J. Physiol. (1967), **189**, *pp.* 63–84
With 12 *text-figures*
Printed in Great Britain

MECHANISM OF STIMULATION OF AORTIC CHEMORECEPTORS BY NATURAL STIMULI AND CHEMICAL SUBSTANCES

BY A. S. PAINTAL

From the V. Patel Chest Institute, Delhi University, Delhi-7, India

(*Received* 6 *July* 1966)

SUMMARY

1. Impulses were recorded in single fibres of aortic chemoreceptors of cats anaesthetized with chloralose. There was no demonstrable difference between the responses of the endings of medullated and non-medullated fibres respectively to any of the natural stimuli, such as hypoxia, reduction in blood pressure, or reduction in O_2 content. This indicates that the generator processes are qualitatively and quantitatively identical at the endings of both types of fibres.

2. Most of the endings were practically silent while ventilating the lungs with air. The maximum frequency of discharge averaged over 4–10 sec while ventilating the lungs with 4 % O_2 ranged from 1·5 to 24 impulses/sec; in most fibres (twenty-one out of twenty-six endings) it was less than 12 impulses/sec.

3. All the chemoreceptors tested were considerably stimulated following administration of 0·2 or 2 % CO at a time when the O_2 content was greater than 4 ml./100 ml.

4. All the chemoreceptors were markedly and rapidly stimulated following circulatory arrest while the cat was ventilated with air. This stimulation fell considerably within 3 min of circulatory arrest. Very little or no excitation followed circulatory arrest while ventilating the cat with pure N_2. These results suggest that excitation following circulatory arrest is not produced by a metabolite.

5. There was a remarkable difference between the sensitivities of endings of medullated and non-medullated fibres to drugs. The former were either unaffected by relatively large doses of ACh (100–200 μg) or phenyl diguanide, or if they were stimulated, the excitation so produced was much less than that produced in endings of non-medullated fibres. This supports the hypothesis that drugs produce their effects by an action at the regenerative regions of the endings, i.e. regions where the nerve impulse is

initiated (Paintal, 1964). It also indicates that ACh is not likely to be a transmitter in the normal processes of excitation of chemoreceptors.

6. A mechanism of stimulation of chemoreceptors not involving metabolites is presented.

INTRODUCTION

Recently it has been suggested that certain drugs sensitize, stimulate or depress sensory receptors by an action on their regenerative regions (i.e regions where the impulse is initiated) and not on the generator regions (Paintal, 1964). It was further suggested that the greater responsiveness of the endings of non-medullated fibres was due to the fact that the regenerative region of these fibres was more susceptible to the effects of certain chemical agents than the regenerative region of endings of medullated fibres (Paintal, 1964). These conclusions were arrived at from a large body of evidence which also showed that the diffusion barrier between the nerve fibre and the blood at the regenerative region is absent or insignificant when compared to the diffusion barrier provided by the nerve sheath (Paintal, 1964). However, since so far no experiments have been done specifically to test the above hypothesis it was considered desirable that this should be attempted.

The aortic chemoreceptors were chosen because some of these endings have medullated and some non-medullated fibres and also because the responses of chemoreceptors with non-medullated fibres to hypoxia are qualitatively similar to those with medullated fibres (Paintal & Riley, 1966). If the hypothesis mentioned above is true then chemical agents should stimulate the chemoreceptors with non-medullated fibres more effectively than those with medullated fibres. Two substances, acetylcholine (ACh) and phenyl diguanide, totally unrelated to each other, were chosen for this purpose since they are known to stimulate chemoreceptors (see Heymans & Neil, 1958; Dawes, Mott & Widdicombe, 1952). The results reported in this paper strongly support the above hypothesis.

METHODS

Experiments were carried out on cats weighing between 2·5 and 4·0 kg. They were anaesthetized with chloralose (75 mg/kg I.V.). This was supplemented with small doses of sodium pentobarbital I.V. whenever required.

The aortic nerve was identified by the presence of volleys of baroreceptor impulses in it. Filaments were dissected from this nerve near the nodose ganglion and impulses recorded in individual fibres according to conventional methods (see Paintal, 1963 for details). Chemoreceptor fibres were identified as such if an irregular discharge of impulses appeared in them during administration of 4% O_2 in N_2, and if this discharge ceased on re-admission of air. The conduction velocities of individual chemoreceptor fibres were determined using techniques and criteria that are now well known (Paintal, 1963). Corrections for small variations in temperature were made using a Q_{10} of 1·6 (Paintal, 1965). The present results

are concerned with observations on single fibres. Because of the irregular pattern of discharge in chemoreceptor fibres proof of unitary activity was obtained from the following evidence: that all the impulses were identical in shape and size (as recorded on a fast sweep), that the impulses never summated, that a random sample of the impulses were proved to belong to a specific all-or-none component of the electrically evoked response (see Paintal, 1963) and that all the impulses were identical in shape and size with this evoked response (Fig. 1*A*, *B*). This was easy because in several filaments there were only 1–3 live fibres as revealed by strong electrical stimulation of the vagus and aortic nerve (Figs. 6*A*, 8*A*). Infrequently, when it was considered desirable to record impulses in two fibres simultaneously, the position of the filament on the recording electrodes was arranged so that the impulses of one fibre could be clearly distinguished from those of the second, e.g. by placing the proximal electrode near the injured end of one fibre in order that its impulses acquired a prominent initial positive deflexion.

Hypoxic mixtures of gases were administered by a Starling Ideal (Palmer) respiratory pump. The stroke volume was kept at 50–60 ml. Gases were administered for 2·5–3 min before recording their effects (Paintal & Riley, 1966). This duration was adequate since under these conditions the N_2 wash-out time (Comroe, Forester, Dubois, Briscoe & Carlson, 1962) was about 1 min or less. Before commencing a study of the responses of the endings to drugs, the cat was put on the pump so as to ensure constant ventilation with air.

In order to study the effects of drugs on chemoreceptors an aortic catheter was introduced through the left common carotid artery and its position adjusted to ensure that its tip lay close to the aortic valves. This was always confirmed post mortem. The aortic blood pressure was also recorded through this catheter which was connected to a pressure transducer (Statham, P 23 G). The mean blood pressure was roughly estimated from the records by adding a third of the pulse pressure to the diastolic pressure (Rushmer, 1961). In a few experiments the mean blood pressure was recorded by electronic integration. Sometimes there were obvious artifacts in the blood pressure recording due to the impact of the tip of the catheter against the aortic valves. These records were discarded. The pressure record was interrupted while injecting solutions into the aorta (Figs. 1*C*, 9). In a few experiments the carotid blood pressure was recorded instead of aortic pressure.

A stock solution of acetylcholine chloride was prepared as described by Harris, Gilding & Smart (1956) from 0·1 mg of dry ACh powder contained in a sealed ampoule (Roche Products). This was done in order to rule out possible variations in the concentration of ACh occurring from one experiment to the next due to various possible factors, such as small variations in the amount of ACh in the ampoules (Boyd & Pathak, 1965). The solution (1 mg/ml. ACh) was stored in 1 ml. sealed ampoules (after sterilizing the sealed ampoules for about 5 min in a boiling water-bath) at a temperature of about 7° C for 9 months. Thereafter studies on the effect of ACh on the chemoreceptors were begun. A fresh solution of ACh was prepared in the case of each receptor. Each time an ampoule containing the stock solution was broken and the ACh diluted with 0·9 % NaCl (w/v) to give a solution containing about 100 μg/ml. just before studying the effect of ACh on the ending. The pH of this solution was about 5·5. The main investigation was completed within 3 months.

The concentration of ACh injected was always the same, i.e. about 100 μg/ml. (actually 93 μg/ml.). The doses were varied by varying the volume of solution injected. Near the end of the whole investigation the stock solution of ACh contained in the ampoules was assayed against freshly prepared ACh solution using the cat's blood pressure method (MacIntosh & Perry, 1950). About 6 months later it was assayed again (by a colleague) using the frog's rectus abdominis. On both occasions the concentration was found to be about 0·93 mg/ml. It is therefore certain that the concentration of ACh used was practically uniform throughout the investigation. The figure of 0·93 mg/ml. has been used for calculating the actual doses of ACh injected. The doses and concentrations are expressed in terms of the salt.

The dead space of the aortic catheter was 0·3 ml. This was taken into account in estimating the amount of drug injected. A foot switch and light source were used to signal the moment

of injection which was completed in about 0·5 sec. In some records, the moment of injection was also indicated by the rise in the aortic pressure trace due to the pressure transducer also being in connexion with the syringe at the time of injection. These records showed that the signal lagged behind the injection by 0·1–0·2 sec (Fig. 9*C*), the average of ten observations being 0·17 sec. The measurements of injection–discharge time have been corrected accordingly.

Atropine sulphate (1 mg/kg) was injected intravenously just before beginning the observations on the chemoreceptors and, if the experiment continued for more than a few hours thereafter, this was supplemented by a further injection so as to ensure that little or no slowing of the heart was caused by ACh. For such intravenous injections a catheter was introduced through the external jugular vein such that its tip lay in the right atrium. Only those cats were atropinized in which the effects of drugs on chemoreceptors were studied.

In all the experiments the chest was closed. For producing permanent circulatory arrest in the animal with intact chest, 30 ml. of air was injected quickly into the right atrium through the catheter. This led to a rapid fall in cardiac output and fall in blood pressure and irreversible and sudden circulatory arrest.

The intratracheal pressure was recorded with a pressure transducer (Statham) and suitable amplifier. The e.c.g. (lead II) was also routinely recorded. The oxygen content and percent O_2 saturation of the arterial blood before and during administration of CO were determined by standard methods (Peters & Van Slyke, 1932). Air analysis was done with a Scholander 0·5 ml. gas analysis apparatus.

RESULTS

Responses of chemoreceptors to natural stimuli. The responses of the aortic chemoreceptors to natural stimuli were, in general, similar to those already described (Paintal & Riley, 1966). Typical responses in single fibres during hypoxia are shown in Figs. 1*D*, 3*D*, 6*C* and 8*C*. In seventeen out of twenty-seven fibres, the frequency of discharge while ventilating the lungs with air was 0·1 impulses/sec or less. During administration of 4% O_2 in N_2 the frequency of discharge (averaged over 4–10 sec) varied considerably in different fibres, the range being 1·5–24 impulses/sec (Table 1); the frequency was less than 12 impulses/sec in twenty-one out of twenty-six fibres. The peak frequency of discharge measured as the reciprocal of the least interval between two impulses ranged from 25 to 125 impulse/sec (Table 1).

The above characteristics are common to chemoreceptors of both medullated and non-medullated fibres and also to 'transitional fibres', i.e. fibres with intermediate conduction velocities (see Paintal & Riley, 1966). In fact as shown in Table 1 there is no significant difference between endings of medullated and non-medullated fibres as far as responses to natural stimuli is concerned. The fibres in Table 1 which were chosen for making specific comparisons between responses of medullated and non-medullated fibres were carefully selected so as to leave no doubt about their identity.

Thus since the diameter of the thinnest medullated fibre is 1 μ (Duncan, 1934) and the ratio of conduction velocity (m/sec) to fibre diameter (μ) may be assumed to be of the order

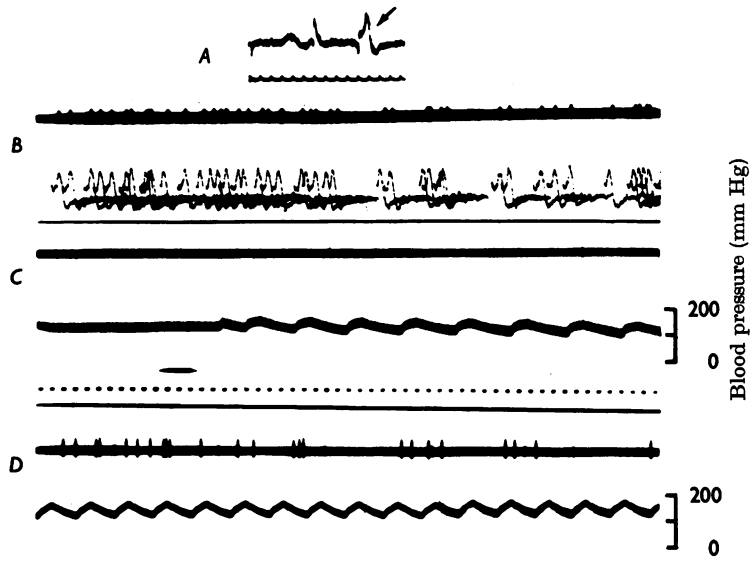

Fig. 1. Responses of chemoreceptors with medullated fibres. *A*, which consists of four superimposed sweeps with msec time marks shows the electrically evoked all-or-none impulse in the fibre at arrow. *B* is a continuous record of activity in the fibre 2·5 min after ventilation with 4 % O_2. *B* also shows the same impulses recorded simultaneously on sweeps (triggered by the impulses) of another beam of the oscilloscope; time marks for these are the same as those in *A*. Note that all the impulses are identical in shape and size with the evoked impulse at arrow in *A*. Conduction velocity of the fibre was 7·2 m/sec. *C* shows complete silence in another medullated fibre (conduction velocity, 6·2 m/sec) while ventilating the cat with air. At signal 150 μg ACh was injected into the aorta; note complete absence of stimulation. *D* shows the discharge in the same ending 3 min after ventilating with 4 % O_2. The 0·1 sec time marks in *C* are common to *B*, *C* and *D*. The second trace in *C* and *D* is that of aortic blood pressure (calibration on the right).

TABLE 1. Comparative responses of chemoreceptors of medullated and non-medullated fibres to natural stimuli. Numbers in parentheses indicate number of fibres

	Non-medullated			Medullated		
	Range	Mean	S.E.	Range	Mean	S.E.
Frequency of discharge with air (impulses/sec)*	0·1–1·5	0·2 (17)	0·09	0·1–3·5	0·8 (10)	0·4
Frequency of discharge with 4 % O_2 (impulses/sec)*	1·5–19·0	7·7 (17)	1·2	2·5–24·0	8·9 (9)	2·2
Peak frequency of discharge during hypoxia (impulses/sec)†	52–100	69 (15)	4·5	25–125	63·0 (9)	10·2
Latency for reduction of discharge on readmission of air (sec)	3·2–11·0	6·1 (12)	0·8	4·3–11·0	7·6 (6)	1·2

* Averaged over 4–10 sec.

† Reciprocal of least interval between two impulses.

of 4·5–5·0 in the lower diameter range (Boyd, 1964, 1965), only fibres with conduction velocities greater than 5 m/sec were selected for inclusion in the medullated group. The range of conduction velocities of fibres of this group was 6–13 m/sec (mean, 9 m/sec). Similarly, since the upper limit of conduction velocity for non-medullated fibres may be taken to be 2·5 m/sec (Gasser, 1950), only fibres with conduction velocities less than 2·5 m/sec were included in the non-medullated group.

The main conclusion that follows from Table 1 is that the responses of chemoreceptors with medullated fibres to natural stimuli are similar to those with non-medullated fibres. This conclusion also applies to other natural stimuli, such as the effect of haemorrhage, CO, fall in blood pressure or circulatory arrest as described below. The speed of responses to natural stimuli is also the same in both groups. Thus the interval between the re-admission of air and the reduction of discharge (produced by 4 % O_2) is the same, being about 6 sec in non-medullated fibres and about 7·6 sec in medullated fibres (Table 1).

The effect of 'breath-holding' was examined in a few endings by recording the discharge after stopping the respiratory pump. Figure 2*B* shows a typical response in which the activity of the ending increased several-fold within 40 sec of 'breath-holding'. As expected, the effects are modified by changes in blood pressure, a rise causing a reduction in the discharge while the breath is 'held' (Fig. 2*B*).

The observations of Lee, Mayou & Torrance (1964) on the relation of blood pressure to the discharge have been confirmed qualitatively (Fig. 2*A*) apart from exceptional endings. However, there are two noteworthy differences. First, that with normal blood pressure (i.e. above 100 mm Hg), the frequency of discharge while ventilating the lungs with air was very much lower than those reported by Lee *et al.* (1964). In fact in seventeen out of twenty-seven fibres it was less than 0·1 impulses/sec (e.g. Fig. 1*C*, 6*B*, 9*A*) and in only one fibre was it as high as 3·5 impulses/sec (Table 1). Secondly, in the present investigation a much greater reduction of blood pressure was necessary in order to stimulate the endings. Thus in certain endings there was only a small or hardly any increase in discharge even though the blood pressure was 50 mm Hg (Fig. 3). These differences can be attributed to differences in techniques used—single fibre preparations in the present case and multifibre ones in the case of Lee *et al.* (1964).

Effects of CO. The responses of four chemoreceptors (with medullated and non-medullated fibres) during ventilation with air containing 0·2 or 2 % CO were recorded in three cats. In all four fibres there followed clear-cut stimulation of the endings in the absence of change in blood pressure (Fig. 4*B*, *C*). Stimulation set in sooner with the higher concentrations of CO. The % O_2 saturation of arterial blood during the time of recording the effects was about 60 % (O_2 content, 9·6 ml./100 ml.) in the first cat, 30 % (O_2 content, 4·4 ml./100 ml.) in the second and 27 % (O_2 content, 4·2 ml./

100 ml.) in the third (two fibres). The blood pressures were 50, 103 and 95 mm Hg respectively in the three cats. Under these conditions the degree of excitation was equal to or a little less than that produced by administering gas mixtures containing 4 or 5 % O_2. In the last two cats it is possible to attribute the excitatory effects of CO to the reduced O_2 content of arterial blood leading to low P_{O_2} in the glomus (see Discussion). In the first cat excitation occurred at higher O_2 saturation (60 %) possibly

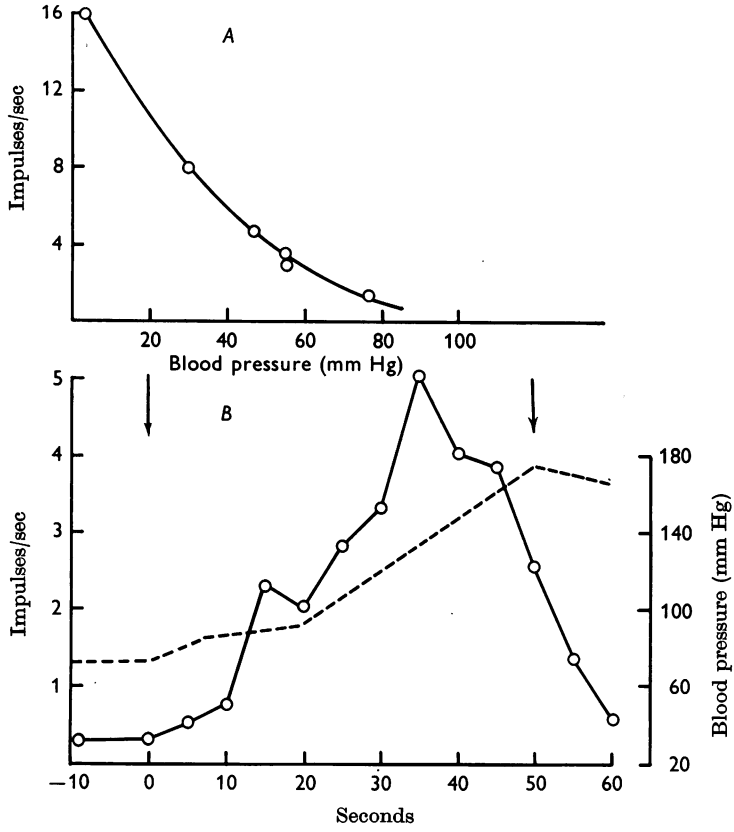

Fig. 2. Responses in aortic chemoreceptors. *A* shows the relation between blood pressure (abscissa) and frequency of discharge in a chemoreceptor with a medullated fibre (conduction velocity, 10 m/sec). Reduction in blood pressure was achieved by withdrawing various quantities of blood. The last point near zero blood pressure pertains to the discharge recorded about 1·5 min after circulatory arrest produced by injecting 30 ml. air into the right atrium. *B* shows the development of the discharge in another chemoreceptor after stopping the respiratory pump at arrow ('breath-holding'). The increase in activity was apparently limited by the simultaneous rise in blood pressure (interrupted trace). At second arrow resumption of artificial respiration reduced the discharge still further. Conduction velocity of the fibre was 1·3 m/sec.

because of the prevailing low blood pressure (50 mm Hg). The values for O_2 saturation and blood pressure during CO administration are of the same order as those recorded by Duke, Green & Neil (1952) in one of their experiments (see Fig. 1*C* in Duke *et al.* 1952). Therefore the present observations on aortic chemoreceptors differ qualitatively from those of Duke *et al.* (1952) who observed that carotid chemoreceptors were not stimulated by CO.

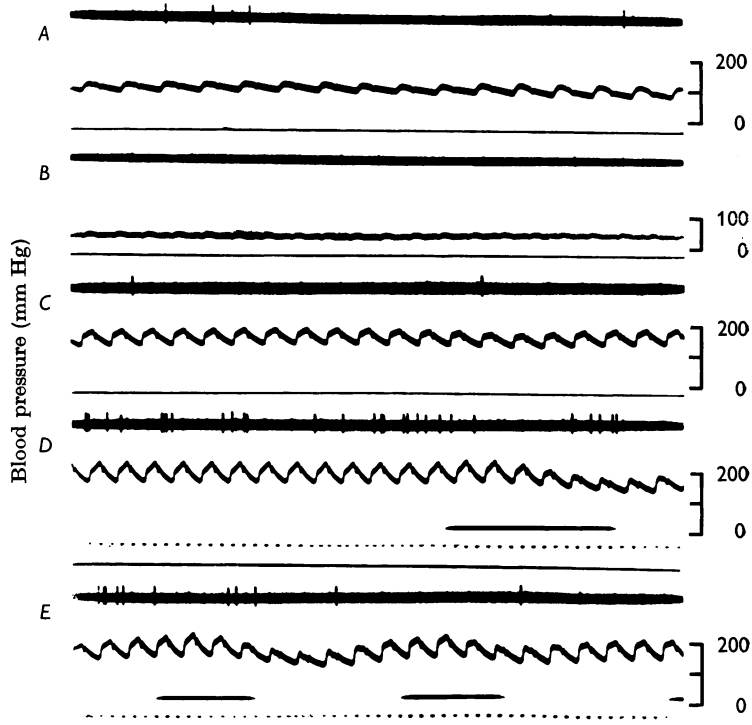

Fig. 3. Effect of reduction of blood pressure on a chemoreceptor with a non-medullated fibre (conduction velocity, 1·5 m/sec). *A* is a record before bleeding the cat. *B* recorded after withdrawing 35 ml. blood; note absence of stimulation even though the blood pressure has fallen to about 40 mm Hg. *C* is a record after injecting back 35 ml. blood. *D* shows the discharge 2·5 min after ventilation with 4·2% O_2. At signal in *D* ventilation with air was resumed and continued in *E* at signals. *C* and *D* are continuous. From above downwards in each record, impulses in a fibre, aortic blood and in *D* and *E*, signal and 0·1 sec time marks which apply to *A*, *B* and *C* as well.

Effects of circulatory arrest. It is known that occluding the common carotid artery stimulates chemoreceptors markedly (see Heymans & Neil, 1958). The only convenient way of making comparable observations on aortic chemoreceptors *in vivo* is by occluding the pulmonary artery. However, occluding the pulmonary artery involves physical interference

in the region of the aortic chemoreceptors and this procedure has itself on some occasions increased the activity in the endings. A functional occlusion of the pulmonary artery was therefore produced by injecting 30 ml. of air quickly into the right atrium. This procedure produced a precipitous and irreversible fall in blood pressure which set in within 3 sec of the start of the injection of air.

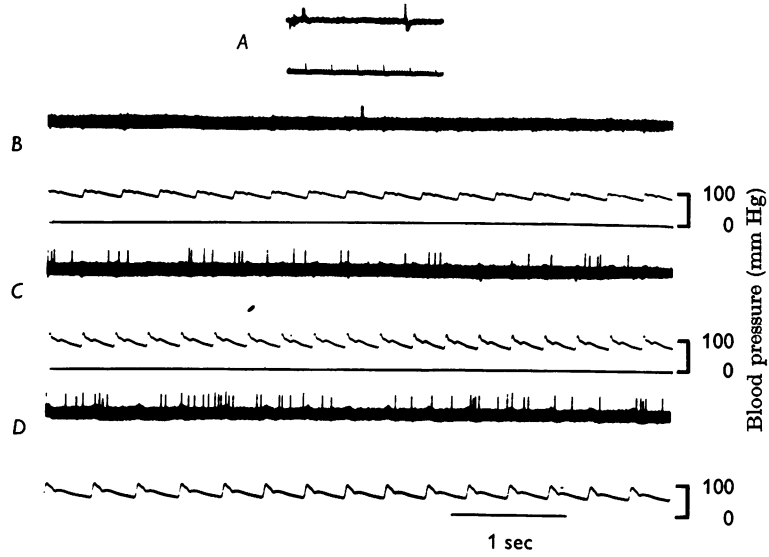

Fig. 4. Effect of CO on a chemoreceptor. *A* is a record of four superimposed sweeps (with 10 msec time marks) showing the evoked impulse of the single non-medullated fibre with a conduction velocity of 1·3 m/sec. *B* is a record of normal activity in the fibre which was taken 1 min after start of ventilation with 2 % CO in air. *C* and *D* show increased activity 2·5 and 4·5 min after ventilation with 2 % CO in air. Note, carotid blood pressure in *C* is nearly the same as that in *B*. The O_2 content of arterial blood taken between *C* and *D* was 4·2 ml./100 ml., O_2 saturation was 27 %. The frequency of discharge produced by 5 % O_2 was 11/sec (not shown).

A typical response following circulatory arrest while the cat was ventilated with air is shown in Fig. 5*A–D*. A marked increase in discharge sets in within 5–10 sec and after reaching a peak there follows a noteworthy reduction in discharge within 1–3 min in different fibres (Fig. 5*D*). This reduction appears much sooner if circulatory arrest is produced while the cat is ventilated with hypoxic mixtures of air. For example, it appeared within 1 min after circulatory arrest in the case of the ending illustrated in Fig. 6. After the initial reduction in discharge, activity in the fibres continues at a considerably reduced rate for 20–30 min after which it ceases altogether. These observations contrast markedly with the belief that the *intense* discharge of chemoreceptors persists for as long as 30 min

after death (Bogue & Stella, 1935; Heymans & Neil, 1958; Joels & Neil, 1962*a*; 1963).

The period of reduced activity is interspersed by periods of complete silence or considerably reduced discharge (Fig. 5*D*). In some fibres the alternation between silence and activity is quite regular (Fig. 5*D*). The time for reduction of the discharge after the initial increase following circulatory arrest varies among different fibres, and the period of silence

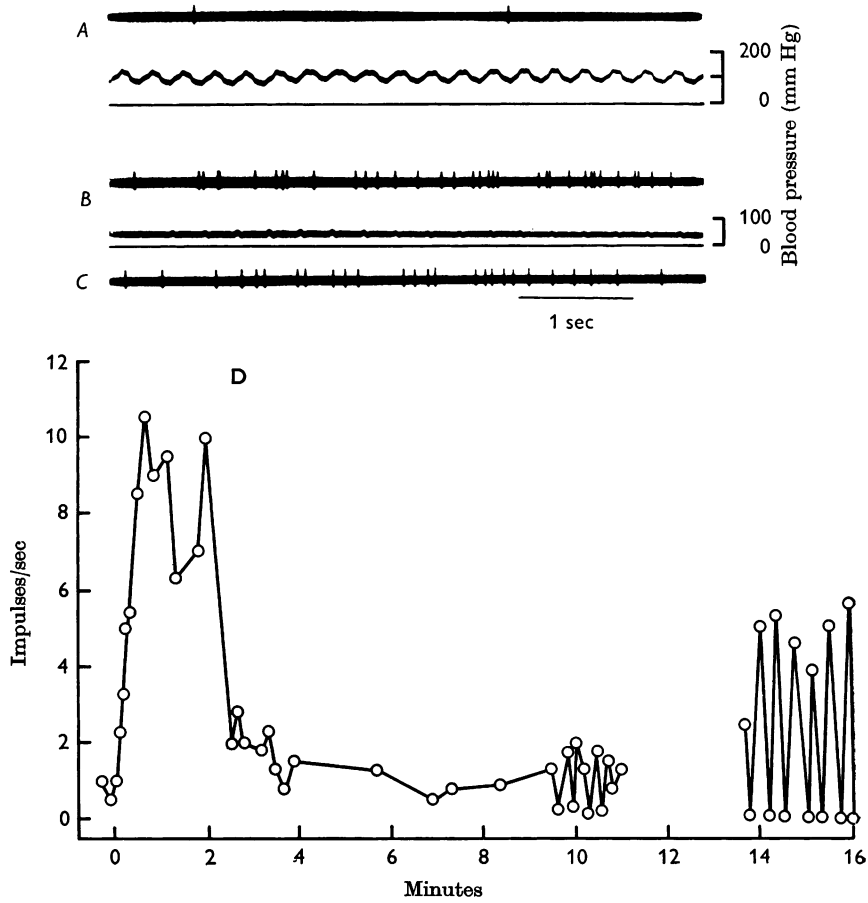

Fig. 5. Effect of circulatory arrest on a chemoreceptor. *A–C*, samples of records from which the graph *D* was plotted. *A* shows the normal activity before circulatory arrest. *B* 21 sec after; *C* (without pressure trace) an excitatory phase of cyclical activity 16 min after circulatory arrest. Conduction velocity of the fibre was 1·4 m/sec. *D* At zero time circulatory arrest was produced by injecting 30 ml. air into the right atrium. The immediate increase in activity is followed by reduction of the discharge within 3 min following circulatory arrest. Note the subsequent cyclical activity at about 10 min and especially between 14 and 16 min. Blood pressure before circulatory arrest was 100 mm Hg.

or reduced activity varies in duration in different fibres. So it is to be expected that no obvious change in the total discharge would be apparent in some experiments when recording from several active chemoreceptors simultaneously. In this connexion it is important to point out that one comes across occasional fibres in the aortic nerve with discharge patterns typical of chemoreceptors but which are not affected by hypoxia. Such

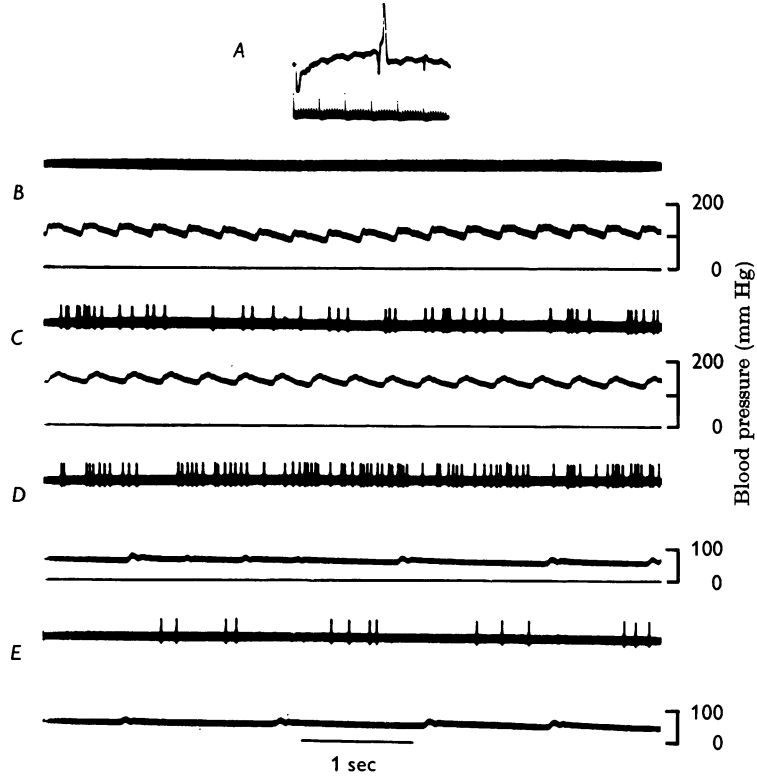

Fig. 6. Effect of circulatory arrest on a chemoreceptor. *A* is a record of four superimposed sweeps showing a single all-or-none impulse. The inflexion on the rising phase is due to recording conditions (cf. Fig. 1). The conduction velocity of the fibre was 1·7 m/sec. *B* shows normal inactivity in the chemoreceptor while ventilating the lungs with air at a blood pressure of about 110 mm Hg. *C* is a record of activity 4 min after ventilation with 3 % O_2. *D* and *E* are records at 20 sec and 70 sec after injecting 30 ml. air into the left atrium while ventilating the cat with 3 % O_2. Note the marked fall in carotid blood pressure (calibration on the right).

activity must form part of the total activity recorded in filaments containing several active fibres.

The initial reduction in the discharge (Fig. 5*D*) and the subsequent periods of silence were quite unexpected, since at the time the present experiments were performed it was assumed that the process of excitation

of chemoreceptors was mediated by some metabolite which would continue to increase in concentration in the vicinity of the chemoreceptors following circulatory arrest (see Heymans & Neil, 1958). Clearly the responses shown in Figs. 5*D* and 6*E* cannot be explained on the basis that excitation of chemoreceptors following circulatory arrest is largely due to the effect

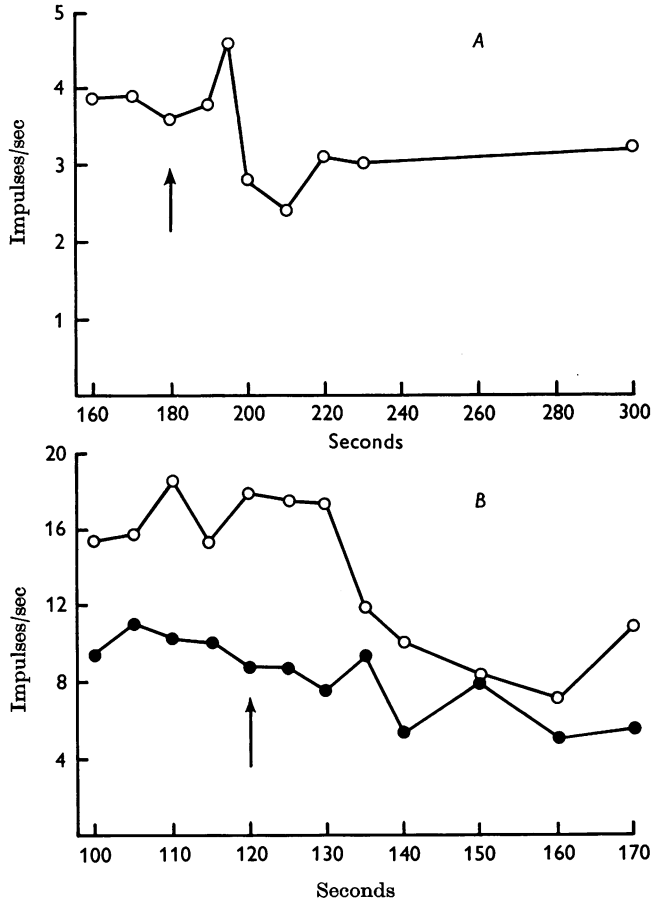

Fig. 7. Effect of circulatory arrest on chemoreceptors. In both *A* and *B* circulatory arrest was produced by injecting 30 ml. air into the right atrium at arrows. Abscissa indicates time after start of ventilation with N_2. The slight increase in activity in *A* is followed by depression and unchanged activity thereafter; Conduction velocity of this fibre was 18 m/sec (exceptionally high). *B* shows no initial increase activity at all in two non-medullated fibres following circulatory arrest. Blood pressure before circulatory arrest in *A* was 60 mm Hg; in *B* it was 120 mm Hg.

of accumulated metabolites. On the other hand, the excitation can be satisfactorily explained as being due to the marked fall in the local tissue P_{O_2} that must occur owing to the high metabolic activity of certain cells

in the glomus (see Discussion). The subsequent fall in discharge in Fig. 5*D* can be attributed to some adaptive process that constitutes an invariable feature of all sensory receptors.

In order to determine to what extent excitation was due to fall in P_{O_2} and to what extent to accumulation of metabolites, circulatory arrest was produced about 1½–3 min after ventilation with almost pure N_2 (99·7 %). The cat was ventilated with N_2 on the assumption that the arterial P_{O_2} would fall to such a low level that a further reduction in the local P_{O_2} inside the glomus (by metabolic usage of O_2) would not take place following circulatory arrest. If, under these conditions, there still resulted a significant increase in discharge following circulatory arrest, it could then be concluded that the increased discharge was produced by some metabolites that had accumulated owing to circulatory stagnation. In three out of seven chemoreceptors on which this procedure was tried, circulatory arrest enhanced slightly the already marked excitation produced by ventilation with pure N_2. This increase which lasted for less than 12 sec was followed by depression (Fig. 7*A*). In the remaining four fibres there was no added increase (Fig. 7*B*): this constitutes firm evidence against the contribution by any metabolite (be it a specific chemical, CO_2 or H^+) to the excitation produced following circulatory arrest. This experiment also shows that no mechanical factor *per se*, e.g. change in intrasinusoidal pressure is involved in the excitation following circulatory arrest. In fact the entire excitation is adequately explained by the fall in the P_{O_2} in the glomus tissue due to stagnation and the high rate of O_2 consumption by the glomus cells (Daly, Lambertson & Schweitzer, 1954).

Occasionally one comes across an ending in which the discharge produced by hypoxia ceases in the presence of maintained hypoxia. Figure 8 shows one such fibre in which the discharge ceased about 110 sec after start of ventilation with pure N_2. This type of response was apparently reproducible since it ceased 115 sec after N_2 in an earlier trial. In the third trial in this fibre, circulatory arrest 85 sec after start of ventilation with N_2 produced only a small short-lived increase in discharge which ceased soon after the increased activity. Such responses with and without superadded circulatory arrest constitute convincing evidence against any significant role by a metabolite in the production of the discharge during hypoxia. In fact it is much easier to explain the behaviour in Fig. 8*C* as a case of fatigue or rapid adaptation of the ending. A peripheral block is unlikely since the frequency of discharge is low.

Effects of ACh. Certain aortic chemoreceptors are stimulated by intra-aortic injections of ACh if the amount injected is greater than 30 μg in adult cats (Fig. 9). The discharge thus produced is of a much higher frequency than that produced by strong natural stimuli, i.e. hypoxia (cf.

Fig. 9*C* with Fig. 9*D*). Because of this higher frequency, the discharge following ACh injection appears more regular in comparison. The duration of stimulation depends on the quantity of ACh injected. In the doses used in the present investigation, it varied from a fraction of a second to a maximum of 4·5 sec in one fibre. In the majority of fibres it was 1–2 sec following 100–150 μg ACh.

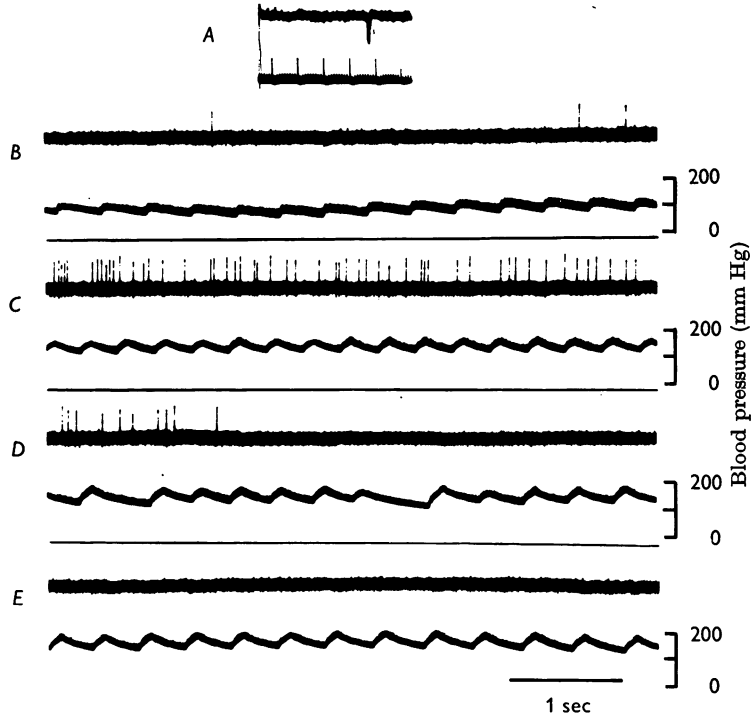

Fig. 8. Spontaneous cessation of discharge in a chemoreceptor during hypoxia. *A* shows a single all-or-none impulse in the fibre (conduction velocity, 1·3 m/sec). *B* is a record (polarity of impulses inverted) while ventilating the cat with air. *C* was taken 105 sec after start of ventilation with N_2; this discharge ceased in *D* for no apparent reason and no impulses appeared for over 20 sec thereafter. An identical response was obtained in an earlier trial on the same ending. Records *C*, *D* and *E* are continuous. This is the same ending whose response to 'breath-holding' has been shown in Fig. 2*B*. The second trace in each record is of carotid blood pressure.

The latency between the beginning of injection and the start of stimulation (injection–discharge time) averaged 1·3 sec (range 0·7–2·3 sec). The speed of response to ACh (or phenyl diguanide, see below) is therefore greater than that following injection of blood with low P_{O_2} (Paintal & Riley, 1966). As a rule, the injection–discharge time was roughly of the same order in different fibres of the same cat. Sometimes it increased in the same ending with repeated injections; in other cases it decreased.

Some endings ceased to respond to repeated injections of ACh. On the other hand there were others in which the response to ACh increased with repeated injections. Tachyphylaxis to ACh is therefore not a significant feature in the present case.

Finally, there was clear evidence of post-excitatory depression following ACh in some endings with a base line discharge. This will be more obvious if the effects of drugs are examined during hypoxic stimulation of chemoreceptors.

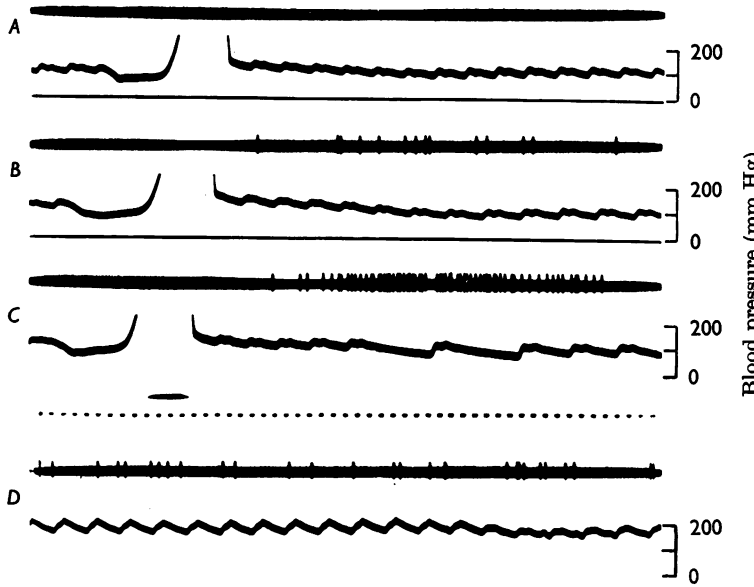

Fig. 9. Response of a chemoreceptor with non-medullated fibre to ACh. *A* shows the normal inactivity in the fibre (aortic B.P. about 100 mm Hg) and the absence of stimulation following injection of 1 ml. 0·9 % NaCl indicated by the abrupt rise in the aortic pressure trace. *B* shows the response following 30 μg ACh and *C*, that following 100 μg ACh; note the marked stimulation in contrast to the total unresponsiveness in the medullated fibre of Fig. 1*C*. *C* also shows the lag between the injection and the signal. *D* shows the response after 3 min ventilation with 4·1 % O_2. The 0·1 sec time marks in *C* apply to *A*, *B* and *D* also. Conduction velocity of the fibre was 1·4 m/sec. The cat weighed 2·6 kg.

Dose–response relationships. In order to make valid comparisons of the response of different endings in different cats with one another, the response of any particular ending to different doses of ACh was recorded. The total number of impulses produced by various doses were determined and these observations plotted graphically for each ending. Typical responses are shown in Fig. 10. The average frequency of discharge (total number of impulses divided by the duration of stimulation) was also plotted (Fig. 10).

However, this can give a misleading impression about degree of stimulation; e.g. a relatively high frequency discharge will be recorded even if only a few impulses are produced, provided they are produced within a very short interval. Consequently emphasis was given to total discharge since this gives a better index of the response to ACh because it takes both duration of stimulation and frequency of discharge into account.

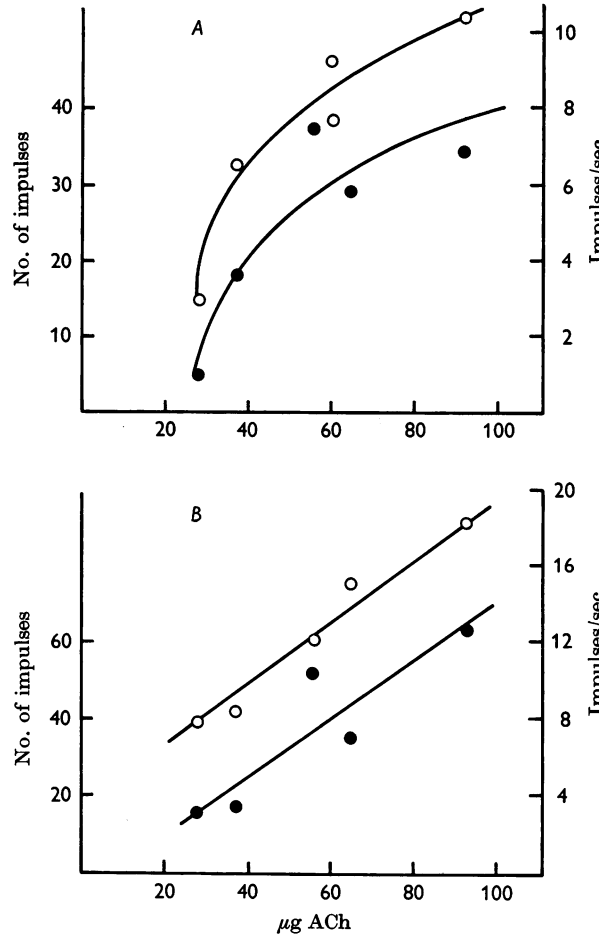

Fig. 10. Relation of dose of ACh (abscissa) to the responses produced in two chemoreceptors. Open circles, average frequency of discharge; closed circles total number of impulses produced by a given dose. Conduction velocity of the two fibres were 1·1 and 2·0 m/sec. The cat weighed 3·1 kg.

In most endings there appeared to be a linear relation between the dose of ACh and the number of impulses and a straight line was therefore drawn to fit the points (Fig. 10*B*). In a few cases, where it was certain that

the relation was non-linear, a curved line was drawn to fit the points (Fig. 10*A*). From such graphs, the number of impulses produced by a particular dose, e.g. 20 μg/kg, was then determined for different endings. The frequency distribution of the different grades of responses is shown in Fig. 11.

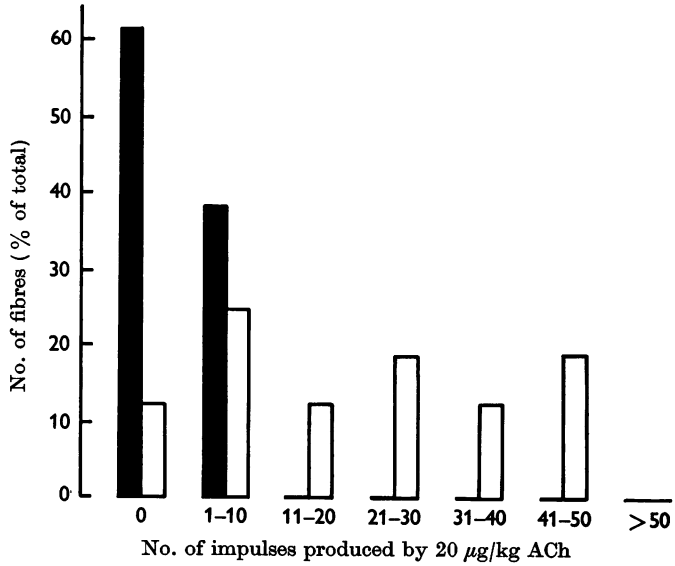

Fig. 11. Histogram showing number of fibres of chemoreceptors (in %) with different intensities of responses to ACh. Abscissa, total number of impulses produced by 20 μg/ml. ACh. The data were obtained from dose-response graphs as in Fig. 10. Total number of fibres in the medullated group (black columns), 13; total number of non-medullated fibres (open columns), 16.

Comparative responses of endings with medullated and non-medullated fibres

In contrast to the responses to natural stimuli, those following ACh depend to a considerable extent on whether the fibre of the ending is medullated or non-medullated. Thus Fig. 11 shows that about half of the endings of non-medullated fibres yielded responses that were 2–5 times the maximum response of endings with medullated fibres. On the other hand about 60 % of the latter were not stimulated at all by 20 μg/kg ACh. In fact not a single impulse was produced in about half the endings of medullated fibres by about 100–200 μg doses of ACh. The average number of impulses produced by 20 μg/kg ACh in thirteen endings was 1·5 (range 0–9; S.E. 0·7). In marked contrast, only one ending out of sixteen with non-medullated fibres failed to respond following 100 μg/ACh. The average number of impulses produced by 20 μg/kg was 21 (range 0–46; S.E. 4·0), i.e. about 14 times the activity in endings of medullated fibres.

The difference between the responses of endings of medullated and non-medullated fibres to ACh is therefore highly significant.

Effects of phenyl diguanide. The responses following phenyl diguanide were similar to those following ACh. Nineteen endings that were stimulated by ACh were also stimulated by phenyl diguanide and six endings that were not stimulated by ACh were also not stimulated by phenyl diguanide. Five of the latter were endings of medullated fibres which again supports the conclusion arrived at above that the endings of non-medullated fibres are far more sensitive to chemical substances than those of medullated fibres. Apart from occasional endings, in general the responses produced by phenyl diguanide were about a third to a half those produced by identical doses of ACh; three endings stimulated by ACh were not stimulated by phenyl diguanide at all. The correlation coefficient between the intensity of the responses to identical doses of ACh and phenyl diguanide was about 0·57; this is highly significant ($P = < 0{\cdot}01$ for 27 degrees of freedom).

DISCUSSION

Since the responses of the endings of medullated fibres to natural stimuli are quantitatively similar to those of non-medullated fibres, it is reasonable to conclude that the generator mechanisms are quantitatively and qualitatively similar in both groups. The marked differences between the responses of the two groups of endings to drugs (ACh and phenyl diguanide) cannot therefore be explained by any action on any of the processes leading up to the production of the generator potential which, so far, is recognized as the essential stimulus for the production of the propagated impulse at the ending (Katz, 1950; Gray, 1959). It follows that ACh and phenyl diguanide probably act on the regenerative region of the ending (Fig. 12). This supports the author's hypothesis regarding the site of action of drugs (Paintal, 1964) on the one hand, and on the other makes the possibility of ACh playing a part during natural stimulation *in vivo* less likely. The possible role of ACh in preparations of the carotid body studied *in vitro* might well be different in view of the recent observations by Eyzaguirre & Koyano (1965*a*, *b*) and Eyzaguirre, Koyano & Taylor (1965).

The role of ACh during natural stimulation of chemoreceptors has been debated in the past and evidence (Douglas, 1954; Liljestrand, 1954; Heymans & Neil, 1958) has been marshalled for and against the respective points of view. Any theory in which a role for ACh is claimed (Eyzaguirre & Koyano, 1965*a*, *b*, Eyzaguirre *et al.* 1965) must account for the marked differences in the relative sensitivities of endings of medullated and non-medullated fibres to ACh, and the fact that many endings of medullated fibres are not at all stimulated by ACh even in relatively large doses (100–200 μg).

The postulate that chemoreceptor excitation is mediated through metabolites (Landgren & Neil, 1951; Neil, 1951) arose because it was believed earlier that the metabolic requirements of the carotid body were low (Comroe & Schmidt, 1938). In recent years, i.e. after the demonstration by Daly *et al.* (1954) that the metabolism of the carotid body is in fact high, this postulate has rested largely on the belief that the *intense* discharge of chemoreceptors persists for as long as 30 min after death (Heymans & Neil, 1958; Joels & Neil, 1963). The persistence of the *intense* discharge after death has not been confirmed in single fibres of aortic chemoreceptors in the present investigation. In fact the opposite has been observed, i.e. a significant fall in the level of activity within 3 min of circulatory arrest

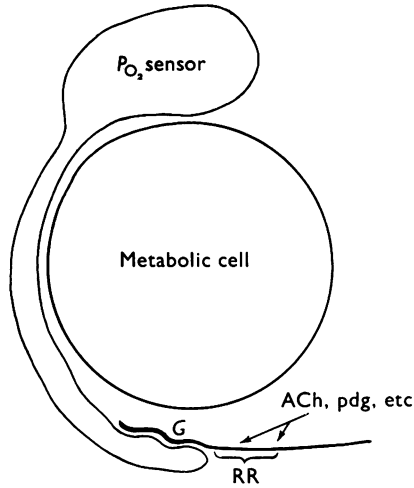

Fig. 12. Schematic diagram showing possible mechanism of initiation of impulses at chemoreceptors. *G*, generator region; RR, regenerative region of ending. Lowering of local PO_2 owing to low oxygen availability is sensed by the P_{O_2} sensor which by producing a suitable physical change at the generator region leads to the production of a generator potential. This initiates the propagated impulse at the regenerative region. Drugs like ACh and phenyl diguanide act on the regenerative region.

followed by alternating periods of silence (or much reduced activity) and activity in the fibres (Fig. 5*D*). Clearly, these observations provide good evidence against the production of the discharge by a metabolite. However, the best evidence that no metabolite is involved is provided by the repeated observation that circulatory arrest does not cause any further increase in the activity of chemoreceptors while the cat is being ventilated with pure nitrogen (Fig. 7).

Since the involvement of ACh or any other excitatory metabolite now appears rather unlikely, other mechanisms of stimulation of chemoreptors

need to be considered including possibly mechanical ones since it is known that the carotid glomus undergoes changes in size during hypoxia (de Castro, 1951). One should expect to find two kinds of cells in the glomus, one which is specifically sensitive to changes in the local P_{O_2}—the P_{O_2} sensor, and another that is responsible for the high rate of oxygen consumption—the metabolic cell. It is conceivable that the P_{O_2} sensor undergoes some physical change when the local P_{O_2} falls and this change (transmitted to the ending) produces the generator potential at the nerve ending (Fig. 12). Fall in the local P_{O_2} will occur whenever there is low *oxygen availability*, e.g. due to low P_{O_2} of the blood, reduced blood flow or reduced O_2 content following administration of CO. Adaptation of the P_{O_2} sensor can account for reduction in the discharge during circulatory arrest (Fig. 5*D*). It should be noted that the metabolite hypothesis rests on the basis that there is little or no adaptation to metabolites.

Two types of cells are now known to exist in the carotid body (Garner & Duncan, 1958; Ross, 1959; Eyzaguirre & Uchizono, 1961; Knoche & Schmitt, 1963). There is evidence that the epithelioid cell probably has a high metabolic rate (Ross, 1959) and so this might well be the metabolic cell. Similar functions have not been assigned to the sustentacular cell which is notable for its processes and also the fact that it is clearly associated with nerve terminations (Ross, 1959). Perhaps this is the P_{O_2} sensor.

The characteristically irregular pattern of discharge during hypoxia has been advanced as evidence favouring the production of the discharge by a metabolite or transmitter (Biscoe & Taylor, 1963; Eyzaguirre & Koyano, 1965*a*). This irregularity could also be accounted for if it were assumed that the P_{O_2} sensor undergoes some physical alteration in an irregular or non-uniform manner with respect to other parts of the same cell.

The results on the effects of CO_2 on aortic chemoreceptors (Paintal & Riley, 1966) and the present ones on the effects of CO and circulatory arrest are obviously inconsistent with existing knowledge, obtained from the numerous studies on carotid chemoreceptors. Since it is hard to believe that the responses of aortic chemoreceptors can be qualitatively different from those of the carotid, there is a strong possibility that the inconsistencies have arisen from differences in techniques used. In the present case all the observations have been made without altering the external environment of the aortic bodies in any way whatever, particularly since it was found that physical interference in the vicinity of the aortic bodies influenced the activity of the ending (see Results). On the other hand, in the case of the carotid chemoreceptors physical interference close to the carotid body is unavoidable. Moreover, there is no method of retaining intact the external natural environment of the carotid body which of necessity has to be exposed to air or physiological solutions (with a P_{O_2} of about 150

mm Hg) while recording impulses from carotid chemoreceptors. The importance of the external environment will be appreciated from the fact that even light has an effect on certain responses of chemoreceptors (Joels & Neil, 1962*b*).

A second possible source of difference is that the present work has been confined to observations on single fibres and it has therefore been possible to observe certain phenomenon, e.g. cyclical activity after circulatory arrest (Fig. 5*C*, *D*), which would otherwise be obscured when recording from several active fibres simultaneously.

I am most grateful to Professor B. Katz, F.R.S., and Professor R. L. Riley for reading the manuscript and for making valuable criticisms. I am thankful to Dr S. S. Deshpande for kindly assaying the stock solution of ACh and to Mr J. P. Bahuguna for making and analysing the gas mixtures.

REFERENCES

BISCOE, H. & TAYLOR, A. (1963). The discharge pattern recorded in chemoreceptor afferent fibres from the cat carotid body with normal circulation and during perfusion. *J. Physiol.* **168**, 332–344.

BOGUE, J. Y. & STELLA, G. (1935). Afferent impulses in the carotid sinus nerve (nerve of Hering) during asphyxia and anoxaemia. *J. Physiol.* **83**, 459–465.

BOYD, I. A. (1964). The relation between conduction velocity and diameter for the three groups of afferent fibres in nerves to mammalian skeletal muscle. *J. Physiol.* **175**, 33–35*P*.

BOYD, I. A. (1965). Differences in the diameter and conduction velocity of motor and fusimotor fibres in nerves to different muscles in the hind limb of the cat. In *Studies in Physiology*, ed. D. R. CURTIS & MCINTYRE, A. K., pp. 7–12. Berlin: Springer-Verlag.

BOYD, I. A. & PATHAK, C. L. (1965). The response of perfused frog hearts to minute quantities of acetylcholine, and the variation in sensitivity with season. *J. Physiol.* **176**, 191–204.

COMROE, J. H., JR., FORSTER, R. E., DUBOIS, A. B., BRISCOE, W. A. & CARLSON, E. (1962). *The Lung*, 2nd ed., p. 66. Chicago: Year Book Publishers Inc.

COMROE, J. H., JR. & SCHMIDT, C. F. (1938). The part played by reflexes from the carotid body in the chemical regulation of respiration in the dog. *Am. J. Physiol.* **121**, 75–97.

DALY, M. DE BURGH, LAMBERTSON, C. J. & SCHWEITZER, A. (1954). Observations on the volume of blood flow and oxygen utilization of the carotid body in the cat. *J. Physiol.* **125**, 67–89.

DAWES, G. S., MOTT, J. C. & WIDDICOMBE, J. G. (1952). Chemoreceptor reflexes in the dog and the action of phenyl diguanide. *Archs int. Pharmacodyn. Thér.* **40**, 203–222.

DE CASTRO, F. (1951). Sur la structure de la synapse dans les chemorecepteurs: leur mécanisme d'excitation et rôle dans la circulation sanguine locale. *Acta physiol. scand.* **22**, 14–43.

DOUGLAS, W. W. (1954). Is there chemical transmission at chemoreceptors? *Pharmac. Rev.* **6**, 81–83.

DUKE, H. N., GREEN, J. H. & NEIL, E. (1952). Carotid chemoreceptor inpulse activity during inhalation of carbon monoxide mixtures. *J. Physiol.* **118**, 520–527.

DUNCAN, D. (1934). A relation between axone diameter and myelination determined by measurement of myelinated spinal root fibres. *J. comp. Neurol.* **60**, 437–462.

EYZAGUIRRE, C. & KOYANO, H. (1965*a*). Effects of hypoxia, hypercapnia, and pH on the chemoreceptor activity of the carotid body *in vitro*. *J. Physiol.* **178**, 385–409.

EYZAGUIRRE, C. & KOYANO, H. (1965*b*). Effects of some pharmacological agents on chemoreceptor discharges. *J. Physiol.* **178**, 410–437.

EYZAGUIRRE, C., KOYANO, H. & TAYLOR, J. R. (1965). Presence of acetylcholine and transmitter release from carotid body chemoreceptors. *J. Physiol.* **178**, 463–476.

EYZAGUIRRE, C. & UCHIZONO, K. (1961). Observations on the fibre content of nerves reaching the carotid body of the cat. *J. Physiol.* **159**, 268–281.

GARNER, C. M. & DUNCAN, D. (1958). Observations on the fine structure of the carotid body. *Anat. Rec.* **130**, 691–709.

GASSER, H. S. (1950). Unmedullated fibres originating in dorsal root ganglia. *J. gen. Physiol.* **33**, 651–690.

GRAY, J. A. B. (1959). Initiation of impulses at receptors. In *Handbook of Physiology*, section I, vol. 1, Neurophysiology, ed. FIELD, J. & MAGOUN, H. W., pp. 123–145. Washington: American Physiological Society.

HARRIS, D. T., GILDING, H. P. & SMART, W. A. M. (1956). *Experimental Physiology for Medical Students*, 6th ed., p. 63. London: Churchill.

HEYMANS, C. & NEIL, E. (1958). *Reflexogenic Areas of the Cardiovascular System*, pp. 185–186, 190. London: Churchill.

JOELS, N. & NEIL, E. (1962*a*). Carotid glomus chemosensory excitation. *J. Physiol.* **164**, 11–12*P*.

JOELS, N. & NEIL, E. (1962*b*). The actions of high tensions of carbon monoxide on the carotid chemoreceptors. *Archs int. Pharmacodyn. Thér.* **139**, 528–534.

JOELS, N. & NEIL, E. (1963). The excitation mechanism of the carotid body. *Br. med. Bull.* **19**, 21–24.

KATZ, B. (1950). Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. *J. Physiol.* **111**, 261–282.

KNOCHE, H. & SCHMITT, G. (1963). Über Chemo- und Pressorreceptorenfelder am Coronarkreislauf. *Z. Zellforsch mikrosk. Anat.* **61**, 524–560.

LANDGREN, S. & NEIL, E. (1951). Chemoreceptor impulse activity following haemorrhage. *Acta physiol. scand.* **23**, 158–167.

LEE, K. D., MAYOU, R. A. & TORRANCE, R. W. (1964). The effect of blood pressure upon chemoreceptor discharge to hypoxia, and the modification of this effect by the sympathetic-adrenal system. *Q. Jl exp. Physiol.* **49**, 171–183.

LILJESTRAND, G. (1954). Transmission at chemoreceptors. *Pharmac. Rev.* **6**, 73–78.

MACINTOSH, F. C. & PERRY, W. L. M. (1950). Biological estimation of acetylcholine. *Meth. med. Res.* **3**, 78–92.

NEIL, E. (1951). Chemoreceptor areas and chemoreceptor circulatory reflexes. *Acta physiol. scand.* **22**, 54–65.

PAINTAL, A. S. (1963). Vagal afferent fibres. *Ergebn. Physiol.* **52**, 74–156.

PAINTAL, A. S. (1964). Effects of drugs on vertebrate mechanoreceptors. *Pharmac. Rev.* **16**, 341–380.

PAINTAL, A. S. (1965). Effects of temperature on conduction in single vagal and saphenous myelinated nerve fibres of the cat. *J. Physiol.* **180**, 20–49.

PAINTAL, A. S. & RILEY, R. L. (1966). Responses of aortic chemoreceptors. *J. appl. Physiol.* **21**, 543–548.

PETERS, J. P. & VAN SLYKE, D. D. (1932). *Quantitative Clinical Chemistry*, pp. 257–260.

ROSS, L. L. (1959). Electron microscopic observations of the carotid body of the cat. *J. biophys. biochem. Cytol.* **6**, 253–262.

RUSHMER, R. F. (1961). *Cardiovascular Dynamics*, 2nd ed., p. 137. Philadelphia: Saunders Company.