dextrose saline solution was given and the urinary bladder was catheterized in order to withdraw urine and maintain a fluid balance. The total volume infused in a 12 hr period was about 200 ml. and a similar volume of urine was withdrawn. With this pro-

cedure, it was found that arterial pressure was satisfactorily maintained during a long experiment. The infusion was especially useful in the experiments in which pH_a and P_{a,CO_2} were varied, for these were often accompanied by substantial changes in arterial blood pressure. If there was a spontaneous tendency for blood pressure to fall, injections of dextran (6 % in 0·9 % sodium chloride, Cutter Laboratories, Berkeley, Calif.) were sometimes substituted for the dextrose saline. The usual dose was 5–10 ml.

Since it has been found that, in a proportion of experiments, chemoreceptor discharge increases as arterial blood pressure is reduced over the range 60–160 mm Hg (Biscoe *et al.* 1970), results in the present series were rejected where unacceptably large changes in pressure occurred. Where O_2 response curves were determined, the accepted limits were a mean arterial pressure above 80 mm Hg and a S.D. less than ± 10 mm Hg about the mean value of mean arterial pressure. Similar criteria were applied to the experiments in which the response curves to changes in P_{a,CO_2} or pH_a were derived. In these, however, too few points were obtained for the S.D. to be calculated. The range over which arterial pressure changed has therefore been given.

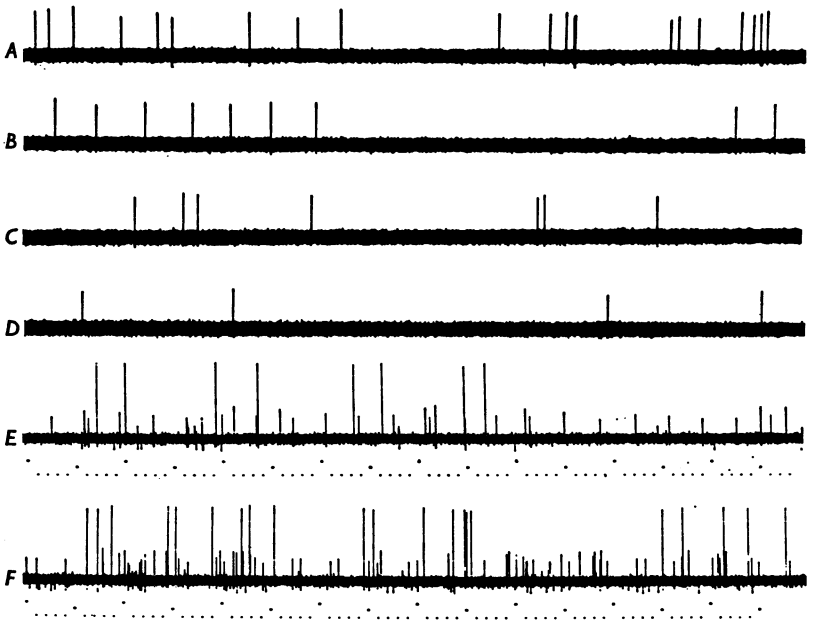

Fig. 1. *A* to *D*, chemoreceptor action potentials recorded from the carotid sinus nerve of one cat. P_{a,O_2} (mm Hg) in *A*, 77; *B*, 103; *C*, 182; *D*, 520. The end-tidal CO_2 (mm Hg) was 32 throughout. *E* and *F* are from another preparation and show effects of increased P_{a,CO_2} at constant pH (7·52) and P_{a,O_2} (280 mm Hg). P_{a,CO_2} (mm Hg) in *E* was 37, and in *F* was 58.

Mean arterial pressure (mm Hg) in *A*, 119; *B*, 119; *C*, 118; *D*, 115; *E*, 89; *F*, 87. The time marks on *E* and *F* are 100 msec apart and apply also to *A* to *D*.

RESULTS

Identification of chemoreceptor fibres

The criteria used to identify chemoreceptor fibres were similar to those given in the preceding paper (Biscoe *et al.* 1970) though with some modifications. The response of chemoreceptor afferent fibres to clamping the carotid artery depended on the efficacy of the vertebral-occipital anastomosis; sometimes no increase in discharge occurred. Sympathetic fibres were not encountered in the sinus nerve in this series of experiments.

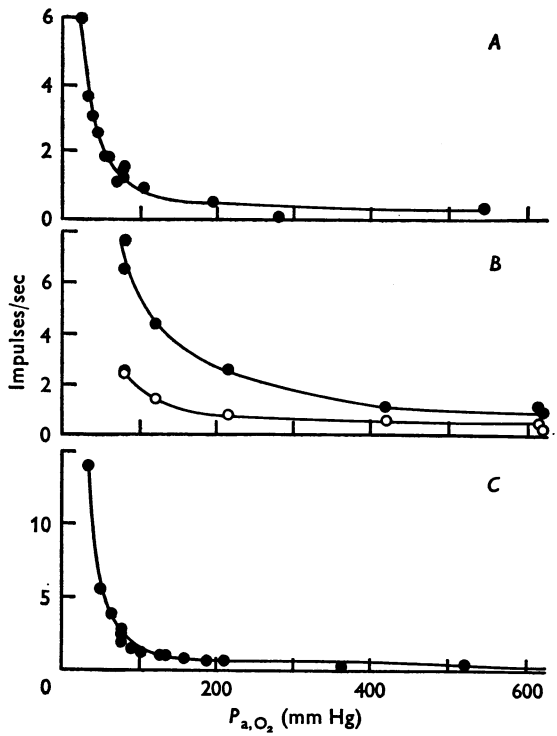

Fig. 2. The rate of chemoreceptor afferent discharges (impulses/second) in single fibres, plotted against the arterial O_2 tension (mm Hg), all from the same cat. *B* shows two fibres (●, ○) from the same strand; *A* and *C* are from single fibres. Arterial P_{CO_2} was in the range for *A*, 28–31 mm Hg; for *B*, 29–31 mm Hg; for *C*, 31–34 mm Hg. M.A.P. ± S.D. in *A*, 95 ± 7; *B*, 97 ± 5; *C*, 123 ± 6.

Two of the types of record used in this study are illustrated in Fig. 1: *A* to *D* are from one experiment and show the activity of a single unit changing with oxygen tension; *E* and *F* are from another experiment where CO_2 was changed and show a large potential with a number of smaller

ones. The activity of a single unit could be studied in such a strand because the large spike was easily identified throughout the experiment.

All the graphs plotted in this paper are made from counts of the number of action potentials that occurred in 20 sec periods of moving film records.

Effect of changing P_{a,O_2}. The responses of single chemoreceptor units at different levels of oxygen tension were recorded from thirty-eight fibres in eighteen strands; there were a number of successful experiments in which two or three fibres could be distinguished over a wide range of O_2 tensions. The shape of each response curve was similar to an hyperbola and examples are shown in Fig. 2. The curves for the four fibres illustrated in Fig. 2 were obtained from the same cat; the curves in Fig. 2*B* were plotted from potentials recorded from two fibres in the same strand. The single unit of Fig. 1, *A* to *D*, was used in the study plotted in Fig. 2*C*. These curves are characteristic for the whole group of results and illustrate a number of features. First, in no case did any unit cease firing when the O_2 tension was raised, although the response curve usually became almost flat. The O_2 tension at which the curve of rate of discharge against O_2 tension departed from the near horizontal could usually be estimated for each fibre with an accuracy of $\pm$ 5–25 mm Hg from these graphs. This estimation was made by drawing a straight line through the points taken at high O_2 tension and noting the O_2 tension at which the remaining points on the curve departed from this line. The mean value obtained from thirty-eight fibres was 190 mm Hg (S.D. $\pm$ 40 mm Hg), and the range was 140–400 mm Hg. The activity of one fibre in Fig. 2*B* was changing even at a P_{a,O_2} up to 400 mm Hg, whereas the activity of the second fibre changed remarkably little over such a wide range. Fig. 2 illustrates the wide variation in the shapes of response curves in one animal and suggests that there is a spectrum of response curves of different shapes, which together make up the whole nerve or multifibre strand response. This view is supported by similar observations in eleven experiments where more than one fibre was observed in one animal. Typically, as the O_2 tension fell below 100 mm Hg the rate of impulse activity increased very rapidly, in some cases to almost 15/sec (Fig. 2*C*). A second feature of the curves is that there was no decline in frequency of action potentials at the low oxygen tensions studied, 25–30 mm Hg. There was no evidence for hysteresis when the P_{a,O_2} was altered in one direction and returned to control though the occurrence of the phenomenon is not excluded by these data.

It was noted that in some experiments the amplitude of the single fibre chemoreceptor potentials declined at low oxygen tension, to recover when the O_2 tension was raised.

Effect of changes in P_{a,CO_2} *and pH*. An increased chemoreceptor afferent discharge in the sinus nerve due to an elevation in arterial CO_2 tension has

been described by many workers (Euler *et al.* 1939; Eyzaguirre & Lewin, 1961; Biscoe & Millar, 1966, 1968). In those experiments, in order to obtain a range of points the P_{CO_2} was varied by changing the artificial ventilation and, thus, the pH was allowed to vary with the CO_2. Such a technique was used in six of our experiments, involving eleven fibres from seven strands, and the results from one are plotted in Fig. 3*A* for an extended range of

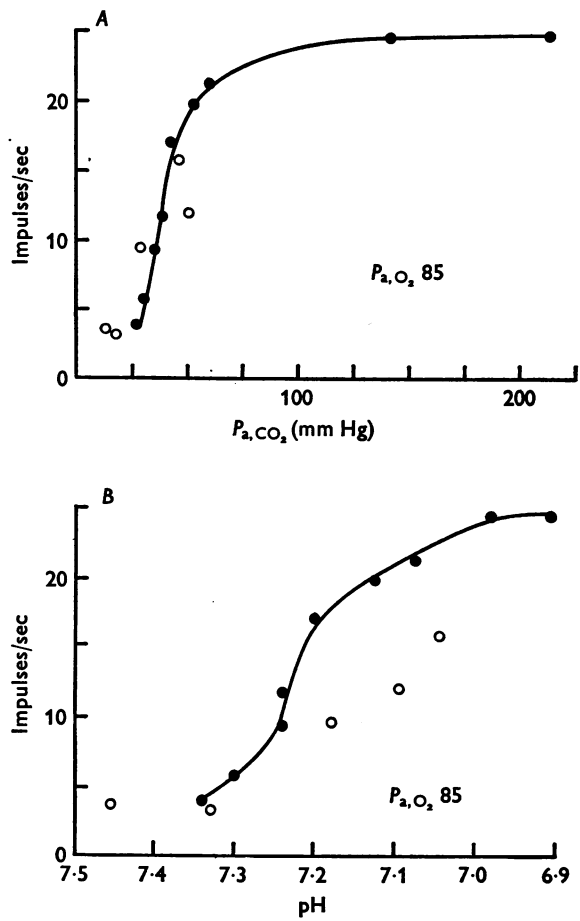

Fig. 3. Chemoreceptor afferent discharge (impulses/sec) against arterial CO_2 tension (*A*) and arterial pH (*B*). Both graphs *A* and *B* are plots of rates of firing of the same single chemoreceptor fibre. The rate and volume of the artificial ventilation were varied to change the P_{a,CO_2} and so also the pH. The P_{a,O_2} was 85 mm Hg throughout. ●, results after independent variable was increased; ○, result after decrease. M.A.P. was 115 mm Hg ± 13. This calculation excludes the two lowest values for chemoreceptor discharge in each graph where the M.A.P. was 84 and 61 mm Hg.

CO_2 tensions. The points lie on a sigmoid curve; the response was more or less linear between CO_2 tensions of 25 and 65 mm Hg.

Effects of changes in P_{a,CO_2} *alone.* In another series of experiments, involving eighteen fibres from twelve strands, the CO_2 tension was changed

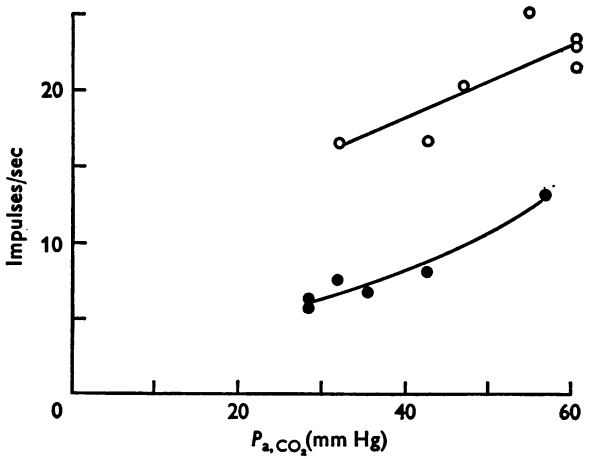

Fig. 4. Nerve discharge (impulses/sec) recorded from a single chemoreceptor afferent fibre and plotted against P_{a,CO_2}. For each curve the pH was kept constant (see text). Open circles, pH = 7·25 units, mean arterial pressure ± S.D. = 91 ± 9 mm Hg. Filled circles, pH = 7·45 units, mean arterial pressure ± S.D. = 85 ± 4 mm Hg. P_{a,O_2} was 80 mm Hg throughout.

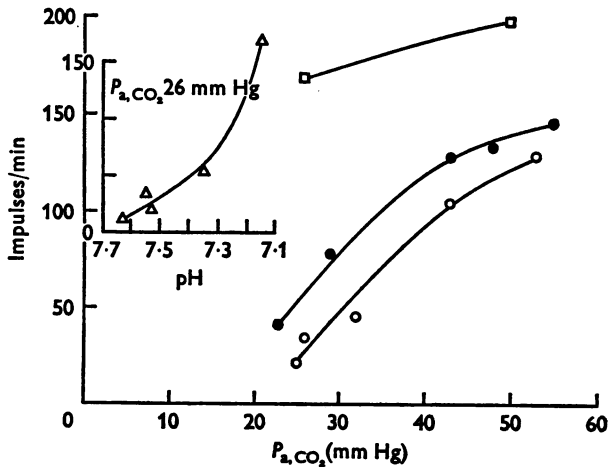

Fig. 5. Discharge rate (impulses/min) recorded from a single chemoreceptor afferent fibre and plotted against P_{a,CO_2} (○, ●, □) and against pH (Δ). ○, pH = 7·51–7·54; ●, pH = 7·35–7·37; □, pH = 7·15–7·17; Δ, P_{a,CO_2} = 26 mm Hg. The P_{a,O_2} was 270 mm Hg. Some additional data not shown on the other graphs were used for the pH plot.

and the arterial pH was kept constant. After infusion of the appropriate acid or alkaline solution, 20–30 min were allowed to elapse until the rate of discharge as monitored on the polygraph remained constant for 5–10 min. Hornbein & Roos (1963) found that 1 hr was required for the discharge to stabilize. In our experiments, 20–30 min were usually sufficient but the doses of bicarbonate which we used (1 M-$NaHCO_3$, 2–3 ml./kg) were probably somewhat smaller than those used by Hornbein & Roos (1963). Clearly, if the bicarbonate is given in one dose the arterial concentration will be changing continuously depending on the rate of renal clearance. We frequently observed that if 1 hr elapsed after infusion, the pH and P_{a,CO_2} were very nearly back to control (pre-infusion) levels. At the time when recordings were taken, 20–30 min after the infusion, the CO_2 and pH were reasonably stable. The results for one experiment are given in Fig. 4 (see also Fig. 1*E* and *F*). P_{a,O_2} was maintained at 80 mm Hg throughout while P_{a,CO_2} was altered over the range 30–60 mm Hg at two levels of pH, 7·25 and 7·45 units. In this and in three other experiments, it was confirmed that at any level of P_{a,CO_2}, chemoreceptor discharge was reduced as pH was raised. Thus it was possible to obtain distinct responses to P_{a,CO_2} at different levels of pH. Secondly, it was possible to confirm in all fibres tested that with pH held constant, chemoreceptor discharge increased with P_{a,CO_2}. This response to CO_2 was not therefore caused by changes in arterial pH. The scatter of points obtained made definition of the response curve to CO_2 difficult; in all fibres tested, the response appeared to be linear over the range 25–65 mm Hg.

In one experiment the P_{a,O_2} was held constant at 270 mm Hg (cf. Hornbein & Roos, 1963, who used a high O_2 tension). Some of the results from this experiment are shown in Fig. 5 where there is an increase in the discharge rate as the CO_2 was raised within the range 20–55 mm Hg. It should be noted that the receptor complex is responding even at this high P_{a,O_2} level and at a pH of 7·52.

Effects of changing pH. Hornbein & Roos (1963) recorded the activity in the sinus nerve as the pH was changed on altering the P_{a,CO_2}. The results of such an experiment are plotted in Fig. 3*B* and are the same data that were plotted in Fig. 3*A* against P_{a,CO_2}. There was a progressive increase in the discharge rate as the pH was reduced from 7·45 to 6·905 units. The relationship between pH and chemoreceptor discharge, like that of CO_2, is not a linear one. However, there is one difference between the curves. When the pH was lowered (open circles) the points did not fall on the same line as when it was raised (filled circles). This may be contrasted with the P_{a,CO_2} plot (Fig. 3*A*) where there was no hysteresis. This was not a constant finding but appeared to be a valid one in this experiment.

In six fibres from five separate strands of five cats data were obtained

from experiments in which the P_{a,O_2} and P_{a,CO_2} were held constant while the arterial H^+ was varied. These experiments were conducted in a way similar to those in which pH was held constant and P_{a,CO_2} varied. Again 20–30 min were required to elapse after the alkaline or acid infusion before the discharge rate became stable for 5–10 min. The results from one such experiment are presented in Fig. 6, which is representative of the data from all fibres studied. In this experiment, H^+ response curves were obtained at two different levels of P_{a,CO_2}. In both cases, the discharge of the single chemoreceptor afferent increased with increasing H^+ at constant P_{CO_2}. It should be noted also that the discharge was uniformly higher at the higher P_{CO_2} for any level of H^+.

The inset graph of Fig. 5 also shows a response to H^+ and it should be noted that the receptor continues to discharge at a P_{a,CO_2} of 26, P_{a,O_2} of 270 and pH_a of 7·62.

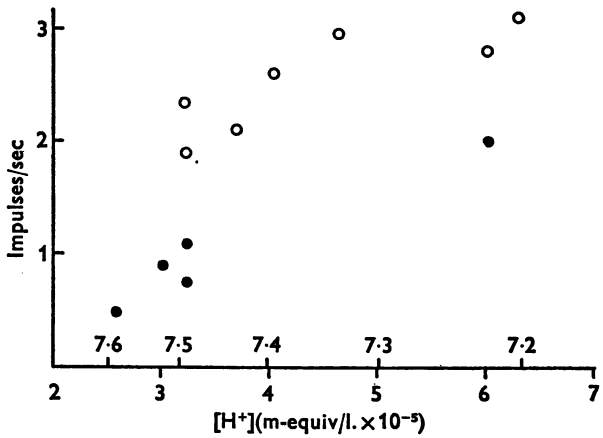

Fig. 6. The discharge of a single chemoreceptor afferent fibre (impulses/sec) plotted against the arterial H^+. ○, P_{a,CO_2} was 40 mm Hg, M.A.P. = 115 ± 11; ●, P_{a,CO_2} was 17 mm Hg, M.A.P. = 152 ± 6; P_{a,O_2} was in the range of 110–115 mm Hg throughout. The upper scale on the abscissa is for pH.

DISCUSSION

The experiments described here comprise a study of the whole carotid body complex and how it reacts to various stimuli and are not simply a study of the *receptor*. The results reported in the accompanying paper (Biscoe *et al.* 1970) indicate that, of the variables involved, carotid body chemoreceptor activity is often independent of physiological changes in mean arterial pressure. However, the fact that in some experiments carotid chemoreceptor activity can be affected by changes in arterial pressure must be borne in mind when these results are interpreted. For example,

the rise in arterial pressure in hypoxia could limit the rise in chemoreceptor activity and similar effects could be seen with the other blood gases.

In general, our results agree with those of previous workers. With respect to the chemoreceptor responses to changes in P_{a,O_2}, we have found the relation to be similar to an hyperbola (Euler *et al.* 1939; Alvarez-Buylla, 1951; Hornbein *et al.* 1961; Eyzaguirre & Lewin, 1961). However, we have not been able to confirm the finding of Witzleb (1963) that the response curve up to a threshold level of O_2 tension is a monotonic decreasing function and above this threshold is parallel to the abscissa.

Our results differ from those quoted above, and from Paintal & Riley (1966) and Paintal (1967) for aortic chemoreceptors, in that we have not been able to show that the carotid body chemoreceptors ever cease to discharge. It follows that we did not find a threshold in the usual sense of the word.

The observation that the amplitude of the action potentials declines at low O_2 tensions could explain the finding by Hornbein *et al.* (1961) that the integrated nerve discharge in multifibre strands declines at these tensions. In their experiments, the amplitude of the individual action potentials as well as the rate would affect the computation of the final count.

The responses of chemoreceptor afferent fibres to changes in P_{a,CO_2} with the pH controlled have been studied previously only by Hornbein & Roos (1963) and Joels & Neil (1960). The former workers were unable to confirm the findings of Joels & Neil that CO_2 was an excitant. Hornbein & Roos (1963) recorded from the whole nerve *in vivo* with the circulation intact, and they measured the activity by integrating the total nerve signal. This technique gives more weight to large nerve impulses than to small ones as recorded at the electrodes. On the other hand, Joels & Neil (1960) perfused the carotid body with a modified Krebs solution at constant pressure and recorded the activity by photographing the nerve impulses from a multifibre strand on moving film. This method does not allow accurate quantitation. Our results confirm the finding of Joels & Neil (1960) that elevated levels of arterial CO_2 increase the discharge of carotid body chemoreceptor afferents at constant pH. They also contrast with the view put forward by Neil & Joels (1963) that, at high P_{O_2}, the response of the chemoreceptors to CO_2 is almost negligible: in our experience, the chemoreceptor discharge approximately doubled when P_{a,CO_2} was raised from 25 to 45 mm Hg, pH being maintained within the physiological range and P_{a,O_2} at 270 mm Hg. It is possible that at this level of discharge the reflex effect of CO_2 may be negligible but it is clear that the receptor responds.

The results reported in this paper are intended to be complementary to those reported by Biscoe *et al.* (1970) and indicate that their methods of isolating the carotid sinus, artificial perfusion of the carotid body and measurement of carotid body blood flow do not affect the qualitative responses of the carotid body. In all respects, the curves relating chemoreceptor activity and the independently varied arterial gas tensions and H^+ in the two series are similar.

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