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OBSERVATIONS ON THE RHYTHMIC VARIATION IN THE CAT CAROTID BODY CHEMORECEPTOR ACTIVITY WHICH HAS THE SAME PERIOD AS RESPIRATION

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SUMMARY

1. The activity in carotid body chemoreceptor afferent fibres in the cat has been recorded and found to have a rhythm with the same period as respiration.

2. This rhythm is not an artifact; it is not due to arterial pressure changes with respiration nor to cyclical changes in pulmonary venous admixture. It is caused by changes in blood gas tensions during each respiratory cycle.

3. The amplitude of the rhythm is modified by transient and long-term changes in inspired oxygen or CO_2 so that a rise or fall in O_2 or CO_2 tensions of arterial blood (P_{a,O_2}, P_{a,CO_2}) from the physiological range reduces it. The ratio of the rhythm amplitude to the mean rate of chemoreceptor discharge increases with P_{a,O_2} over the range 40–240 mm Hg.

4. The rhythm is modified by changes in respiratory frequency and volume.

5. The fluctuations of arterial oxygen tension which have the same period as respiration are shown to be conducted up the vertebral artery at least as far as the vertebro-occipital anastomosis.

6. It is proposed that the chemoreceptor rhythm reflects the moment to moment changes in blood gas tensions.

INTRODUCTION

The tension of CO_2 in alveolar gas is known to fluctuate within the respiratory cycle, being at its lowest at peak inspiration and rising throughout the expiratory phase (Haldane, 1915; Haldane & Priestley, 1935). These workers also demonstrated that the amplitude of this fluctuation

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was affected by the inhalation of CO_2, by alterations in the frequency of respiration and during voluntary exercise. Similarly, fluctuations of alveolar oxygen tension having the opposite phase were observed by Haldane (1915), were characterized theoretically by Chilton & Stacy (1952) and were deduced from breath-holding experiments by Dubois, Britt & Fenn (1952). The rate of capillary blood flow through the lungs makes it likely that these fluctuations are transmitted to pulmonary venous blood (Roughton, 1945) and there is some experimental evidence that they survive passage through the left side of the heart and can be measured in the main arteries. Nims & Marshall (1938) and Marshall & Nims (1938) recorded changes in pH in arterial blood which were related to respiration. Bjurstedt (1946) also recorded changes in arterial pH and oxygen saturation which varied with respiration. Åstrom (1952) illustrated similar though less well marked changes while Buhlman (1960) measured a difference of a few mm Hg P_{a,CO_2} between full expiration and inspiration in man. Bergman (1961) also showed that oxygen saturation varied with respiration and Purves (1965) showed a similar fluctuation of P_{a,O_2}.

These observations prompted the question whether fluctuations in the rate of discharge with a similar period could be recorded from the carotid body chemoreceptors, for a change in P_{a,O_2} might be assumed to affect the peripheral chemoreceptors especially if accompanied by a change in P_{a,CO_2} having the opposite phase. We have therefore investigated the chemoreceptor discharge from the carotid body of cats and new-born lambs. Some of the results have been recounted briefly in an earlier communication (Biscoe & Purves, 1965) and the results from the lambs are reported in another paper (Biscoe & Purves, 1967*b*).

METHODS

Fourteen cats were anaesthetized with pentobarbitone sodium, 30 mg/kg injected intraperitoneally. Subsequent injections were given through a radial vein cannula. The trachea was cannulated and the carotid sinus approached from the medial side by reflexion of the larynx and pharynx in the mid line. In five cats, a loop of polyethylene tube, 20–25 cm long, was inserted into the carotid artery on the same side from which carotid body recordings were to be made. Into this loop was placed a small, rapidly responding electrode the properties of which are described in detail elsewhere (Purves, 1966). The capacity of the loop and cuvette was 0·25 c.c. The electrode had a lag of not more than 0·05 sec; its response to a change in P_{O_2} of 0–150 mm Hg in blood or saline was 95% complete in 0·35–0·42 sec. A pulse pressure of 150/50 mm Hg caused a change in electrode current output equivalent to not more than 0·5 mm Hg P_{O_2} and the electrode was not sensitive to changes in flow. From a T piece in the same loop on the cardiac side of the oxygen electrode, carotid arterial pressure was continuously measured with a Statham P23AD strain gauge. In some experiments, the pressure in the left brachial artery was measured instead. Respiration was monitored with a pneumotachograph using a Statham differential strain gauge; the flow signal was integrated to give tidal volume. End-tidal P_{CO_2} was recorded continuously through a needle inserted into the tracheal cannula with an infra-red CO_2 analyser (Beckman LB1).

The outputs of the recording devices were simultaneously presented on a multi-channel recording oscilloscope (Electronics for Medicine) from which permanent records were obtained.

Nerve recordings. The chemoreceptor activity in the sinus nerve was monitored from the distal end of the cut nerve. In early experiments the sinus nerve was dissected until strands containing chemoreceptor fibres only were found. This was unrewarding since the elimination of unwanted potentials is often very difficult or, if successful, the resulting strand contains too few fibres for a rhythm to be observed superimposed upon the random discharge found in single chemoreceptor units (Biscoe & Taylor, 1963). However, success was occasionally achieved by this method (Fig. 1) and the results were then unequivocal.

In later experiments, the adventitia was stripped off the carotid sinus region to remove the baroreceptor nerve endings (Alvarez-Buylla, 1951). In these preparations, the activity recorded from the sinus nerve was then only of chemoreceptor origin.

The nerve was placed on a stainless steel plate and covered with warm liquid paraffin maintained at 38° C by radiant heat. The plate was fixed to the steel frame to which the animal was clamped and was earthed to the pre-amplifier. Action potentials were recorded through bipolar platinum wire electrodes connected to a high impedance a.c. pre-amplifier (Tectronix type 122) coupled to an oscilloscope. The nerve activity could be photographed from a slave oscilloscope and the signa from one vertical deflexion plate of a pair was fed to a pulse height selector.

The pulse height selector. The pulse height selector operated over a range of 0–30 V which was equivalent to a vertical deflexion of 5 cm on the oscilloscope tube face. The selector could be set to exclude the noise which was usually 40–50 μV peak-to-peak amplitude and which represented on the oscilloscope face a vertical deflexion of 0·5 or 0·25 cm depending on the gain setting. The output of the device was a square pulse of constant amplitude and duration which was used to reset a step function, millisecond, time scale to zero or which was viewed on the oscilloscope and was continuously counted on a rate-meter with a variable integrating time constant. The output of the rate-meter gave a permanent continuous paper record on a Texas Instruments Servo-Riter and on the Electronics for Medicine recorder. Any of the output signals referred to could be photographed together with the nerve recording while audiomonitoring was possible at all stages in the recording and counting system. The fact that noise was excluded by the selector was shown in the following ways; (1) zero output when no spikes were being seen on the oscilloscope; (2) a step-like increase in output when noise was counted as the lower level of the selector was adjusted; (3) monitoring the output of the pulse height selector on the oscilloscope below the action potential record to show that selector output square waves matched the recorded spikes. This technique has been used by Andersen & Curtis (1964); (4) using the output of the pulse height selector to reset a step function millisecond time scale to zero (Biscoe & Taylor, 1963); the resetting was often found easier to watch and observe with action potentials when the count rate was high. In addition, inhalation of 100 % oxygen reduced chemoreceptor activity in the majority of experiments to zero; occasionally a small amount of residual activity was observed (see Fig. 2*A*). This response is further discussed in connexion with Fig. 9*C*. Some or all these tests were always used.

The apparatus used for nerve recording in this work was also used in a demonstration to the Physiological Society on another subject (Biscoe & Millar, 1964) and is discussed later in this paper. The temperature of the cats was maintained at 37–38° C with an electric heating blanket.

RESULTS

Rhythmic changes in carotid body chemoreceptor rate have been recorded having a constant relation to the respiratory fluctuations of P_{a,O_2}. The precise relation to a phase of respiration is dependent on the lung-to-

carotid circulation time. Figure 1 is a film record of action potentials recorded from a baroreceptor-free strand of the sinus nerve. The sympathetic supply to the carotid body from the superior cervical ganglion was intact. The potentials can be seen discharging with the same period as respiration and the activity in this strand was rapidly depressed when the cat was given 100 % oxygen to breathe.

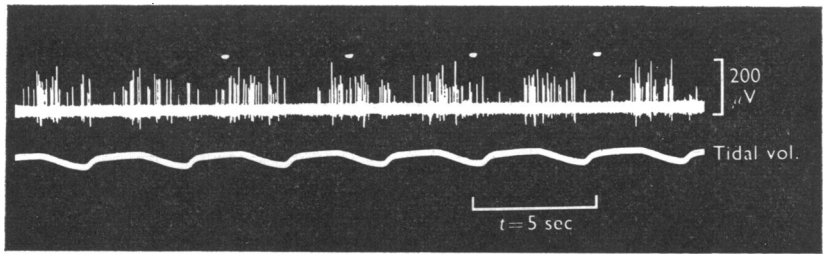

Fig. 1. Chemoreceptor activity (upper trace) measured in a baroreceptor-free strand of the sinus nerve and respiration (lower trace), inspiration downwards. Cat, pentobarbitone sodium. Cervical sympathetic intact. Each chemoreceptor discharge group is probably related to changes in alveolar gas tensions in the previous respiratory cycle.

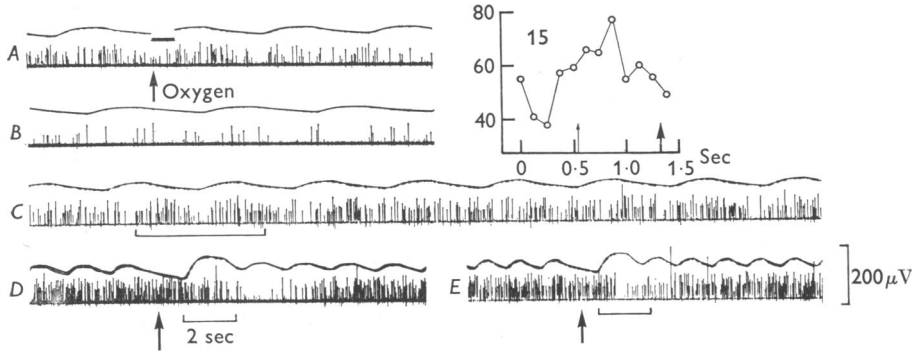

Fig. 2. Each record shows, above, the respiratory gas flow recorded by a thermister in the tracheal cannula and, below, chemoreceptor action potentials from the left sinus nerve. Spontaneous ventilation, inspiration downwards. The left preganglionic cervical sympathetic was cut before records *A*, *B*, *C* and *E* were taken.

A. Control, at the arrow the inspired gas was changed from air to 100 % oxygen.

B. After the oxygen had been on for 4 sec.

C. Control record showing the relationship of the chemoreceptor discharge to respiration. The graph plots the summed distribution of nerve impulses within 15 breaths for the record of which *C* is a part. The ordinate is the number of impulses, the abscissa is time, the small arrow marks the onset of inspiration and the large arrow the onset of expiration.

D. The effect of a spontaneous gasp, on the nerve activity before cutting the cervical sympathetic.

E. The effect of a spontaneous gasp after cutting the cervical sympathetic.

Calibration 200 μV. Each time scale is 2 sec.

Often, the rhythmic activity was not so well marked as this and another example is given in Fig. 2 in which the preganglionic cervical sympathetic on the recording side had been cut. Records *A* and *B* illustrate depression of activity by oxygen and *C* is a long continuous record. There is a tendency for the frequency of the potentials to increase during inspiration (which is downwards on the trace) a feature which is most obvious during the first four breaths and in the penultimate one. This impression is confirmed if the distribution of potentials within each respiratory cycle is plotted and summed for 15 cycles of identical duration. Each respiratory cycle was divided into 12 equal parts and the number of potentials counted from the record. The counts for 15 breaths were then added to give the graph. If the potentials were distributed within the breath according to chance, they should lie evenly distributed around the mean of 15 breath totals. This mean was 56·25. The graph reveals that the number of potentials reaches a peak during inspiration and a trough during expiration. Furthermore, the departures from this mean are greater than would be expected by chance according to the t test where $t = 3{\cdot}679$, $\phi = 11$ and $0{\cdot}005 > P > 0{\cdot}001$. Further evidence in support of the thesis that the chemoreceptor potentials are not randomly distributed within a breath is shown in Fig. 3, where a single unit exhibited a rhythm in time with respiration. This is a rare occurrence though here the animal was artificially ventilated at a slow rate of 16–17/min which would serve to make the effect more pronounced (see Fig. 13).

Figure 3 shows control activity when the cat was ventilated with air (*A*), the effect of raising (*B*) and then of lowering (*C*) the inspired oxygen tension. The activity of both the large and small units shows the expected decrease and increase in rate respectively. Records *D* and *E* are a continuous sequence where the rhythmic behaviour is well shown. The graphs were constructed in the same way as for Fig. 2 and here show the results after summing 20, 30, 40 and 50 breaths. After 20 breaths, the mean per group was 141 nerve impulses and the deviations were significant ($t = 5{\cdot}5$, $\phi = 11$, $0{\cdot}005 > P > 0{\cdot}001$) as were the deviations after 30 and 40 breaths. Similarly, after 50 breaths, the mean was 354 per group and the deviations remained significant ($t = 4{\cdot}16$, $\phi = 11$, $0{\cdot}001 > P$). Similar evidence was obtained from another cat both before and after sympathetic nerve section.

The temporal relationship between the chemoreceptor rhythm and the breath to which it was attributed varied between animals and in the same animal at different points in the experiment. This delay was presumably a measure of the lung-to-carotid circulation time and a method used to clarify this is shown in Fig. 4. The upper trace, *A*, shows a sequence of two respiratory cycles taken from a long series used for the results given in Fig. 3. It will be noted that after the respiratory pump had been turned off,

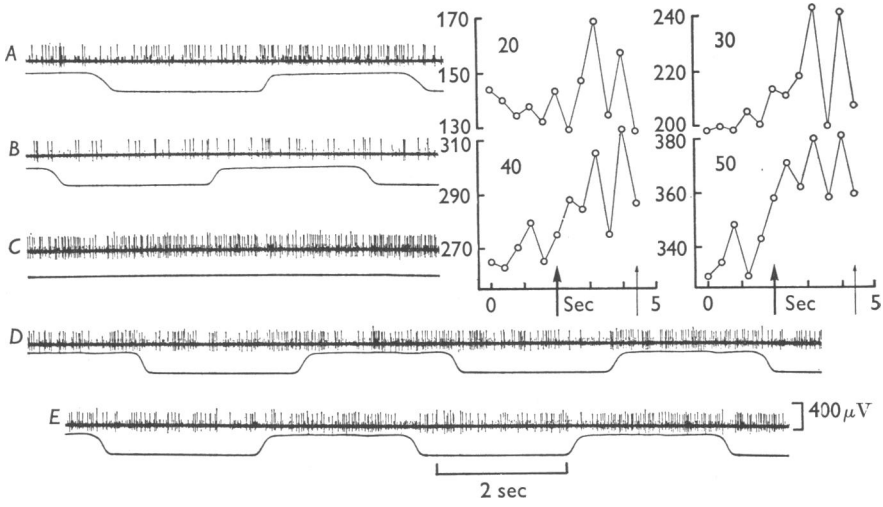

Fig. 3. Each record shows, above, chemoreceptor action potentials recorded from the left sinus nerve of a cat and, below, end tidal P_{CO_2} with inspiration indicated by a downwards excursion. Gallamine, artificial ventilation, sympathetic intact. *A*, Control sequence, the cat ventilated with air. *B*, 6 sec after changing the inspired gas to 100% oxygen. *C*, 20 sec after switching off the respiratory pump. *D*, *E*. A continuous sequence (*E* overlaps the end of *D*) showing variation in rate with each breath. The graphs are plots of the summed distribution of action potentials within successive breaths of which *E* and *D* are a part. The number of breaths in each case is indicated above each graph. The ordinate is the number of action potentials while the abscissa is time. The large arrow marks the onset of expiration, the small arrow the onset of inspiration. Calibration is 400 μV. Time scale, 2 sec.

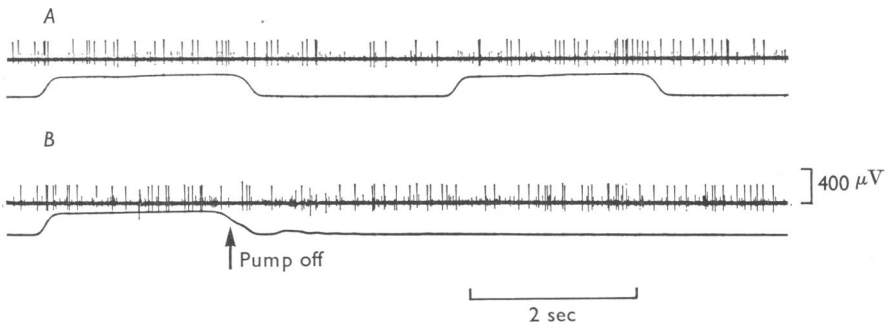

Fig. 4. Chemoreceptor action potentials from the same experiment illustrated in Fig. 3. The upper trace shows action potentials while the lower trace shows end-tidal P_{CO_2}, inspiration downwards. *A*, is a section of control record in which the rhythmical variation in chemoreceptor rate is shown over two respiratory (pump) cycles. *B*, shows the effect of turning off the respiratory pump on the rhythmical chemoreceptor discharge and illustrates that the lag in its production is due to the circulation time. Trace *C* in Fig. 3 follows shortly after this trace.

trace *B*, there was a decline in activity as in *A*; thereafter, there was a slow rise in chemoreceptor activity to control levels, i.e. those seen during inspiration, and then the frequency continued to increase. Trace *C* in Fig. 3 shows the discharge 25 sec after turning off the pump. It is not certain at what point peak inspiration took place but, assuming that it was halfway through the respiratory cycle, then the delay of approximately 2·5–3·0 sec before the decline in chemoreceptor activity is consistent with the lung-to-carotid circulation time measured by Purves (1966). Inspection of Figs. 3 and 4 reveals that there is considerable variation between breaths with regard to the point at which the minimum rate of discharge occurred. This is to be expected when a single unit is studied in view of the highly irregular discharge pattern which such units exhibit.

Recognition of chemoreceptor activity. It is well known that rhythmic changes in action potential rate in time with respiration may be recorded which are in fact not related to the supposed source. This situation could easily arise in the neck and, in our experiments, several different types of rhythmic activity were, in fact, seen. The most obvious in the sinus nerve is that which may be recorded from baroreceptors if the arterial pressure shows well marked variations in time with respiration. Such variations may be distinguished from chemoreceptor fluctuations, first, by the changes in rate in time with the pulse; secondly, by the fall in rate from baroreceptor afferents which usually though not always accompanies clamping the carotid artery; and, thirdly, especially by the response to a few breaths of oxygen. The last test is very reliable and also demonstrates whether the blood supply to the carotid body has been compromised or not. In addition, when the carotid loop was *in situ* for recording $P_{a_{O_2}}$ and carotid artery pressure, the carotid artery and sinus region could be flushed with 0·9% NaCl solution, equilibrated with room air. This sudden increase in pressure at once excited baroreceptors which had survived either nerve dissection or stripping the adventitia from the sinus region, whereas chemoreceptor activity was transiently abolished. Audio-monitoring of the pulse height selector output proved to be a useful method for detecting unwanted baroreceptor potentials. On some occasions, these were smaller than the chemoreceptor potentials and were also firing irregularly when near threshold. However, the pulse height selector converted all input signals to a constant size so that the baroreceptor signal was relatively amplified and its rhythm could at once be detected. The dissection was always continued until no baroceptors responded.

Another source of activity which may mislead is that recorded from pharyngeal muscles when these contract in time with respiration. This was seen on two occasions and was only recorded if the sinus nerve was inadequately earthed since such artifacts were eliminated with the stainless-

steel plate. The rhythm was deceptively like that seen from the chemoreceptors, since there were no peaks of activity with the increase in pulse pressure. Movement artifacts in time with respiration could simulate rhythmic activity but these were usually readily identifiable and could be eliminated.

The cause of the rhythmic variation in chemoreceptor rate

If chemoreceptor afferents were certainly identified, a possible source of rhythmic fluctuation in rate might have been a change in arterial blood pressure with each breath which could have modified blood flow through the carotid body. This was tested by producing a bilateral open pneumothorax with elimination of the respiratory induced changes in arterial blood pressure. Figure 5*B* illustrates such an experiment in a cat which was artificially ventilated at a slow rate, 13/min, and a large tidal volume, 70 ml. In *A*, well-marked variations in chemoreceptor rate, carotid P_{a,O_2} and mean arterial blood pressure were recorded. After the bilateral pneumothorax was prepared, shown in *B*, the chemoreceptor rhythm was enhanced as were the fluctuations of carotid P_{a,O_2} while the arterial pressure variations were considerably reduced, though not abolished.

It may be argued that the chemoreceptor fluctuations followed the rhythmic changes in sympathetic rate which are known to occur with respiration. This cannot entirely be the case since, in Fig. 5*D*, they are seen to persist even though the cervical sympathetic nerve on the same side had been cut. However, it was observed that if the cervical sympathetic nerves supplying the carotid body were divided, the mean rate of chemoreceptor discharge could vary by up to 15–20 % during a control period of steady respiration; the respiratory variations in rate could usually be seen to persist. Further, we have found that after cutting the cervical sympathetic it is very difficult to obtain stable conditions under which to observe the effects of various stimuli. In Fig. 5, between *B* and *C* when the vagi were cut, the rhythmic changes persisted, suggesting that if these changes were originating through the sympathetic, there could be no afferents in the vagus concerned in their generation. Figure 5*D* shows the results after cutting the sympathetic on both sides. In this figure, there is supporting evidence that these changes in the sinus nerve are not arising from the baroreceptors for it will be noted that the increase in the discharge rate occurred as the pressure fell and the peak rate was usually reached when the pressure was lowest.

Support for this view that the variations in chemoreceptor rate are determined by changes in blood gas tensions is given by the records shown in Fig. 6. The cat had taken a spontaneous deep breath which is shown in the tidal volume and end-tidal CO_2 tracings. The mean carotid arterial

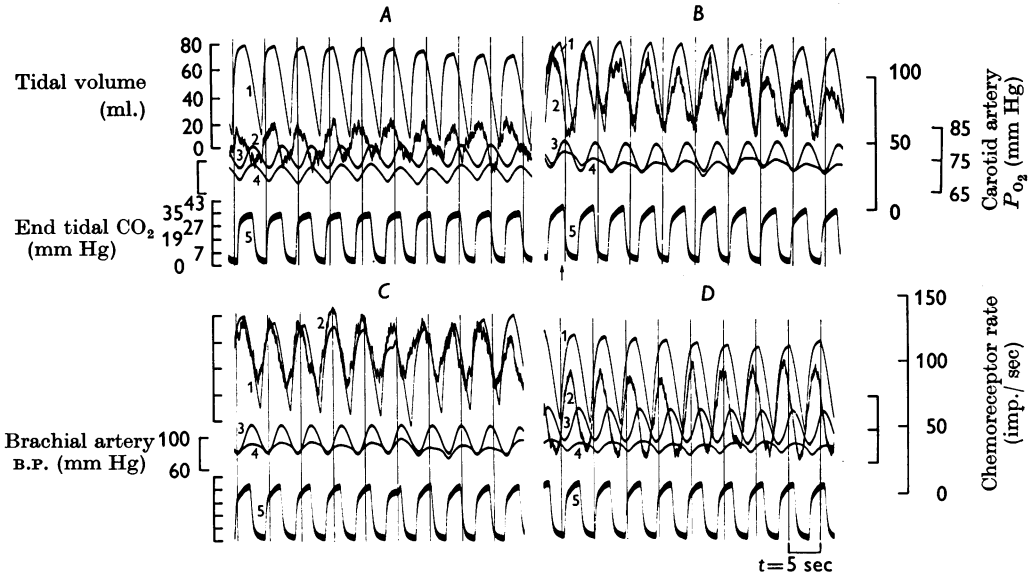

Fig. 5. Cat, pentobarbitone sodium, gallamine triethiodide, artificial ventilation. Effect of thoracotomy, vagotomy and sympathectomy on chemoreceptor rhythm. In each panel, from above down: 1. Respiration, tidal volume—70 ml.; frequency—13/min. 2. Rate-meter output showing chemoreceptor activity, impulses/sec. 3. Carotid artery oxygen tension, mm Hg. 4. Mean brachial artery blood pressure, mm Hg. 5. End-tidal P_{CO_2}, mm Hg. *A*, control. *B*, after bilateral thoracotomy showing increase in amplitude of chemoreceptor and $P_{a.O_2}$ fluctuations. *C*, after bilateral vagotomy. *D*, after bilateral preganglionic cervical sympathectomy. t = 5 sec.

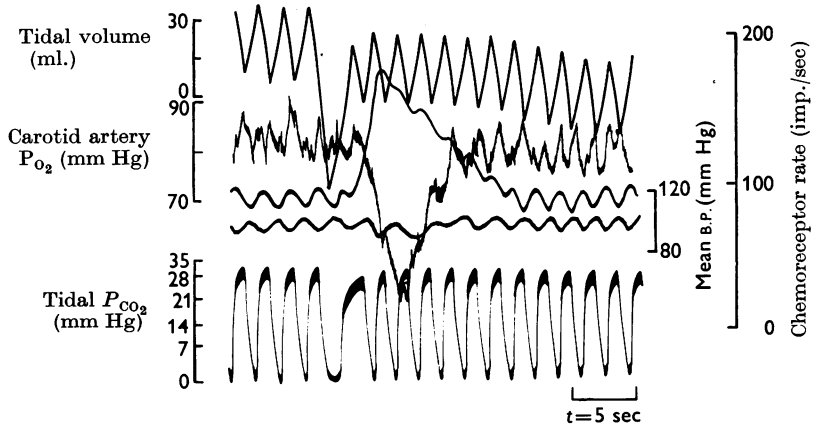

Fig. 6. Cat, pentobarbitone sodium. Effect of a spontaneous deep breath. Records from above down; tidal volume (ml.), rate-meter output recording chemoreceptor activity (impulses/sec), rate-meter time constant 0·33 sec; carotid P_{a,O_2} (mm Hg), mean carotid blood pressure (mm Hg) and end-tidal P_{CO_2} (mm Hg). t = 5 sec.

pressure fell by a small amount over the succeeding 5 sec. The rise in carotid P_{a,O_2} occurred when the arterial pressure was at its lowest and, shortly after this, chemoreceptor activity fell from 130 to 25 impulses/sec as P_{a,O_2} reached almost 100 mm Hg. Carotid P_{a,O_2} then fell in opposite phase to the rise in chemoreceptor rate.

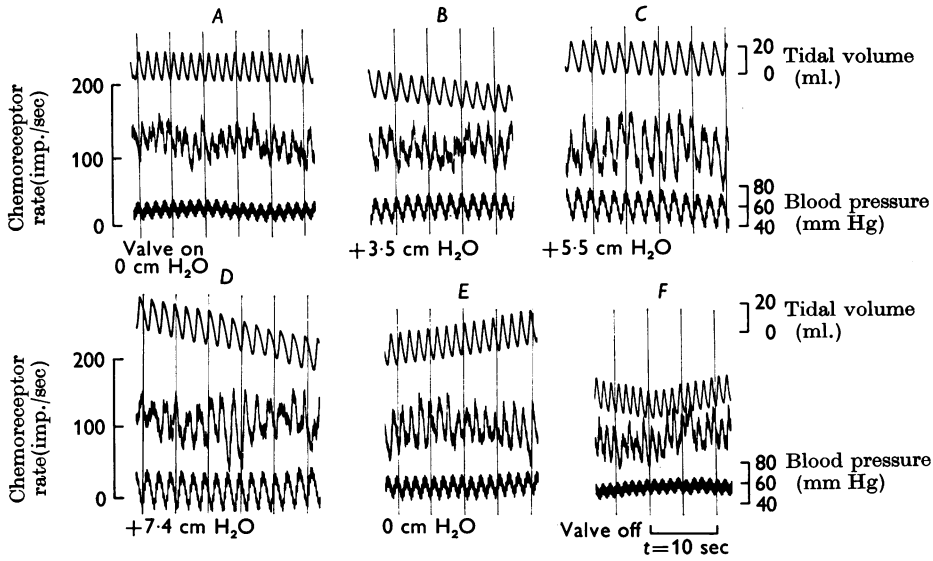

Fig. 7. Cat, pentobarbitone sodium, spontaneous ventilation. Effect of increasing mean expiratory pressure upon chemoreceptor discharge. In each panel, records from above down: tidal volume (ml.), chemoreceptor activity in impulses/sec (rate-meter time constant 0·1 sec), carotid artery blood pressure, mm Hg. *A*, breathing air, respiratory valve only. *B*, mean expiratory pressure, +3·5 cm H_2O. *C*, +5·5 cm H_2O. *D*, +7·4 cm H_2O. *E*, respiratory valve only. *F*, respiratory valve removed. t = 10 sec.

The effect on the nerve discharge of a spontaneous deep breath is also shown in Fig. 2. In the record *D*, the sympathetic was intact on the recording side and there was complete suppression of the larger action potentials. When the preganglionic cervical sympathetic nerve on the same side had been cut (record *E*), a deep breath still produced complete suppression. Thus the effect is not dependent on an intact sympathetic supply.

The evidence presented so far suggests that the variations in chemoreceptor rate follow upon changes in arterial gas tensions which themselves have the same period as respiration. Such changes in blood gas tensions could theoretically be produced by alterations in the ventilation/perfusion ratio or by cyclical changes in venous admixture with each breath which might be especially likely in an anaesthetized animal lying supine. Areas of collapse could change in size and account for arterial gas fluctuations.

That possibility was checked by raising the mean expired pressure since this would tend to eliminate areas of collapse and would diminish the amplitude of the blood gas fluctuations which arose from that cause. The results from one such experiment are shown in Fig. 7 *A–F*. In *A*, the expired pressure was normal and the chemoreceptor fluctuations were well marked. Progressive increase in the mean expiratory pressure, *B*, *C* and *D*, led to a slowing of respiration and an increase in the magnitude of the variations in rate, not a decrease. There was a parallel increase in arterial pressure fluctuations. In *E*, the mean expiratory pressure was lowered to normal with some recovery of respiratory frequency and decline of fluctuations of discharge and, in *F*, the respiratory valve was removed showing that, by itself, it offered a resistance to respiration. This finding was repeated in two other cats; in no case did we observe a decrease in the variations of the chemoreceptor rate. In practice, this procedure was found to be a useful method of enhancing otherwise small variations.

The effects of changes in inspired gas mixtures

Oxygen. When the inspired oxygen tension was increased above that in room air, there was a fall in the mean rate of chemoreceptor discharge and a fall in the amplitude of the variation in rate. When the inspired oxygen was lowered, there was a rise in the mean rate of discharge and, again, a fall in the amplitude of variations. Figure 8 shows the changes in a spontaneously breathing cat when the inspired oxygen tension (P_{I,O_2}) was lowered (*A*, *B* and *C*) and then raised to that of room air again (*D* and *E*). These changes in chemoreceptor discharge were accompanied by an increase in tidal volume and respiratory frequency and a decrease in end-tidal P_{CO_2} which will themselves affect the behaviour of the chemoreceptors (see below). Thus comparisons of the chemoreceptor discharge are better made during artificial ventilation. Under these conditions, the same qualitative result is obtained and in Fig. 9 the relation between the mean chemoreceptor rate and its variations with P_{a,O_2} are shown. Figure 9*A* shows that the amplitude of the discharge fluctuation is at its greatest within the physiological range, i.e. 75–95 mm Hg P_{a,O_2}. This contrasts with the relation between mean P_{a,O_2} and the amplitude of its fluctuation with respiration which has been shown to be approximately linear over the range 60–200 mm Hg (Purves, 1966). In our experiments, it was found that, if P_{a,O_2} was increased above 100 mm Hg, the mean chemoreceptor rate started to fall and variations in rate were more difficult to discern. Another comparison is made in Fig. 9*B* for the same results as in *A*. The ratio of amplitude to mean level of chemoreceptor activity is plotted against P_{a,O_2} and there is an approximately linear relation over part of the range of P_{a,O_2}. Figure 9*C* shows the relation between the mean level of chemo-

receptor activity (expressed as per cent of the activity at low P_{a,O_2}) and arterial P_{O_2}. It was our experience that raising the arterial oxygen tension always reduced chemoreceptor activity and on many occasions the tonic activity observed in the normoxic range was virtually or completely extinguished. On other occasions, as in Fig. 9*C*, there was clear activity at high P_{a,O_2}. We have not examined this point in detail in the present series of experiments but we have discussed it in the context of results from newborn lambs (Biscoe & Purves, 1967*b*).

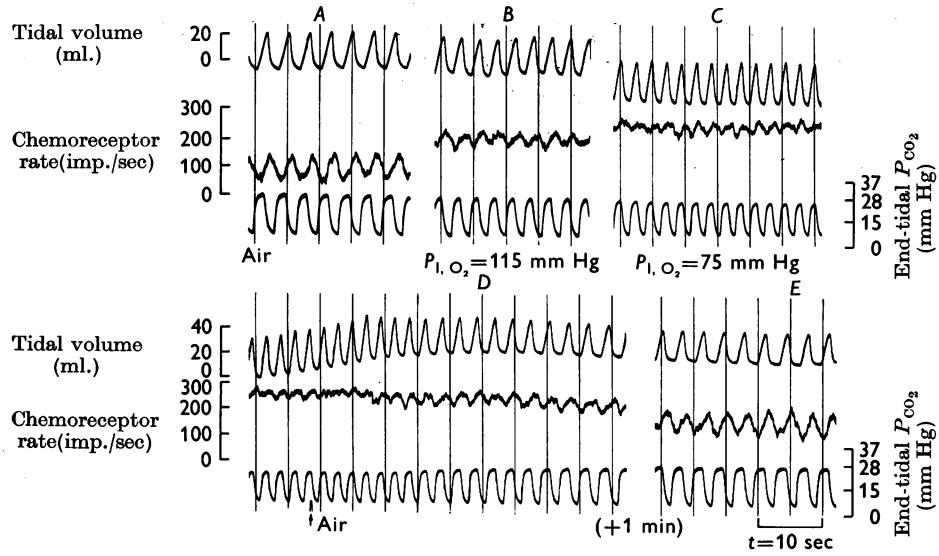

Fig. 8. Cat, pentobarbitone sodium, spontaneous ventilation. Effect of lowering inspired oxygen tension on carotid chemoreceptor discharge. In each panel, from above down: tidal volume (ml.), chemoreceptor activity in impulses/sec (rate-meter time constant 0·3 sec) and end-tidal P_{CO_2} (mm Hg). *A*, breathing air. *B*, P_{I,O_2}= 115 mm Hg. *C*, P_{I,O_2} = 75 mm Hg. *D*, 3 min after *C* showing the return to air. *E*, 1 min later, still breathing air. t = 10 sec.

Carbon dioxide. When inspired CO_2 was raised in steps, no obvious change in the mean chemoreceptor discharge or in minute volume of ventilation ($\dot{V}$) was noted until the CO_2 concentration in air was approximately 2 %. This was possibly due, in part, to the fact that in many experiments, control end-tidal P_{CO_2} was low (30–34 mm Hg) and thus some way below a CO_2 'threshold'. Figure 10 shows the response to the inhalation of 1 % CO_2 in air (upper trace, *A*) and 2 % CO_2 in air (lower trace, *B*). With 1 % CO_2, there was no measurable change in the mean rate of chemoreceptor rate or of its variations: with 2 % CO_2, there was a small increase in the mean level of chemoreceptor activity, an increase in end-tidal P_{CO_2} and an increase in $\dot{V}$ which was therefore inadequate to hold end-tidal P_{CO_2} con-

stant. There was no measurable change in the amplitude of the fluctuations of chemoreceptor discharge. Figure 11*A* shows that when 4 % CO_2 in air was given, there was a well-marked increase in the mean chemoreceptor rate and a definite suppression of the rate variations. End-tidal P_{CO_2} increased and the increase in $\dot{V}$ was therefore again inadequate. These changes were more clearly seen when 5 % CO_2 in air was given (lower trace, *B*). The arterial blood pressure and pulse rate were unaffected by the lower CO_2 mixtures but the pulse pressure increased after 20–30 sec on 2 % CO_2. With the higher concentrations of CO_2, pulse rate was unaffected, but arterial pressure showed a steady rise to a peak after 30 sec and the

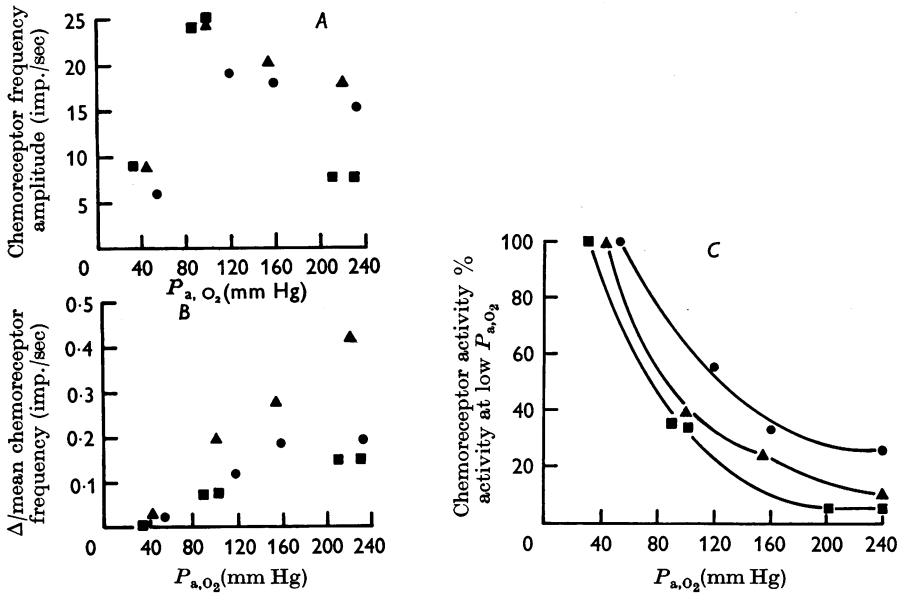

Fig. 9. *A*. The relation between the amplitude of chemoreceptor frequency fluctuation in impulses/sec (ordinate) and P_{a,O_2} (mm Hg; abscissa). Three cats, (●, ■, ▲) each given pentobarbitone sodium and gallamine triethiodide with controlled ventilation: P_{A,CO_2} 30–34 mm Hg.

B. The relation between the ratio, amplitude of chemoreceptor rate fluctuation to mean chemoreceptor rate and arterial P_{O_2} mm Hg. Three cats, as in *A*.

C. The relation between mean chemoreceptor rate expressed as % chemoreceptor rate at low P_{a,O_2} and arterial P_{O_2}. Three cats as in *A*.

pulse pressure was again increased. Figure 6 effectively illustrated the response of the chemoreceptors to a brief rise in P_{a,O_2}. In Fig. 12, the response of the chemoreceptor discharge to transient inhalations of 5 % CO_2 in air is shown. Again, as in Fig. 6, there is a brief delay, the circulation time following the breath before the change in mean chemoreceptor rate. Each increase in rate was followed by recovery and the rate variations

persisted throughout; their amplitude was not markedly damped at the peaks of the increase in mean rate. The 'off' transients with CO_2 inhalation, shown in Fig. 11, also shows the resumption of variations in rate similar to that seen in Fig. 8 when the cat breathed air after a period of hypoxia.

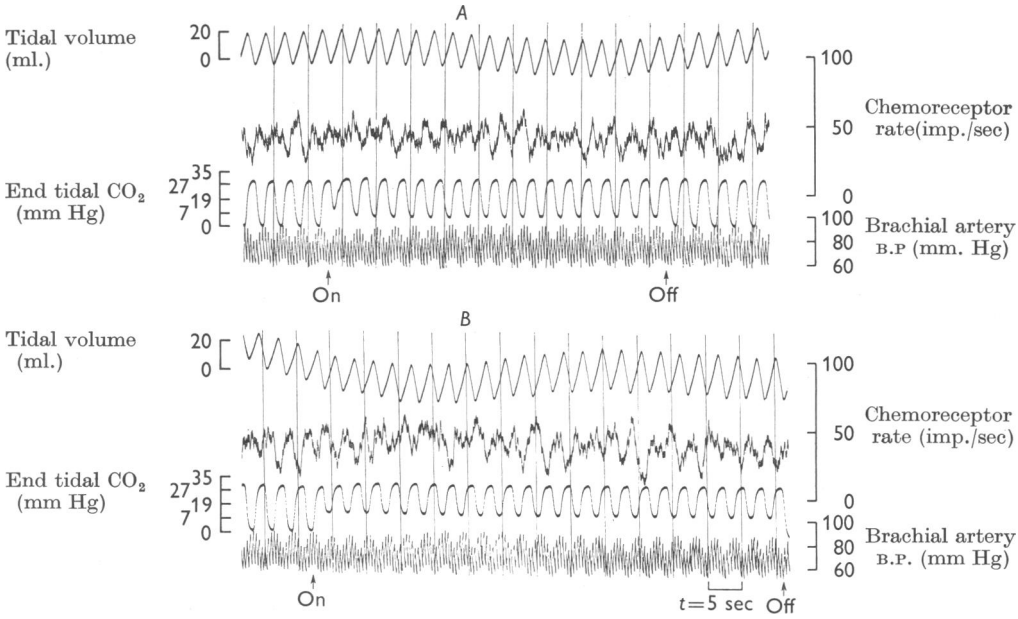

Fig. 10. The effect of increasing inhaled CO_2 upon chemoreceptor activity. Cat, pentobarbitone, spontaneous respiration.

A. From above down: tidal volume (ml.), chemoreceptor rate (rate-meter time constant 0·33 sec), end-tidal P_{CO_2} and brachial artery blood pressure (both mm Hg). 1 % CO_2 was added to the inspired air between the arrows.

B. As in *A*; 2 % CO_2 added to the inspired air between the arrows.

The effect of changing the frequency and depth of respiration

If the variations in chemoreceptor rate were related to changes in arterial blood gas tensions, themselves dependent on the frequency and depth of respiration, it would be predicted that these variations should be modified by changes in respiration. This was investigated in cats given gallamine triethiodide and ventilated artificially. Figure 13*A* shows well-marked variations in chemoreceptor rate under these conditions. When the tidal volume was increased at the same respiratory (pump) frequency (Fig. 13*B*), the mean chemoreceptor rate was depressed but the fluctuations were only slightly reduced. When the respiratory (pump) frequency was then increased (Fig. 13 *C*), the mean rate of discharge doubled and the fluctuations disappeared. They returned in Fig. 13*D* when the respiratory

frequency was lowered but the tidal volume remained the same. Now with the respiratory parameters different from the original level (Fig. 13*A*) but with a similar end-tidal P_{CO_2}, the mean rate of firing was much the same and the rate variations had a similar amplitude. Several points should be noted. In trace *B*, the chemoreceptor rate variation is much

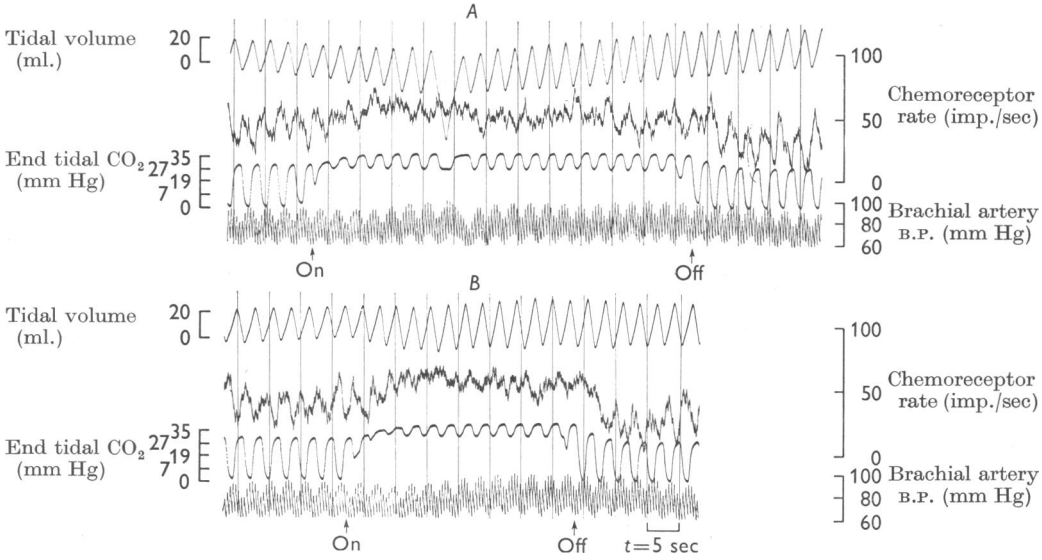

Fig. 11. Cat, as in Fig. 10. *A*. 4% CO_2 and *B*, 5% CO_2 added to the inspired air between the arrows.

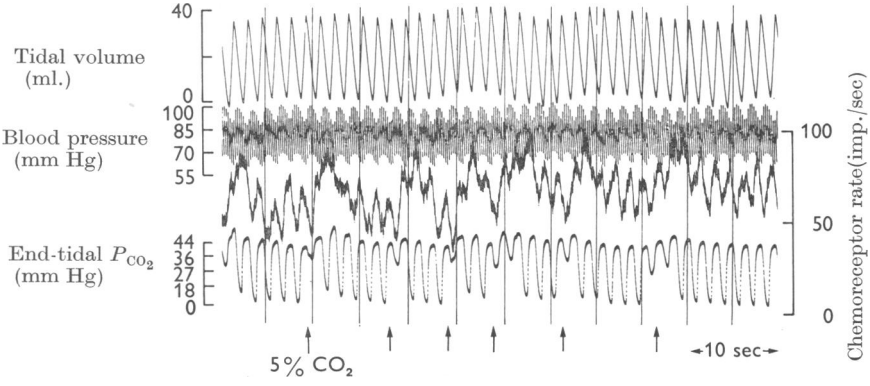

Fig. 12. Effect of adding 5% CO_2 intermittently to the inspired air on chemoreceptor activity. Cat, pentobarbitone sodium. Records from above down: tidal volume (ml.), brachial artery pressure (mm Hg), chemoreceptor activity in impulses/sec (rate-meter time constant 0·33 sec), end-tidal P_{CO_2} mm Hg. 5% CO_2 was added for 1 or 2 breaths at each arrow. t = 10 sec.

the same as in *A* and of the same frequency: therefore the rate of change of chemoreceptor discharge within the variations is altered very little although the mean rate is depressed. Again in *D*, the variations in rate have a similar amplitude but their frequency is slower: therefore the rate of change of discharge within variations is slower. It will be observed that the end-tidal CO_2 alters markedly through the series and the chemoreceptor changes are, of course, associated with changes in blood concentrations since these are uncontrolled.

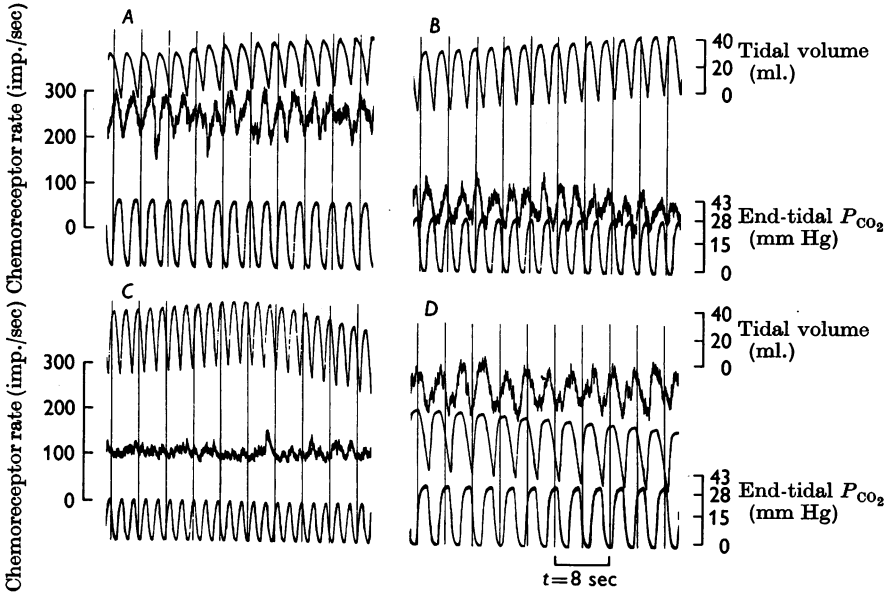

Fig. 13. Effect of changing the rate and depth of respiration on chemoreceptor activity. Cat, pentobarbitone sodium, gallamine triethiodide, artificial ventilation. *A*. From above down: tidal volume (ml.), chemoreceptor activity in impulses/sec (rate-meter time constant 0·33 sec) and end-tidal P_{CO_2} (mm Hg). Respiratory frequency (f) = 24/min; tidal volume (V_T) = 33 ml. *B*. f = 24/min; V_T = 45 ml. *C*. f = 33/min; V_T = 45 ml. *D*. From above down; chemoreceptor activity, tidal volume and end-tidal P_{CO_2}. f = 20/min; V_T = 45 ml. t = 8 sec.

Clamping the carotid artery. Frequently, when the carotid artery was clamped on the same side on which the chemoreceptor rate was being recorded, there was an increase in the mean rate. In two cats this increase was very small and Fig. 14 shows the changes which occurred when this was done. Immediately, there was a small increase in chemoreceptor activity possibly due to a change in blood flow through the carotid body which would then be derived from vertebro-occipital anastomoses. The chemoreceptor rhythm, however, was seen to persist.

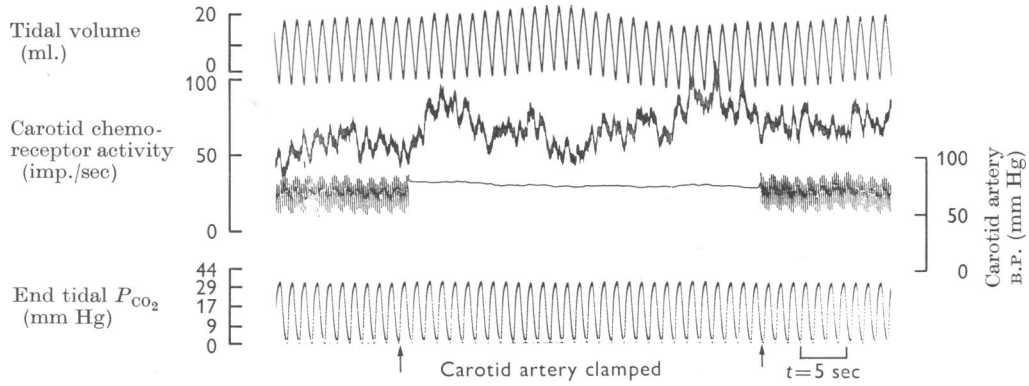

Fig. 14. Cat, pentobarbitone sodium, spontaneous respiration. Effect of clamping the common carotid artery on chemoreceptor activity. From above down: tidal volume (ml.), output of rate-meter showing chemoreceptor activity in impulses/sec (rate-meter time constant 0·33 sec), carotid artery blood pressure (mm Hg) and end-tidal P_{CO_2} (mm Hg). Between the arrows, the cardiac end of the polyethylene loop placed in the carotid artery was clamped and the tap to the pressure transducer turned out. Note the persistence of the chemoreceptor fluctuations. $t = 5$ sec.

DISCUSSION

The method. The pulse counting system senses the presence or absence of an action potential above the lower gate level of the pulse height selector. If a spike increases in size, it will still only be counted as one spike: if it decreases in size, it may be lost if the amplitude declines below the lower gate level. It is possible that variations in rate recorded on the rate-meter could be due to a fluctuation in spike size above and below the lower gate setting in the selector or due to a fluctuation in noise above and below this setting. Such changes could be produced by movement artifacts but against this postulate must be set the following points.

1. The nerve was always laid on to a stainless-steel back plate and then led to the electrodes so that it hung loosely from them. After a time, it appeared to adhere to the plate. The electrodes were inspected through the operating microscope to ensure that movement could not be seen.

2. The peak of the fluctuation in rate did not bear the same relation to the tidal volume record in different experiments, and the increase in rate often reversed while respiration (tidal volume) was continuing in the same direction. That is, the change in chemoreceptor rate was not in phase with respiration. It is difficult to explain reversal of the rate record if it is a movement artifact while movement continues in the same direction.

3. Thoracotomy which diminished the movement of the tissues and sometimes eliminated it in the neck was often associated with an increase in the amplitude of the rhythm—not with a decrease or disappearance.

4. The changes in chemoreceptor rate bear a close relation to the fluctuations in arterial oxygen tension.

5. The production of turbulence by incorrect alignment of the oxygen electrode in the carotid loop abolished chemoreceptor rate changes. This was not rigidly tested but it was noticed that often after flushing out the electrode cuvette the chemoreceptor fluctuations returned.

6. We were able to produce artifact free records while the animal's hind limbs were being moved vigorously (Biscoe & Purves, 1967*a*), a much more severe source of movement artifacts than respiration.

7. Increasing the frequency of respiration in the paralysed animal led to the disappearance of the fluctuations in rate. If these had been due to artifacts, they would have been expected to continue at the higher rate.

As a further precaution, the oscilloscope face was always inspected to ensure that action potentials were not getting smaller as, for example, when carotid artery oxygen tension fell and that the noise did not increase.

It was possible that part of the changes in activity in the sinus nerve was due to the effects of alterations of P_{a,O_2} or of pH_a upon axons. Where such measurements have been made (Lehmann, 1937*a*, *b*; Wright, 1946), the chemical changes have been large and prolonged and we consider that, under our experimental conditions in which the sinus nerve was desheathed and sometimes dissected, this possibility was remote.

We therefore conclude that a change in action potential rate was counted and that this change originated within the carotid body. The results have been presented and discussed on this basis.

The rhythmical changes in chemoreceptor rate. The presence of rhythmical changes in the total integrated chemoreceptor activity in the sinus nerve having the same period as respiration has previously been reported by Hornbein, Griffo & Roos (1961). In addition, Siminoff (1964) observed a group of action potentials in the cat aortic nerve using his cross-correlation technique which exhibited a change in rate having the same period as respiration. He suggested that these may have been chemoreceptor in origin but could not exclude the possibility that they were arising from thoracic stretch afferents. In our view, the variations in rate will be extremely difficult to observe without some form of electronic monitoring.

The precise time relationship between the chemoreceptor rhythm and fluctuations of blood gas tensions and the respiratory cycle is determined by the circulation time. This will account for the variation in their timing between experiments. In addition, if Siminoff (1964) was observing a chemoreceptor rhythm from the aortic bodies, this information will arrive at the medullary centres before the signals observed here since the circulation time to the aortic bodies will be shorter than to the carotid bodies. If this is the case, a mechanism is provided by which the central nervous

system may compute the aortic-carotid circulation time for blood gases while the baroreceptors will provide similar information about the pulse pressure wave. Indeed Green (1954) and Boss & Green (1956) have demonstrated the presence of baroreceptors over the entire length of the carotid artery which would provide much more exact information on this point.

The observation that in two cats we were able to see the chemoreceptor rhythm persisting at the same mean level, although the carotid artery was clamped off suggests a remarkably good vertebro-occipital anastomosis. More important, it shows that the fluctuations in gas tensions are carried in the vertebral arteries, certainly down branches to the anastomosis. It may be reasonable to conclude that they reach as far as the arteries supplying the medullary chemoreceptors (Mitchell, Loeschke, Massion & Severinghaus, 1963).

The question arises as to what is the effective stimulus in arterial blood which gives rise to the fluctuating rhythm in the chemoreceptor discharge. Some evidence on this point may be obtained from a figure published previously (Biscoe & Purves, 1965; Fig. 1) and Fig. 8 in the present paper. In the former figure, it would appear at first sight that, if the chemoreceptor rhythm seen in the control period was caused by the 1–2 mm Hg fluctuations of P_{a,O_2}, the increase in mean chemoreceptor discharge in response to a 25 mm Hg fall in P_{a,O_2} when the lamb inhaled 10 % oxygen in N_2 for a few breaths is disproportionately small, even allowing for the fact that with the transient hyperventilation, end-tidal P_{CO_2} fell and, presumably, pH_a rose. It is further unlikely that the chemoreceptor rhythm was caused by these small fluctuations of P_{a,O_2} since it is known that, in the new-born lamb, the chemoreceptors are at their least sensitive to changes in P_{a,O_2} in or just below the normoxic range (Purves, 1966). However, it is now clear that pH_a fluctuates with the same period as respiration (Band & Semple, 1966) and it is probable that this is mainly due to a similar fluctuation of P_{a,CO_2} which may be expected to be in opposite phase to fluctuations of P_{a,O_2} and whose amplitude in relation to the latter will be determined by the respiratory quotient and arterial bicarbonate. If it can be assumed that, as in the steady state, the fluctuations of these blood gas tensions and pH are acting not merely additively but by potentiating one another, then it is clear that the change in the total chemical signal with each respiratory cycle is considerable: and it is probable that the contribution of fluctuations of P_{a,O_2} may be quite small.

Thus the changes observed in Fig. 8 of the present paper could be interpreted as follows. When inspired oxygen was reduced from ambient to 115 mm Hg, the increase in chemoreceptor discharge was principally due to the fall in P_{a,O_2} but was, to some extent, offset by the accompanying fall in end-tidal P_{CO_2}. The reduction in amplitude of the chemoreceptor

rhythm was due to the reduction in amplitude of fluctuations of P_{a,O_2} as mean P_{a,O_2} fell and of P_{a,CO_2} as respiratory frequency rose. Further, when the cat again breathed room air, the decline in chemoreceptor activity to control levels and the return of the rhythm appears to be more closely related to the slowly rising end-tidal P_{CO_2} (records *D* and *E*) and fall in respiratory frequency than to the relatively rapid rise in P_{a,O_2} (see for example Fig. 6).

The time course of changes in chemoreceptor activity. The transient changes in chemoreceptor discharge following a deep breath (Fig. 7) show that the chemoreceptors have a very short latency, very much less than 1 sec when measured from the rise in carotid P_{a,O_2}. This argument is reinforced when it is remembered that the oxygen electrode is in a polyethylene loop and is usually 10–15 cm on the cardiac side of the carotid body. A similar record from an experiment on a new-born lamb (Biscoe & Purves, 1965) shows a similarly rapid response for this species when the carotid P_{a,O_2} was transiently lowered. Further evidence for a rapid response was seen when CO_2 was given as a transient stimulus. Although no electrode fast enough to monitor changes of this gas in blood is available, the chemoreceptor rate increase occurred very soon after the stimulus was applied. The arterial pressure changes following a deep breath have a time course which suggests that the baroreceptors would have a different activity pattern from that of the chemoreceptors: in addition, the fall in pressure would by itself presumably cause an increase in chemoreceptor activity (Lee, Mayou & Torrance, 1964). Both points support the view that the changes seen during a gasp are in the discharge of the chemoreceptors and are mediated by blood gases.

It was only possible to make approximate estimates of the response time of the carotid chemoreceptors to changes in P_{a,O_2} since we did not apply square wave stimuli. In the majority of experiments, the changes were transient, and we had no means of detecting precisely when blood with a changed gas tension reached the chemoreceptors. However, in some experiments in which cats were ventilated artificially, and in which the frequency of respiration was altered, we confirmed that carotid P_{a,O_2} and chemoreceptor activity changed with the same time course as that shown in Fig. 6. The changes in end-tidal P_{CO_2} were small, i.e. 5–7 mm Hg and carotid P_{a,O_2} had changed to a new steady level in 7–12 sec. On the sixteen occasions on which satisfactory measurements were made, the corresponding changes in chemoreceptor activity were 95% complete in 20–32 sec. Since the changes in carotid P_{a,O_2} were probably not square wave, it is to be presumed that the chemoreceptors could respond even more rapidly than this. This response is more rapid than that observed by Lee *et al.* (1964) where the stimulus was a change in systemic blood

pressure and this suggests that, in their experiments, other factors were involved, e.g. redistribution of blood flow within the carotid and aortic bodies.

The effects of changing inspired gas tensions. The modification of the chemoreceptor rhythm by change in P_{I,O_2} suggests that, in the spontaneously breathing animal, a departure from control in either direction diminishes the amplitude of the rhythm. Further, the results with the artificially ventilated animal show that a peak occurs in the rhythm amplitude and that the ratio of this amplitude to mean chemoreceptor discharge rate is linearly related to P_{a,O_2}, at least over part of the range and especially below the oxygen tension in air at sea level. The information in this signal could thus form part of a control system.

The changes seen when inspired CO_2 was raised show that there is no increase in mean chemoreceptor rate when the end-tidal level is maintained constant by increased ventilation. Whenthis was not the case, usually when the inspired concentration of CO_2 was 2–3 % or above, there was an increase in the mean chemoreceptor rate and the amplitude of the fluctuations diminished. Provided that the volume of expired CO_2 per unit time remains constant, the amplitude of alveolar fluctuations of CO_2 should remain unaffected by addition of CO_2 to the inspired air: only the mean (P_{A,CO_2}) will change. The amplitude of the fluctuations of P_{A,CO_2} within each respiratory cycle will be principally determined under such conditions by the frequency of respiration. There are no satisfactory data on this point where the frequency of respiration was controlled. The expiratory–inspiratory alveolar CO_2 differences, measured by Haldane & Priestley (1935) when CO_2 was added to the air, vary very considerably and presumably reflect the great difficulties which they encountered in sampling alveolar gas satisfactorily at high respiratory frequencies with the apparatus then available. However, we would predict that, in the spontaneously breathing animal, the amplitude of P_{A,CO_2} and P_{A,O_2} fluctuations within each respiratory cycle and thus the fluctuations of these gas tensions in blood should diminish when the animal inhales CO_2. Our own observations on the behaviour of the chemoreceptor fluctuations are consistent with these predictions.

The significance of chemoreceptor rate fluctuations. The results presented here are of some interest in the general problem of the regulation of respiration because if, as has been shown, the chemoreceptors can follow small fluctuations of blood gas tensions, it means that they can respond fast enough to the small changes brought by physiologically normal fluctuations in respiration and various types of breath-holding such as occur in talking, drinking, etc. But a more topical problem is that posed by Yamamoto (1960, 1962): do the arterial fluctuations of P_{a,O_2} and P_{a,CO_2} merely

but inevitably follow the corresponding fluctuations in alveolar gas tensions, or are they made use of in the regulation of respiration? In particular, does the presence of a cycle in the stimulus to the chemoreceptors increase the magnitude of the response?

Recently, attempts have been made to answer this question by enhancing or imposing variations of P_{a,CO_2}, either by 'injecting' CO_2 at constant rate into the inspired air (Fenn & Craig, 1963; Cunningham, Lloyd & Patrick, 1964), or by intermittent administration of high CO_2 mixtures to human subjects or dogs (Dutton, Chernick, Moses, Bromberger-Barnea, Permutt & Riley, 1964). The conclusions drawn from these experiments have been contradictory, since the former two sets of authors were unable to show any difference in the change in $\dot{V}$ from that predicted from the more orthodox 'CO_2 response' curves in which CO_2 mixtures of fixed concentration were inhaled. Dutton *et al.* (1964) on the other hand, found that the intermittent breathing of CO_2 caused a greater increase in respiration than the increase in mean alveolar P_{CO_2} could account for.

Our results throw some light on but cannot solve the problem posed by Yamamoto. Thus we would predict that the manoeuvres carried out by Dutton *et al.* (1964) caused the same large and rapid increase in chemoreceptor discharge with the same type of recovery which we have found (Fig. 12), while in the experiments of Cunningham *et al.* (1964), very large fluctuations of chemoreceptor activity would be expected which would, to some extent, be modified by the increase in respiratory frequency. To determine how the mean chemoreceptor frequency was affected will require sophisticated methods of analysis and, in all the experiments cited above, it is probable that the chemoreceptor afferent signals were severely modulated or integrated with signals from other pH sensitive receptors before reaching the respiratory centres.

For the moment, we can only conclude from our results that the peripheral chemoreceptors can and do respond to small changes in blood gas tensions in arterial blood and that, in this way, information about changes in inspired gas tensions and in the frequency and volume of respiration can be relayed to the respiratory centres.

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