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THE RELATION BETWEEN CAROTID BODY CHEMORECEPTOR DISCHARGE, CAROTID SINUS PRESSURE AND CAROTID BODY VENOUS FLOW

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SUMMARY

1. Activity in forty-two single chemoreceptor afferent fibres from the carotid body in thirty-nine cats was measured when the carotid body was naturally and artificially perfused. In nine of these cats, carotid body venous flow was also measured.

2. When pressure within the carotid sinus segment was suddenly raised or lowered, chemoreceptor activity changed in the opposite direction within the first 5–10 sec by an amount which was significantly greater than the variation of activity in the control period. Thereafter activity stabilized at a level which was not different from control.

3. Whether the carotid body was naturally or artificially perfused, carotid body chemoreceptor activity, following this initial transient change, was unaffected in eight out of twelve fibres by alterations in carotid sinus pressure within the range 60–160 mm Hg and carotid body venous flow 10–60 μl./min, blood gas tensions and pH being maintained constant. In the four remaining fibres, chemoreceptor activity increased slightly but significantly as pressure was lowered in this range. Chemoreceptor activity increased in all fibres tested when pressure was lowered below 50–60 mm Hg.

4. Chemoreceptor response curves to changes in P_{a,O_2} (30–450 mm Hg), P_{a,CO_2} (27–62 mm Hg) or $[H^+]_a$ ($3–7 \times 10^{-5}$ m-equiv/l.) were not significantly different whether the carotid body was perfused (*a*) naturally at the prevalent systemic pressure, (*b*) artificially at the same pressure, or (*c*) artificially at one higher pressure, 130 or 140 mm Hg.

5. These results indicate that the carotid body chemoreceptors are relatively unaffected by sustained changes in arterial pressure or in total carotid body flow within the physiological range.

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INTRODUCTION

Lee, Mayou & Torrance (1964) showed that the discharge from aortic chemoreceptors in the cat increased as arterial pressure was lowered. Similarly, Landgren & Neil (1951) demonstrated a rise in carotid body chemoreceptor activity with hypotension caused by haemorrhage. It has been assumed that the rise in chemoreceptor activity is a consequence of a fall in blood flow since it is known that carotid body blood flow falls linearly with arterial pressure (Daly, Lambertsen & Schweitzer, 1954; Purves, 1968).

There are no quantitative studies on the response of the carotid body chemoreceptors to changes in blood pressure or blood flow and it has not therefore been possible to assess how far the changes in arterial pressure which accompany systemic hypoxia or hypercapnia affect the chemoreceptor response. We have therefore measured activity in single carotid body chemoreceptor afferent fibres under conditions where one of the variables P_{a,O_2}, P_{a,CO_2}, pH_a or mean arterial pressure was varied while the others were held constant. In some experiments, carotid body blood flow was also measured. The results presented in the present paper are intended for comparison with those obtained from carotid body chemoreceptors in the cat in which blood pressure was not controlled (Biscoe, Purves & Sampson, 1970).

METHODS

Thirty-nine cats weighing 1·9–3·6 kg were studied. They were anaesthetized with pentobarbitone sodium (Nembutal, Abbott) 30 mg/kg given intraperitoneally. Additional pentobarbitone was given at the rate of approximately 4 mg/kg.hr through a femoral vein cannula. The temperature of the cats was kept at 37–38° C.

The trachea was cannulated and the pharynx and larynx retracted in the mid line to expose the carotid bifurcations from the medial sides.

Perfusion of the carotid body. The method of isolating the carotid sinus region was similar to that of Joels, Neil & Vaughan Hudson (1961) and the volume of blood flow through the carotid body was measured using a technique similar to that of Daly *et al.* (1954). The method of perfusing the carotid body with blood at known pressures or blood gas tensions was that described by Purves (1970) but modified so that the external carotid artery could be temporarily occluded without danger of movement artifacts in the sinus region (Fig. 1).

The cat was given heparin (Pularin, Evans), 15 mg/kg, intravenously and a 15 cm loop with side arm of polyethylene tubing (Portex 204) was inserted into the common carotid artery. The dead space of the side arm and the half of the loop towards the head was approximately 1 ml. All the branches of the common and external carotid arteries between the loop and the external carotid artery catheter some 2 cm rostral to the carotid sinus were tied as far distal from the carotid body as possible to avoid any effects from retrograde clotting. The external carotid artery was cannulated with a catheter which formed a loop to the femoral vein. It could be closed by stopcock B. Pressure in the external carotid loop was continuously measured with a Statham strain gauge attached to stopcock C. When necessary, clamps were applied

to the external carotid artery–femoral vein loop to increase the resistance to flow and so maintain carotid sinus pressure 5–10 mm Hg below that of mean femoral artery pressure (measured with a second Statham P 23 AC transducer). Mean pressures were obtained electronically.

When cannulation was complete, the carotid sinus segment could be isolated by clamping the cardiac side of the common carotid loop and by closing the tap B in the carotid–femoral loop. Simultaneously, the tap A was turned so that any desired pressure from a sphygmomanometer could be applied to the side arm of the carotid loop and hence within the carotid sinus segment. An example of the change in carotid sinus pressure so produced is shown in Fig. 4*C*. In all experiments to be described, perfusion pressure refers to pressure within the isolated carotid sinus loop.

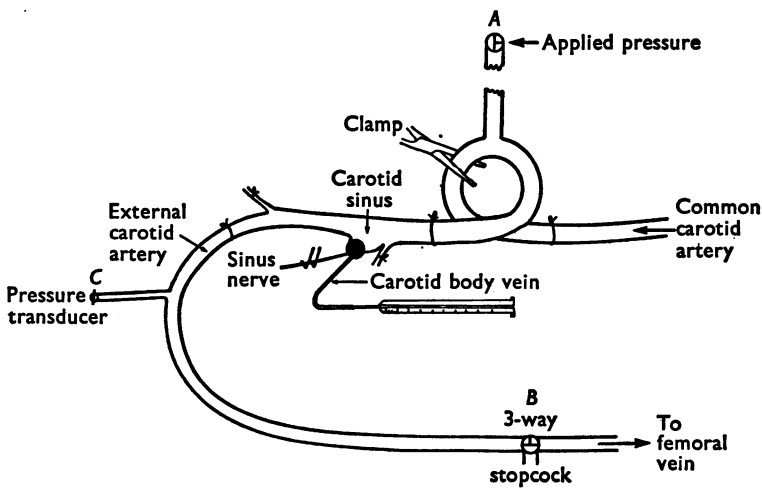

Fig. 1. Diagram to show the method for isolating the left carotid sinus region of the cat. A is a three-way stopcock which closes the side arm of the common carotid loop, allows blood samples to be withdrawn or pressure to be applied within the carotid sinus segment from a sphygmomanometer. B is a stopcock which can close the external carotid–femoral vein loop. When this stopcock is turned and the cardiac end of the common carotid artery loop clamped the carotid sinus segment is isolated. C is a stopcock connecting the side arm of the external carotid–femoral vein loop to a pressure transducer which measures pressure in the carotid sinus segment.

Arterial P_{O_2}, P_{CO_2} and pH were measured from 0·4 ml. femoral artery samples of blood with appropriate Radiometer electrodes at 38° C. The oxygen electrode was calibrated with dithionate and air; the CO_2 electrode with CO_2 in air mixtures of accurately known composition and the pH electrode with standard phosphate buffers. Alveolar (end-tidal) CO_2 was continuously monitored from the trachea with a Beckman LB 1 infra-red CO_2 analyser calibrated at intervals with CO_2 in air mixtures. Arterial and carotid body venous haematocrit was measured as required from 40 μl. samples with a small centrifuge at 5000 rev/min for 5 min.

Nerve recording. The connective tissue and adventitia were removed from the carotid sinus wall, a procedure which eliminated the greater part of the discharge of baroreceptors and made the subsequent isolation of the sinus nerve and search for a

single chemoreceptor afferent fibre somewhat easier than it would otherwise have been. The sinus nerve was cut at its junction with the glossopharyngeal nerve and strands were separated under liquid paraffin. The activity in fine strands (5–25 μ diameter) was monitored on a Tektronix Type 565 oscilloscope, and the signal of the vertical deflexion amplifier was led to an audio-monitor, to another oscilloscope with a P 2 phosphor, to a storage oscilloscope, and to a pulse height discriminator. The nerve potentials displayed on the oscilloscope with the P 2 phosphor were photographed on moving film. The number of nerve impulses occurring in 20 sec periods was read from the film in some experiments. In others the number occurring in 30 sec periods was read from a Hewlett Packard Counter whose input was the output of the pulse height selector. Some records were stored and photographed with a Polaroid camera so that changes in height and shape of the action potentials during a long experiment could be more easily observed at the time of their occurrence. The pulse height discriminator was used to select action potentials and convert them to pulses of standard size, which were then led to a rate-meter.

The animals were given gallamine triethiodide (Flaxedil, May & Baker), 20 mg/kg, intravenously and ventilated mechanically with a pump. The frequency of the pump was fixed; the stroke volume was adjusted to give, under control conditions, an end-tidal P_{CO_2} of 28–32 mm Hg.

Experiments were designed to study the relation between carotid body chemoreceptor discharge and (1) perfusion pressure at constant blood gas tensions and pH; (2) P_{a,O_2} at constant P_{a,CO_2}, pH and pressure; (3) P_{a,CO_2} at constant P_{a,O_2}, pH and pressure and (4) pH at constant P_{a,O_2}, P_{a,CO_2} and pressure.

Each measurement was made as follows. When a steady state with respect to blood gas tensions and pH had been achieved as judged by serial measurement of blood samples, a sample of carotid arterial blood was drawn into the side arm of the common carotid loop, the carotid sinus segment was isolated and a constant pressure, usually between 120 and 150 mm Hg, applied. The frequency of chemoreceptor discharge was measured over the second 30 sec of the 1 min perfusion period (see Results). Most measurements were made in duplicate, the manoeuvre being repeated 1–2 min later.

P_{a,O_2} was altered in random steps by feeding different O_2/N_2 mixtures to the pump. A new steady level was usually achieved in 6–8 min. P_{a,CO_2} was altered by adjusting the stroke volume of the pump or by adding CO_2 to the inspired gas. P_{a,O_2} was then adjusted by adding O_2 or N_2 to the inspired gas and pH_a was maintained at control levels by infusing M-$NaHCO_3$ or M-HCl intravenously. Following the infusion, an interval of 25 min was allowed while any changes in P_{a,CO_2} were adjusted by altering the stroke volume of the pump. A blood sample was taken for analysis and if no further adjustment was necessary, a second blood sample was taken 5 min later to check that a steady state existed. A similar procedure was adopted when pH_a was changed and P_{a,CO_2} and P_{a,O_2} held constant.

RESULTS

Identification of chemoreceptor fibres

The criteria which follow were used to determine the origin of the different groups of fibres found in the sinus nerve.

Chemoreceptor afferent fibres were defined as those in which an appropriate response to changes in the inspired gas occurred within 5 sec. These responses were a decrease in the rate of discharge to very low levels follow-

ing an increase in the P_{I,O_2}, and an increase in rate of discharge when the P_{I,O_2} was lowered or the P_{I,CO_2} was raised. The response of these fibres to clamping the common carotid artery was a rise in the discharge rate, never a fall.

Baroreceptor afferents showed a discharge which was synchronized with the pulse and sometimes with respiration. Their rate of discharge fell when the carotid artery was clamped, and they did not show short latency changes in rate when the inspired gases were altered, only changes which were secondary to variations in arterial pressure.

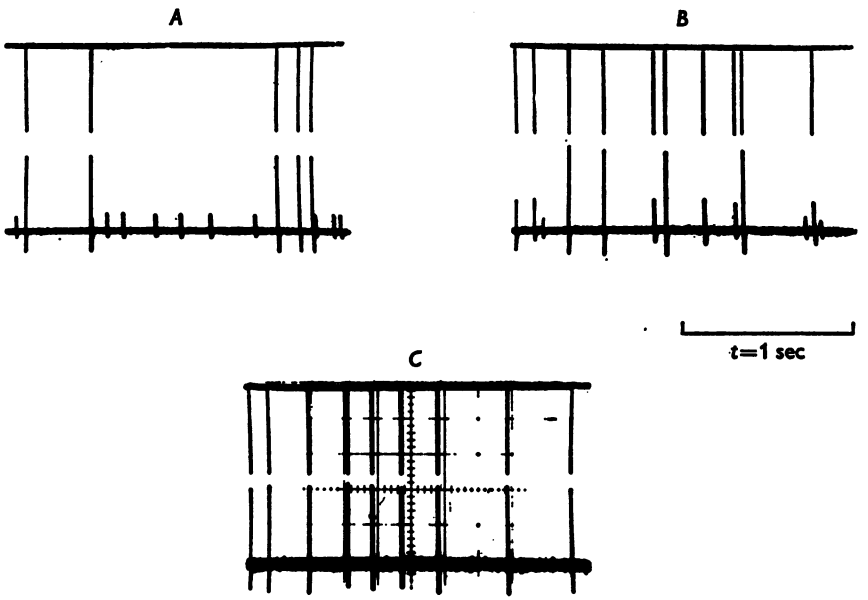

Fig. 2. *A*, *B* and *C*. Oscilloscopic tracings to show the relation between, below, the activity in chemoreceptor fibres and, above, the standard pulses triggered by selected action potentials. For description of traces, see text.

Sympathetic post-ganglionic fibres were encountered twice and showed the classical highly synchronized rhythm in time with ventilation (Adrian, Bronk & Phillips, 1932). They did not respond with a short latency to transient changes in blood gas tension (Biscoe & Purves, 1967), nor to carotid clamping. In addition they showed a cardiac rhythm.

It was usual to dissect the nerve until a strand was found which contained only one active fibre. The nerve strands were large enough to contain several myelinated fibres and many non-myelinated fibres of the types found in the sinus nerve (De Castro, 1951; Ask-Upmark & Hillarp, 1961;

Eyzaguirre & Uchizono, 1961), and in some strands there was a clear separation between two, or more rarely three, action potential amplitudes. Sometimes the shape and size of the potentials changed with the passage of time; if it became impossible to identify the separate potentials the strand was discarded. Constant surveillance of the signal was necessary and was considerably aided by the use of the storage oscilloscope. Usually the most rapid changes in amplitude of the action potential occurred during the first 10–15 min of recording. Therefore, this period of time was allowed to elapse before any experimental sequence was commenced. The 2- or 3-fibre strands were particularly interesting, for they allowed comparison of different single afferents under identical conditions.

In some experiments a discriminator was used so that selected potentials only were counted. Examples of the method are shown in Fig. 2. In *A*, one of two fibres was counted; in *B* two out of three and in *C* a single fibre.

The assumption that potentials arose from single units was based on the following criteria: the amplitude of the potentials was seen to be relatively constant when they were photographed on slowly moving film; the amplitudes varied by an amount no greater than the amplitude of the noise; the nerve impulses were similar in shape and size when viewed on a fast time base; there was never summation of a supposed single nerve potential. To ensure that each spike could be easily distinguished from the others in the 2- or 3-fibre records, the potentials were observed on the storage oscilloscope and photographed with a Polaroid camera so that they could be compared with the potentials obtained from the same strand later in the experiment.

Effects of haematocrit on chemoreceptor discharge

Because a fall in haematocrit is associated with a rise in carotid body blood flow at constant perfusion pressure (Purves, 1970), the effect of changing arterial haematocrit upon chemoreceptor discharge was tested. In the present series of cats the arterial haematocrits at the start of the experiment were in the range of 25–41 %, the majority being between 30 and 35 %. Two methods of altering haematocrit were used. In the first, 20 ml. heparinized blood was taken at the start of the experiment, the red cells separated by centrifuging, and reconstituted with normal saline to give mixtures with approximately 20, 30 and 40 % haematocrit. These mixtures were then placed in small tonometers and equilibrated at 38° C with gas mixtures so as to have blood gas tensions and pH values which differed by no more than 2 mm Hg or 0·03 pH unit from those in the cat. Samples (1 ml.) were taken from the tonometers and injected into the dead space of the side arm of the common carotid loop and the measurement of chemoreceptor discharge was made in the usual way at constant pressure,

130 mm Hg. This method gave inconsistent results and led to a marked sustained rise in chemoreceptor discharge which became continuous on repetition of the perfusion. The offending property of this reconstituted blood was not identified but may have been due to the method of centrifugation, reconstitution or tonometry.

In the second method, used successfully with three fibres in two cats, red cells were obtained by sedimentation from blood from a donor cat. After measurement of the chemoreceptor discharge at control haematocrit in the experimental cat, between 10 and 15 ml. red cells were injected slowly and, after an interval of 20–30 min, a second measurement was made at the new level of haematocrit at the same blood gas tensions and perfusion

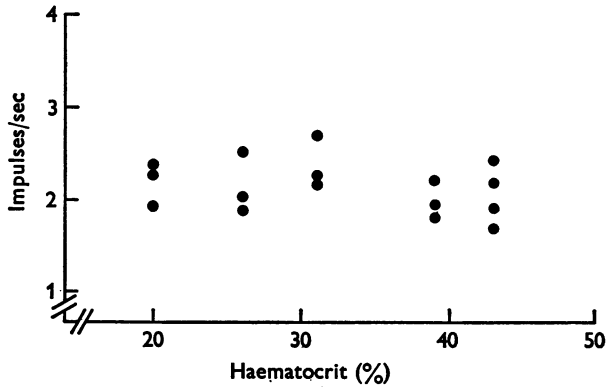

Fig. 3. The relation between the discharge from a single chemoreceptor afferent fibre (impulses/sec) and the arterial haematocrit. For method of altering haematocrit, see text. P_{a,O_2} 91–93 mm Hg, P_{a,CO_2} 29 mm Hg, pH 7·38, perfusion pressure 130 mm Hg. Cervical sympathetic nerve intact.

pressure. Subsequently, blood was removed in 20 ml. steps and was replaced by an equal volume of dextran. At each level of haematocrit, over the range 20–43 %, at least three measurements of chemoreceptor discharge were made. The results for one fibre are shown in Fig. 3 and indicate that, over this range of haematocrit, the frequency of discharge was not affected; similar results were obtained with the other two fibres.

This Figure also gives an indication of the reproducibility of measurements made under steady conditions with respect to blood gas tensions and pressure. In this example, the coefficient of variation varied between 8·5 and 16·7 %.

Serial measurements of haematocrit in experiments in which fluids were injected, e.g. $NaHCO_3$ and HCl solutions in the chemoreceptor/pH experiments, showed that haematocrit fell by no more than 3 % during the experiment.

Changes in temperature with perfusion

The withdrawal of blood into the side arm of the carotid loop and the slow perfusion of the carotid sinus segment with blood over a period of 1 min might have resulted in cooling of the blood and hence have affected chemoreceptor discharge since it is known that the chemoreceptors are sensitive to changes in temperature (Bernthal & Weeks, 1939; Witzleb, 1952; Eyzaguirre & Lewin, 1961). The temperature of blood within the carotid sinus was measured by inserting a calibrated constantin thermocouple by the side of the external carotid arterial cannula so that it lay in the carotid sinus. On the seven occasions on which the temperature was so measured, no change in temperature was observed as blood was withdrawn into the side arm of the carotid loop or for the first 40–50 sec after the carotid sinus segment was isolated and blood perfused. On three occasions, the temperature started to fall towards the end of the 1 min perfusion period, the greatest change noted being 0·4° C. On the other four occasions, the temperature within the sinus did not change. When the clamp was removed from the carotid loop and normal carotid blood flow was resumed, temperature within the sinus fell by up to 4·5° C over the next 5–10 sec, indicating that cooling of the blood had taken place in the exposed part of the loop.

Effects of altering perfusion pressure upon carotid body chemoreceptor activity

Transient changes. The response of the carotid body chemoreceptors to sudden changes in carotid sinus pressure was studied in ten fibres in six cats. On three of these occasions, carotid body blood flow was also measured. One result is shown in Fig. 4 where, in *A*, the frequency of nerve impulses is plotted against time elapsed for 20 sec of the control period and 90 sec of perfusion. In *B*, carotid body blood flow measurements are plotted, here the time taken for 5 μl. blood to flow is the ordinate and the sequence of the measurements is the abscissa. At the arrow in Fig. 4*A* and *B*, carotid sinus pressure was abruptly lowered from 155 to 55 mm Hg. During the control period of 1 min (of which 20 sec are shown), chemoreceptor activity varied between 5·8 and 8·3 impulses/sec, mean 7·15, S.D. $\pm$ 0·82, $n = 15$. During the first 5 sec after the pressure change, chemoreceptor activity rose to a maximum of 10·6 impulses/sec and thereafter fell to a level which did not significantly differ from control. Figure 4*B* shows the accompanying fall in carotid body blood flow which occurred over the same period, indicated by an increase in the ordinate. In approximate terms, blood flow fell from 50–75 μl./min in the control period to 17 μl./min in the first 4–10 sec after the pressure change, remaining at this level for the next 50 sec.

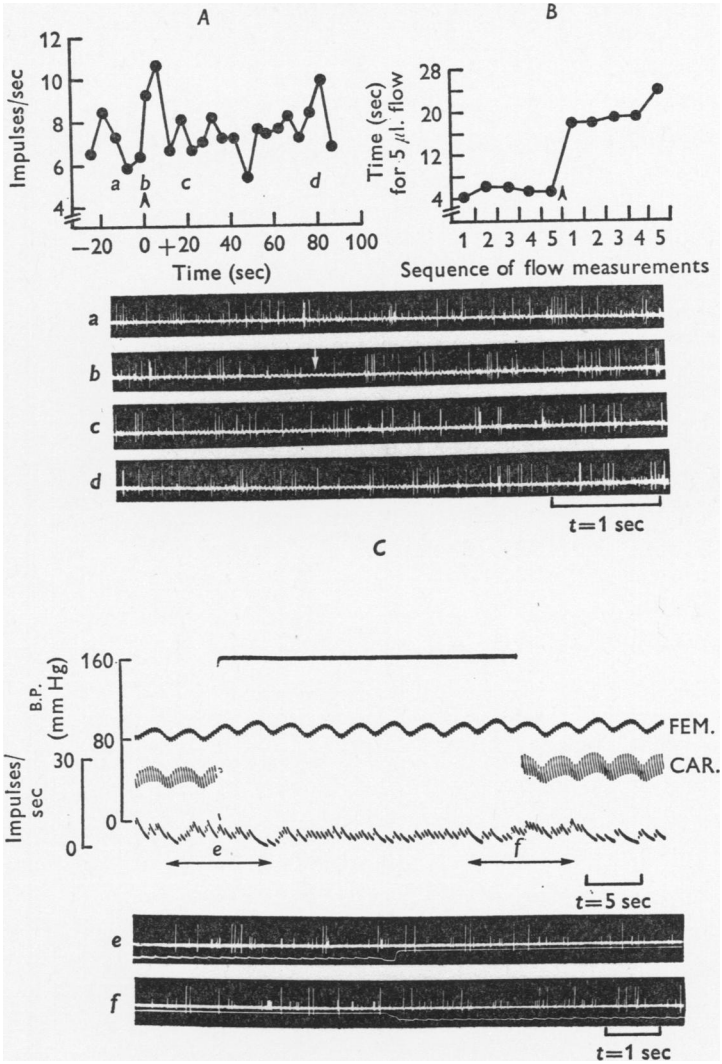

Fig. 4. *A*. The discharge in a single chemoreceptor fibre (impulses/sec) before and after the reduction of perfusion pressure within the carotid sinus from 155 to 55 mm Hg at the arrow $t = 0$. The traces *a–d* were recorded at the points indicated in *A*.

B. The changes in carotid body venous flow, expressed as the time taken in seconds for 5 μl. blood to flow, which occurred as pressure was reduced in *A*.

C. The changes in carotid body chemoreceptor activity which occurred when carotid sinus pressure was abruptly raised from 50 to 160 mm Hg for approximately 20 sec, and then lowered. From above down, mean femoral artery pressure (FEM., mm Hg) and on the same scale, carotid sinus pressure (CAR., mm Hg), rate-meter output of the discharge from a single chemoreceptor fibre (impulses/sec). The filmed records *e* and *f* were recorded at times indicated below the rate-meter record; upper trace in each shows nerve impulses, lower trace carotid artery pressure.

Towards the end of the 90 sec perfusion period, in this and other fibres, there was a rise in activity similar to that shown in Fig. 4*A*, whether the perfusion pressure had been raised or lowered. Whatever its cause, it was concluded that 60–70 sec represented the length of safe perfusion without artifact. Similarly the transient alterations in chemoreceptor discharge immediately following the pressure change indicated that the second 30 sec period after the change best reflected steady conditions.

In six other fibres, similar changes were seen, that is within 5 sec of the abrupt reduction in perfusion pressure, chemoreceptor activity altered by more than twice the standard deviation of values in the control period. In three other fibres, no such early increase in chemoreceptor discharge was noted after similar changes in pressure. On the other hand, when pressure and flow became steady, the level of chemoreceptor discharge appeared to be independent of pressure over the range tested (55–160 mm Hg) and of blood flow (10–75 μl./min). Chemoreceptor activity was similarly measured on seven occasions when carotid sinus pressure was abruptly raised from the range 50–60 mm Hg to 145–160 mm Hg. An example of this manoeuvre is shown in Fig. 4*C* in which neurograms recorded at the on (*e*) and off (*f*) transients are also shown. In this example and in others in which chemoreceptor activity during the control period was low, the discharge was not significantly altered by the change in pressure. In other fibres with a higher resting frequency of discharge, the abrupt rise in pressure was followed by a transient fall in discharge, occasionally to zero, for 5–10 sec after which the discharge rose to control levels for as long as pressure was raised. However, when the changes in pressure were greater than those described above, then much greater changes in chemoreceptor discharge were seen. For example, in one cat, the response of two chemoreceptor fibres to a reduction in pressure from systemic to 20 mm Hg and then to an abrupt rise in pressure to 140 mm Hg was measured. In one fibre, the discharge at systemic pressure of 110 mm Hg was 2·8 impulses/sec; at 20 mm Hg, 24·3 impulses/sec. In the other fibre, the discharge at 120 mm Hg was 1·9 impulses/sec and at 20 mm Hg was 32 impulses/sec. Within 5 sec after the carotid sinus pressure was raised to 140 mm Hg, the discharge fell to zero and 2·2 impulses/sec respectively and thereafter rose to steady levels of 1·5 and 3·8 impulses/sec for the next 40 sec.

Steady-state responses. The chemoreceptor discharge at different steady levels of carotid sinus pressure and total blood flow was measured in twelve fibres from five cats (Figs. 5 and 6). The steady levels of pressure were selected by using a table of random numbers. In the eighteen tests made, two types of response were seen. In the first, seen in thirteen tests on eight fibres, the afferent discharge was not altered over the range of perfusion pressure 60–160 mm Hg. This is illustrated for two levels of P_{a,O_2} in the

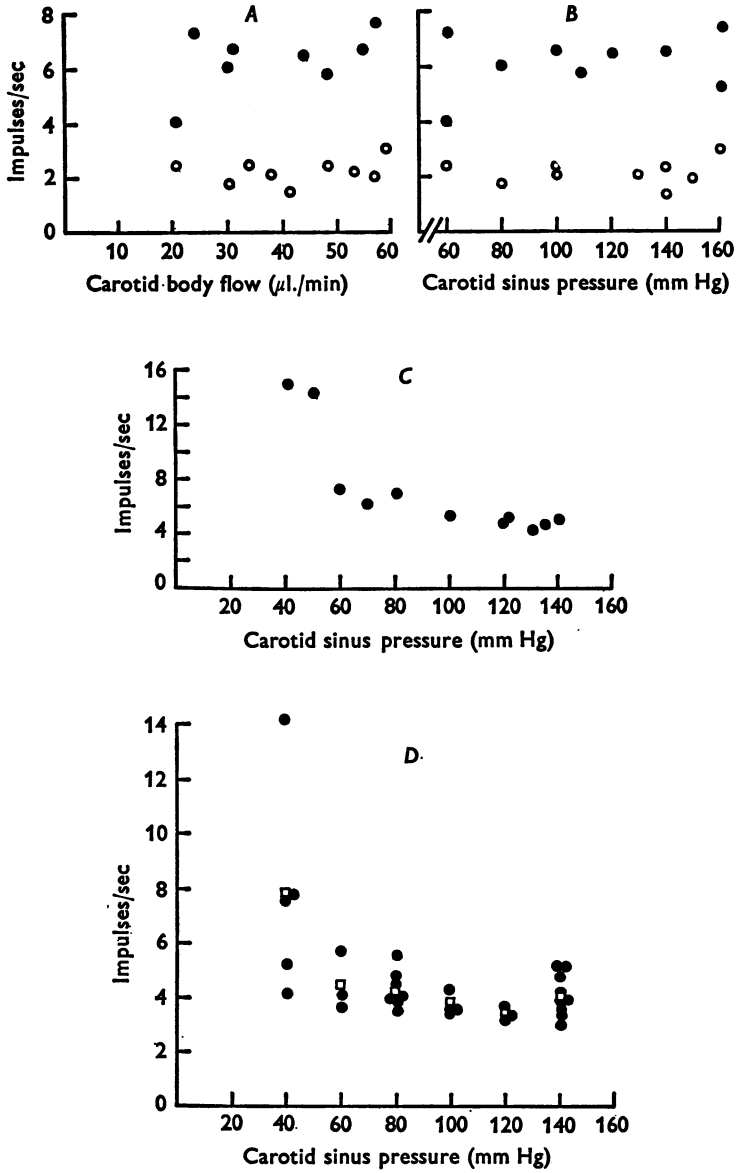

Fig. 5. The relation between activity in a single chemoreceptor fibre and *A*, carotid body venous flow and *B*, carotid sinus pressure. In each, filled circles P_{a,O_2} 87 mm Hg, open circles P_{a,O_2} 240 mm Hg. P_{a,CO_2} 29–30 mm Hg, pH 7·39 units throughout. Cervical sympathetic intact.

C. The relation between activity in a single chemoreceptor fibre and perfusion pressure altered in random steps over the range 40–140 mm Hg. P_{a,O_2} 92 mm Hg, P_{a,CO_2} 30 mm Hg, cervical sympathetic intact. *D*. The relation between the activity in a single chemoreceptor fibre and perfusion pressure altered randomly in multiples of 20 mm Hg steps, filled circles. The mean discharge at each pressure is shown (open squares). P_{a,O_2} 95, P_{a,CO_2} 29 mm Hg, cervical sympathetic intact.

experiment of Fig. 5*A* and *B* which confirms the fact that although chemoreceptor activity was independent of flow and pressure at each level of P_{O_2}, chemoreceptor activity was reduced by a rise in P_{O_2} over the range of pressure tested. The flow may also be varied by small amounts at constant perfusion pressure, by changing the P_{a,O_2}. For example, in one cat when the

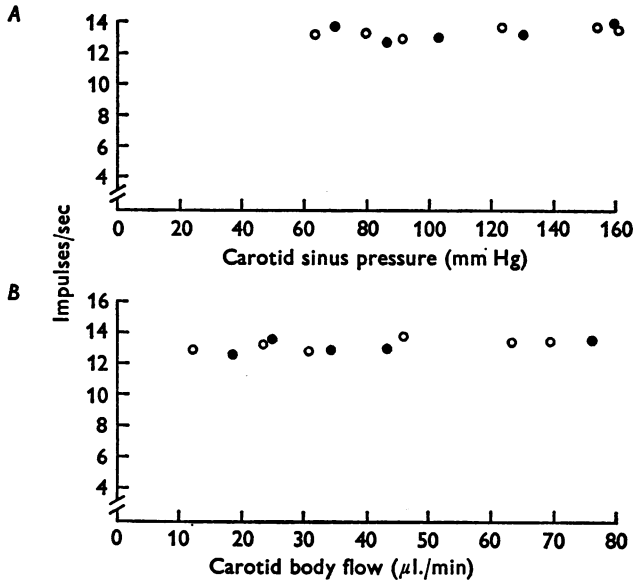

Fig. 6. The relation between activity in a single chemoreceptor fibre and carotid sinus pressure *A*, altered randomly, and the accompanying changes in carotid body venous flow *B*. Filled circles, cervical sympathetic intact, open circles, following section of the post-ganglionic sympathetic fibres to the carotid body.

perfusion pressure was held constant at two levels, 70 and 140 mm Hg, and P_{a,O_2} reduced from 350 to 45 mm Hg there was an increase in carotid body total blood flow from 10 to 17 μl./min at the lower perfusion pressure and 48–54 μl./min at the higher. At the same time and at both levels of perfusion pressure, chemoreceptor discharge increased by an order of magnitude. Such changes in flow would not of themselves alter the chemoreceptor rate according to our results (see Figs. 5*A* and 6*B*). Thus the large changes in chemoreceptor rate which occur cannot be ascribed to the associated changes in total blood flow which are too small and have an inappropriate direction. The absence of an effect of changing perfusion pressure (and total blood flow) above 60 mm Hg is also shown in Fig. 5*D* where the individual readings were taken in random order as usual. The regression line for the points lying between 60 and 140 mm Hg was calculated and

was not significantly different from zero ($t = 0{\cdot}25$, $P > 0{\cdot}1$). In six of these thirteen tests the carotid body was perfused naturally at systemic pressure and also artificially perfused at the same pressure. Changes in systemic pressure were brought about by bleeding the cat or replacing the blood and adjusting the inspired gas mixtures so that blood gas tensions were constant. At no level of pressure over the range 60–160 mm Hg in any test did chemoreceptor activity differ by more than 1·2 impulses/sec between the two methods of perfusion.

The second type of response, seen in four fibres, consisted of a significant increase in chemoreceptor discharge ($P < 0{\cdot}01$) as the pressure and flow decreased (Fig. 5*C*). This rise in chemoreceptor discharge was not due to deterioration of the preparation with time since the measurements were made after a pressure change that was randomly directed to the new steady level.

In both groups of fibres the discharge increased in rate at perfusion pressures below 50–65 mm Hg, a result which was seen in eight tests on five single fibres from three cats. This increase is illustrated in Fig. 5*C* and *D*. In Fig. 5*C* the level of chemoreceptor discharge at these low pressures is approximately double that at higher pressures and in Fig. 5*D* the mean chemoreceptor activity at 40 mm Hg was significantly raised ($t = 3{\cdot}12$, $P < 0{\cdot}025$); but note the wide range of the observations at 40 mm Hg.

The chemoreceptor response to changes in carotid sinus pressure was measured in two fibres in two cats before and after cutting the preganglionic cervical sympathetic nerve on the recording side. Fig. 6*A* and *B* shows the lack of effect on chemoreceptor activity of the parallel changes in carotid sinus pressure (60–160 mm Hg) and carotid body flow (11–78 μl./min). There was no difference in the level of activity whether the cervical sympathetic was intact (filled circles) or cut (open circles). Similar results were obtained in the second cat.

Carotid body chemoreceptor response to blood gas tensions and pH

The responses to changes in blood gas tensions and pH were tested formally in a further series of experiments. The results should be compared with those obtained in the accompanying paper (Biscoe *et al.* 1970) in which the carotid body was naturally perfused at systemic pressures and in which no dissection beyond that necessary for exposure of the sinus nerve was carried out.

Chemoreceptor response to changes in P_{a,O_2}. The changes in activity in response to alterations of P_{a,O_2} were measured in eleven fibres of the sinus nerve in six cats. In two cats (three fibres), activity was measured as carotid sinus pressure was altered over the range 50–160 mm Hg and as P_{a,O_2} was altered over the range 35–420 mm Hg. One of these results is shown in Fig. 7.

Figure 7*A* shows that chemoreceptor activity did not obviously change until carotid sinus pressure was lowered below 70 mm Hg. In Fig. *B*, the response of the same fibre as in Fig. 7*A* is shown when P_{a,O_2} was altered over the range 35–420 mm Hg at constant sinus pressure, either 70 mm Hg

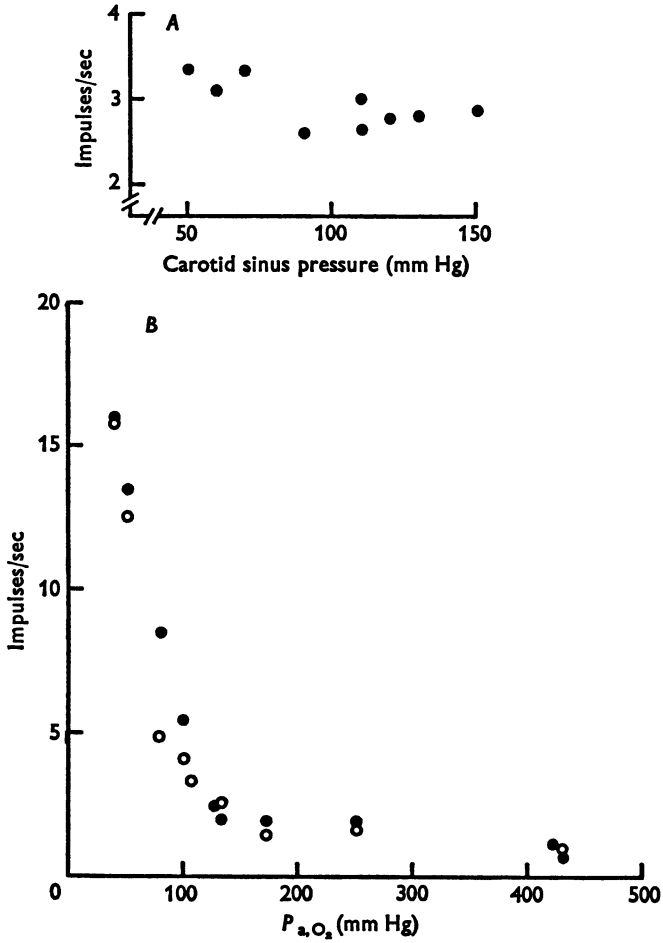

Fig. 7. *A*. The relation between activity in a single chemoreceptor fibre and carotid sinus pressure altered randomly over the range 50–150 mm Hg at constant P_{a,O_2} and P_{a,CO_2}. *B*. Activity in the same fibre as in *A* plotted against P_{a,O_2} over the range 35–420 mm Hg. P_{a,CO_2} 30 mm Hg, carotid sinus pressure 70 mm Hg (open circles), 140 mm Hg (filled circles). Cervical sympathetic intact.

(open circles) or 140 mm Hg (filled circles). In this and in the two other experiments, chemoreceptor activity at the higher sinus pressure did not significantly differ from that at the lower pressure, in each case $P > 0{\cdot}5$,

and further, at any given level of P_{a,O_2} chemoreceptor activity at the two pressures did not differ by more than the error of measurement.

In eight fibres in four cats, the response to alterations of P_{a,O_2} was measured while the carotid body was naturally perfused at systemic pressure, artificially perfused at the same pressure and at another constant pressure. The results from one such experiment are shown in Fig. 8. From this and the other series of response curves obtained, the following points emerge.

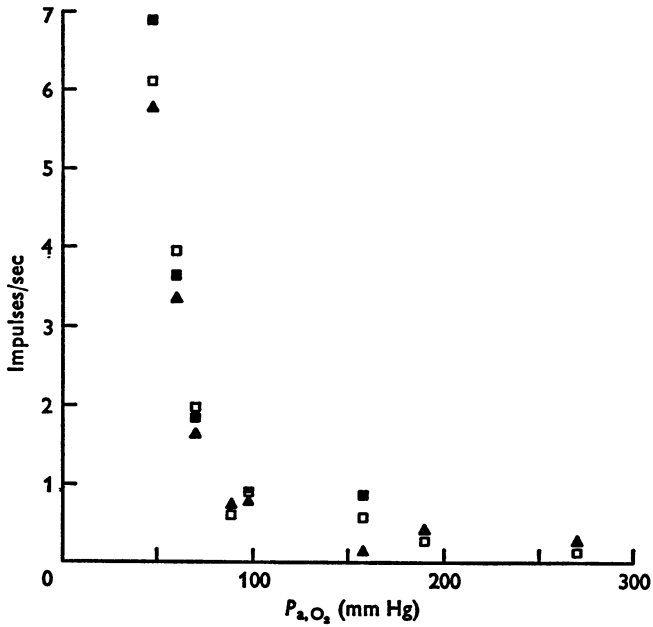

Fig. 8. The relation between activity in a single chemoreceptor fibre and P_{a,O_2} at P_{a,CO_2} 29–30 mm Hg. Open squares, carotid body naturally perfused at systemic pressure, range 95–125 mm Hg; filled triangles, carotid body artificially perfused at systemic pressure; filled squares, carotid body artificially perfused at 130 mm Hg. Cervical sympathetic intact.

1. The frequency of discharge at high O_2 tension, 200 mm Hg, varied between 0·2 and 2·3 impulses/sec.

2. The range over which chemoreceptor activity obviously started to increase as P_{a,O_2} was lowered varied between 110 and 200 mm Hg.

3. In no experiment was chemoreceptor activity extinguished at high P_{a,O_2}, above 400 mm Hg.

4. There was no statistically significant difference between values for chemoreceptor activity whether the carotid body was naturally perfused at systemic pressure, artificially perfused at systemic pressure or whether the

carotid body was artificially perfused at pressures in the range 90–160 mm Hg.

Response to changes in P_{a,CO_2}. Three fibres in two cats were studied. The responses of two fibres in one cat are shown in Fig. 9. As P_{a,CO_2} was altered over the range 24–75 mm Hg, pH_a was adjusted by the infusion of 5–10 ml. M-$NaHCO_3$ or M-HCl. When steady states (as defined in Methods) with

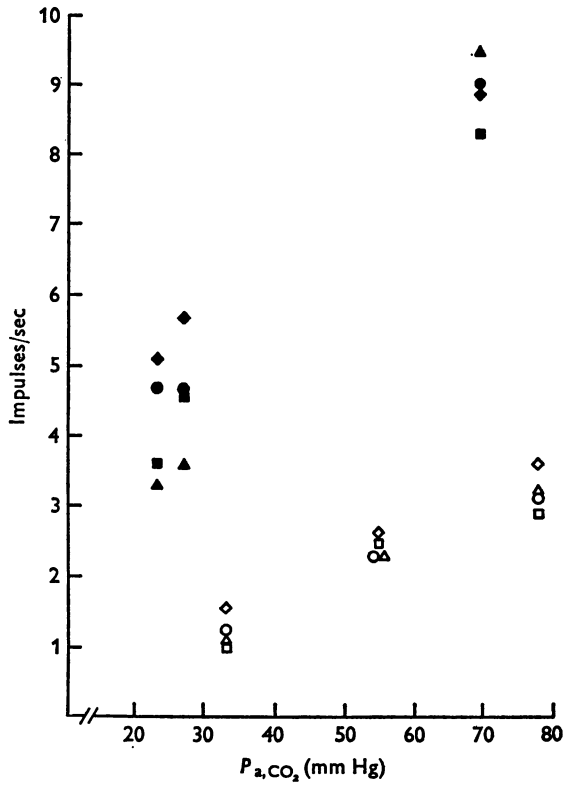

Fig. 9. The relation between activity in two single chemoreceptor afferent fibres and P_{a,CO_2}. Filled symbols, one fibre, pH maintained at 7·27–7·29 units; open symbols, one fibre, pH maintained at 7·32 units. In each experiment, the carotid body was perfused naturally at systemic pressure, range 85–100 mm Hg (circles), artificially at the same pressure (squares), artificially at 110 mm Hg (triangles) and artificially at 140 mm Hg (diamonds). P_{a,O_2} 91–95 throughout; cervical sympathetic intact.

respect to P_{a,CO_2} and pH_a had been achieved, the carotid body was perfused under four conditions: naturally at the cat's systemic pressure; artificially at this pressure; artificially at 110 and at 140 mm Hg. In one fibre (filled symbols), pH_a was maintained at 7·27–7·29 units; in the other fibre (open symbols), pH_a was maintained at 7·32 units. The results from these

fibres and from the third fibre studied confirm first that at any level of P_{a,CO_2}, chemoreceptor activity was not significantly affected by the method of carotid body perfusion nor by the range of perfusion pressure, which varied between 70 and 140 mm Hg. These experiments also provide evidence that the carotid body chemoreceptors respond to changes in P_{a,CO_2} independently of changes in pH_a.

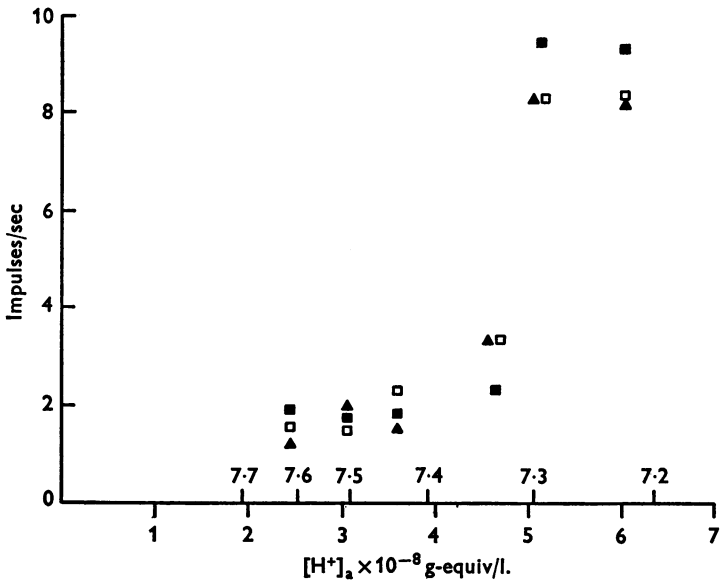

Fig. 10. The relation between activity in a single chemoreceptor afferent fibre and arterial hydrogen ion concentration (g-equiv/l.). For explanation of symbols, see text. Cervical sympathetic intact. P_{a,CO_2} was maintained at 28–29 mm Hg, P_{a,O_2} at 91–93 mm Hg throughout. The upper scale on the abscissa is for pH.

Response to changes in pH_a. Experiments were carried out in ten fibres in six cats in which the relation between chemoreceptor activity and pH_a was studied under various conditions. In general, for any one method of perfusion over the range $2–4{\cdot}5 \times 10^{-8}$ $[H^+]$ g-equiv/l., chemoreceptor activity changed by not more than 1 impulse/sec for a change of 2×10^{-8} g-equiv/l. $[H^+]_a$. When $[H^+]_a$ was raised to 5×10^{-8} g-equiv/l. or more, chemoreceptor activity at least doubled. The results from one experiment are illustrated in Fig. 10. The carotid body was perfused naturally at the cat's systemic pressure which varied between 80 and 110 mm Hg (filled triangles), artificially at the same pressure (filled squares) and artificially at 140 mm Hg (open squares). The results of this experiment show first that changes in chemoreceptor discharge over the range 2·4 to $3{\cdot}7 \times 10^{-8}$ g-equiv/l. were

small but as $[H^+]_a$ increased, chemoreceptor discharge increased. Secondly, there was no evidence of any difference in the level of discharge depending on the method of perfusion of the carotid body. Similar results were obtained from the nine other fibres studied. Altogether forty-three sets of triple readings were obtained under steady-state conditions in which the carotid body was naturally perfused, artificially perfused at systemic pressure and at a pressure within the range 140–160 mm Hg. The greatest difference between any two of the three readings obtained under the various conditions given above and at any level of $[H^+]_a$ was 3·4 impulses/sec. As in Fig. 10, there was no significant difference between the discharge when the carotid body was perfused naturally or artificially at systemic pressure, in each case $P > 0{\cdot}25$, or between the discharge when the carotid body was perfused at systemic pressure or at 140–160 mm Hg. $P > 0{\cdot}5$. In each experiment, the response was similar to that shown in Fig. 10.

DISCUSSION

The method. The carotid body or its blood supply might have been affected in one of two ways: there might have been injury to the carotid sinus and the carotid body vein, or the carotid body could have been affected by the separation of blood in the isolated segment during the period of artificial perfusion.

The following points serve to validate the method of artificial perfusion used. First, when the carotid body had been damaged during dissection either no chemoreceptor activity could be found in the sinus nerve or, if it was, the activity was unusually high at normal blood gas tensions and arterial pressure and was unresponsive to changes in blood gases. In the cats which formed this series, active chemoreceptor fibres were no more difficult to find or to record from than in those cats in which the carotid sinus had not been isolated. Secondly, there was close correlation between chemoreceptor activity recorded when the carotid body was naturally perfused and when it was artificially perfused under the same conditions. This correlation was found over a wide range of blood gas tensions, pH and perfusion pressure. Further, the rate of discharge and pattern of response were similar whether the carotid body was naturally perfused as in the present experiments or as in those reported in the accompanying paper (Biscoe *et al.* 1970) where there was no cannulation of blood vessels in the neck. Thirdly, good reproducibility was obtained when measurements of chemoreceptor activity were separated by an hour or more. Finally, the activity in each fibre responded to high O_2 with a reduction in rate.

The responses to changes in perfusion pressure. In eight out of twelve chemoreceptor afferent fibres tested, activity was not affected by changes

in perfusion pressure over the range 60–160 mm Hg. In the remaining fibres, as perfusion pressure was lowered over this range activity increased by up to 30 % of the value at the highest pressure. In all fibres tested, activity increased sharply as perfusion pressure was lowered below 60 mm Hg. This comparative independence of chemoreceptor discharge and perfusion pressure contrasts with the response seen with abrupt changes in pressure where, in the majority of tests, chemoreceptor activity briefly increased as pressure was lowered and fell as pressure was raised.

The response of the carotid body chemoreceptors to changes in perfusion pressure is different from that seen by Lee *et al.* (1964) with the aortic chemoreceptor discharge. They found an inverse relation between discharge and pressure and the relation which they illustrate operates within the physiological range of pressure. The difference in sensitivity to changes in pressure is unlikely to be explained by the methods used by these workers to alter systemic pressure, by haemorrhage or by ligation of the aorta or by the fact that P_{a,CO_2} was not controlled. Other differences between carotid body and aortic chemoreceptors have been demonstrated in their reflex responses, e.g. the response to nicotine (Comroe & Mortimer, 1964) and the response to mixed venous blood (Daly & Ungar, 1966), and these indicate that different sensitivities to systemic pressure need not be considered remarkable.

It is more difficult to reconcile our results with those obtained by Landgren & Neil (1951). They show examples of an increase in activity when the pressure fell spontaneously from 130 to 90 mm Hg, and again, when pressure was raised from 50 to 90 mm Hg chemoreceptor activity was noticeably higher than under control conditions at 130 mm Hg. It should be remarked that the results of Landgren & Neil (1951) are in no sense quantitative, depending as they do upon recording from multifibre preparations. Further, little indication is given of the time course of events following spontaneous pressure changes. We have found under such conditions that chemoreceptor activity may vary considerably with spontaneous fluctuations in pressure. Similarly, we noted that when pressure was raised after the animal had been bled and become hypotensive chemoreceptor activity was commonly higher at any given level of pressure than prior to the bleed. This was interpreted as being due to a change in the preparation with severe hypotension. Finally, Landgren & Neil (1951) placed reliance on the constancy of blood gas tensions during hypotension since the animal was artificially ventilated. Under these circumstances end-tidal CO_2 may fall, P_{a,CO_2} rise and variable changes in P_{a,O_2} together with a rapid increase in arterial $[H^+]$ occur. These changes may be due to inadequate perfusion of the lungs and tissues. When blood is replaced, end-tidal and arterial CO_2 may increase rapidly and exceed control levels for up to

5 min. For these reasons, we consider that the conclusion reached by Landgren & Neil (1951) that carotid body chemoreceptor activity is affected by changes of pressure within the physiological range should be modified.

Chemoreceptor response to changes in blood gas tensions and pH. The chemoreceptor responses to changes in P_{a,O_2} observed in the present series are similar to those obtained in the naturally perfused carotid body preparation by Biscoe *et al.* (1970) and by previous workers, e.g. Hornbein, Griffo & Roos (1961); Eyzaguirre & Lewin (1961). The response curves were found to vary considerably in shape and in position. It is noteworthy that some curves, as for example that illustrated in Fig. 7*B*, indicated a considerable increase in chemoreceptor discharge as P_{a,O_2} was lowered over the physiological range 105–85 mm Hg. The response to changes in P_{a,CO_2} was approximately linear over the range tested and this also is similar to that seen in the naturally perfused carotid body (see Figs. 3 and 4, Biscoe *et al.* (1970)). It also corresponds to the linear portion of the sigmoid curve described by Eyzaguirre & Lewin (1961) and by Biscoe & Millar (1966, 1968). We have not tested the effect of different levels of P_{O_2} upon the sensitivity of the chemoreceptor response to CO_2 but it is clear that if P_{a,O_2} was held constant within the physiological range 85–105 mm Hg, and P_{a,CO_2} was raised from 25 to 35 mm Hg, the increase in chemoreceptor discharge was not negligible, varying by between approximately 25 and 75% of the value at the lower P_{a,CO_2}. This finding is in contrast to the accepted idea that the carotid body chemoreceptors are relatively insensitive to changes in P_{a,CO_2} in the physiological range and in the absence of hypoxia, e.g. Torrance (1968), Daly (1968). The responses found in the present study also demonstrate that CO_2 exerts an effect upon chemoreceptors which is independent of changes in pH, thus confirming the findings of Joels & Niel (1960).

The curves obtained in response to $[H^+]_a$ also confirm the finding of Joels & Neil (1960) that the chemoreceptor response to $[H^+]_a$ is independent of changes in P_{CO_2}.

The results of the present study provide no direct evidence about the mode of excitation of the carotid body chemoreceptors. They indicate that, within the physiological range, there is little or no relation between chemoreceptor discharge and either the perfusion pressure or total carotid body blood flow. The possibility arises that the distribution rather than the total volume of blood flow is important and this question has been raised elsewhere (Purves, 1970).

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