J. Physiol. (1955) 130, 513–532

OBSERVATIONS ON THE EXCITATION BY ACETYLCHOLINE AND BY PRESSURE OF SENSORY RECEPTORS IN THE CAT'S CAROTID SINUS

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(*Received* 2 *March* 1955)

A variety of sensory receptors appear to be sensitive to acetylcholine in that the application of this drug to the region of the sensory endings has resulted in the appearance of impulses in the corresponding sensory nerves. In mammals this acetylcholine-sensitivity has been demonstrated for chemoreceptors of the carotid body (Euler, Liljestrand & Zotterman, 1941), mechanoreceptors in the skin (Brown & Gray, 1948; Douglas & Gray, 1953) and thermal receptors in the tongue (Dodt, Skouby & Zotterman, 1953); and also for stretch receptors in crustacean muscles (Wiersma, Furshpan & Florey, 1953). There is suggestive evidence that the phenomenon extends to pain endings also (Skouby, 1951; Armstrong, Dry, Keele & Markham, 1953). Some of the receptors studied showed further pharmacological features similar to those found at autonomic ganglia, in that the effects of acetylcholine were paralleled by nicotine and were prevented by previous application of drugs of the ganglion-blocking type; such block did not, however, appear to interfere with the normal functioning of the receptors concerned (Brown & Gray, 1948; Douglas & Gray, 1953; Douglas, 1952). The experiments to be described, performed on the isolated perfused carotid sinus of the cat, show that the pressure receptors also behave in this way. Quantitative evidence is presented which supports the view that acetylcholine has no role at pressure endings analogous to that at autonomic ganglia and motor end-plates.

A preliminary account of this work has already been published (Diamond, 1953). A paper by Landgren, Skouby & Zotterman (1953) on the acetylcholine-sensitivity of pressure receptors has since appeared; these workers do not describe the effects of blocking agents, and in some respects their interpretation of the effects of acetylcholine differs from the present one. A further report has appeared by Dontas (1954).

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METHOD

Dissection. The experiments were performed on forty-one carotid sinuses taken from cats anaesthetized with chloralose (0·08–0·1 g/kg) or a mixture of chloralose (0·05 g/kg) and urethane (0·5 g/kg). The carotid sinus nerve was dissected free in the usual manner, and the central end cut. Near the carotid body the nerve divides into a number of branches, one or two of which can be followed up to the sinus wall. By sectioning all but one of these branches a preparation with only one or a few functional pressure receptor fibres was obtained. When this was not practicable the conventional technique of progressive nerve thinning with frequent testing was used. In order to isolate the common and external carotids and sinus region completely from the surrounding tissue, all unwanted vessels, including many small ones emerging from the carotid body, were divided between ligatures. In a few experiments, after dissection of a nerve branch right up to the sinus wall, the carotid body itself was ligated and removed, leaving a functioning pressure receptor preparation.

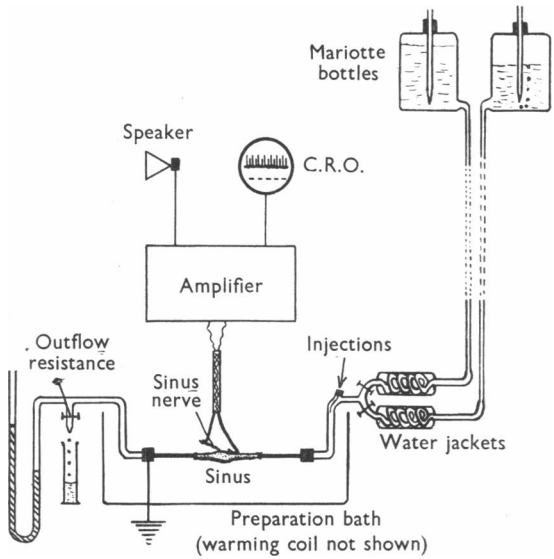

Fig. 1. Diagram of the apparatus.

It was usual to administer heparin (500–1000 i.u./kg) to the animal about ½–1 hr before cannulation. The common and external carotids were cannulated with cannulae made from hypodermic syringe needles. The cannulae formed part of the perfusion system described below, and were clamped in special holders attached to the arm of a Brown-Schuster myograph stand. The sinus and its nerve were then freed from the neck and transferred to a bath containing physiological saline and liquid paraffin.

Perfusion system. The perfusion fluids were contained in two Mariotte bottles suspended from a pulley (Fig. 1). The fluid passed through glass coils surrounded by water jackets just before entering the sinus, and escaped beyond the sinus through one arm of a T-piece whose other arm was connected to a mercury manometer. The pressure and rate of flow were varied by altering the height of the bottles and by controlling the size of the outflow resistance by means of a screw clip. It was found that the frequency of discharge of a single sensory unit at a given perfusion pressure was not significantly different from that when flow was stopped, the pressure being adjusted to the same value.

The temperature, which was recorded with a mercury thermometer placed alongside the sinus, was controlled by means of a constant temperature reservoir from which water was pumped round a circuit including the water jackets and a coil lining the preparation bath. Using this method variations in temperature did not exceed $\pm 1{\cdot}0°$ C.

Recording. The procedure for recording action potentials was the same for both whole and reduced nerves. The cut end of the desheathed nerve was laid on one of a pair of bright platinum electrodes, the other of which lay alongside the sinus wall. The preparation was earthed through the metal outflow cannula. The electrodes were attached to the arm of the myograph stand as were the cannulae; the preparation and electrode assembly were raised into the liquid paraffin layer when recording and lowered into the saline when the nerve required moistening. This arrangement also allowed any leakage from the sinus to drain downwards, thus preventing shunting between the electrodes. Some leakage usually occurred after a few hours' perfusion, but results were not affected at those times.

The action potentials in the sinus nerve were amplified with a balanced amplifier having an input resistance of 2 MΩ; the frequency band width was reduced until the signal to noise ratio was maximum. The signals were displayed on the screen of a cathode-ray oscilloscope which was photographed on moving paper.

Injections. Injections were made into a short side arm near the inflow cannula (Fig. 1). They varied in volume from 0·5 to 1·2 ml., and were completed in less than 1 sec. The bulk of the injected material took 2–20 sec to reach the sinus, according to the rate of flow and rapidity of injection; the oscillations of the mercury column due to the pressure pulse caused by the injection ceased in less than 3 sec.

Solutions. The perfusion fluid used was based on that of Krebs & Henseleit (1932), and made up as follows: KCl, 0·426 g; $CaCl_2$, 0·282 g; $MgSO_4.7H_2O$, 0·294 g; $NaH_2PO_4.2H_2O$, 0·183 g; NaCl, 6·87 g; $NaHCO_3$, 2·1 g; glucose, 1·0 g; H_2O to 1 l.: the solution was equilibrated with oxygen or nitrogen containing 5–6 % CO_2, giving a pH of 7·4.

In many experiments the calcium content was halved, since that of plasma is considered to be only half in a free diffusable state (Updegraff, Greenberg & Clark, 1926). A few preliminary experiments indicated that although quantitatively the responses of the pressure receptors increased as the calcium was reduced, qualitatively the effects of both pressure and drugs were unchanged.

Drugs were dissolved either in the same, or in 0·9 % NaCl solution, and injected at the temperature of the perfusion fluid. The drugs used were: acetylcholine iodide; nicotine tartrate; hexamethonium bromide; tubocurarine chloride; eserine sulphate; atropine sulphate; adrenaline hydrochloride; dihydroergotamine methanesulphonate.

RESULTS

Characteristics of the preparation. In most preparations the receptors remained active for 6–12 hr. The only obvious change in their behaviour with time was a tendency for the threshold pressure—i.e. the lowest steady pressure producing a maintained impulses discharge—to rise. This did not occur in all preparations. In a few preparations the behaviour of receptors altered early in the experiment and only rapid pressure rises excited impulses; these preparations were discarded.

The impulse frequency of single sensory units adapted to a constant value within 1–1½ min after the pressure became constant, and altered only slightly whilst the steady pressure was maintained; this sometimes exceeded 1 hr. Their behaviour has been studied only during this state of equilibrium. The relation between the intrasinusal pressure and the impulse frequency from a

single sensory unit is shown by the pressure-frequency graph for that unit. When such graphs were repeated at different times for the same sensory unit there were usually variations between them. These variations were not related to time, and there are indications that they were due to slow variations in the tone of the sinus wall.

The range of pressure-frequency graphs and the behaviour of the receptors during maintained constant pressures agree with the results obtained by Landgren (1952*a*) from blood-filled sinuses *in situ*.

Fig. 2 shows the pressure-frequency graphs of a single sensory unit at four different temperatures. The effect of reducing the temperature was to increase the threshold pressure and flatten the curve. At room temperature most

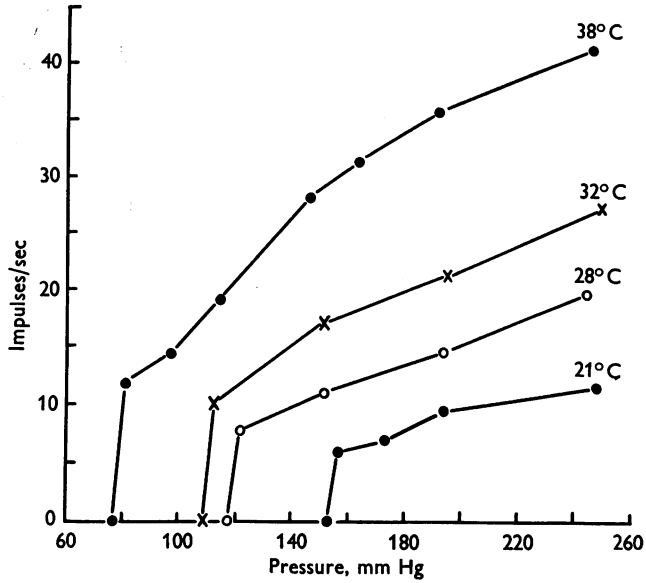

Fig. 2. Effect of reduction of temperature on the pressure-frequency graph of a single sensory unit. Ordinate, impulse frequency. Abscissa, pressure.

receptors did not respond to steady pressures. The effects were fully reversible.

Tests for chemoreceptor activity. The technique of isolating the sinus was such as to produce severe trauma of the carotid body. Chemoreceptor activity was tested for before cannulating by occluding the common carotid below the sinus during asphyxiation of the animal. A continued absence of nerve activity indicated freedom from chemoreceptor interference.

If chemoreceptor activity was revealed, nerve branches to the carotid body were crushed until subsequent tests were negative.

Occasionally the carotid body was removed completely, and later identified histologically. This does not however exclude the possibility of glomus tissue

being present in the sinus wall, an occurrence which has been demonstrated histologically in some species (Meijling, 1938). The behaviour of the pressure receptors was not detectably altered by severe O_2-lack.

Effects of excitant drugs

Acetylcholine. Injections of acetylcholine in adequate doses increased the activity in the sinus nerve. Apart from a momentary increase during the injection the intrasinusal pressure remained constant. Fig. 3 (*a*) and (*b*) show

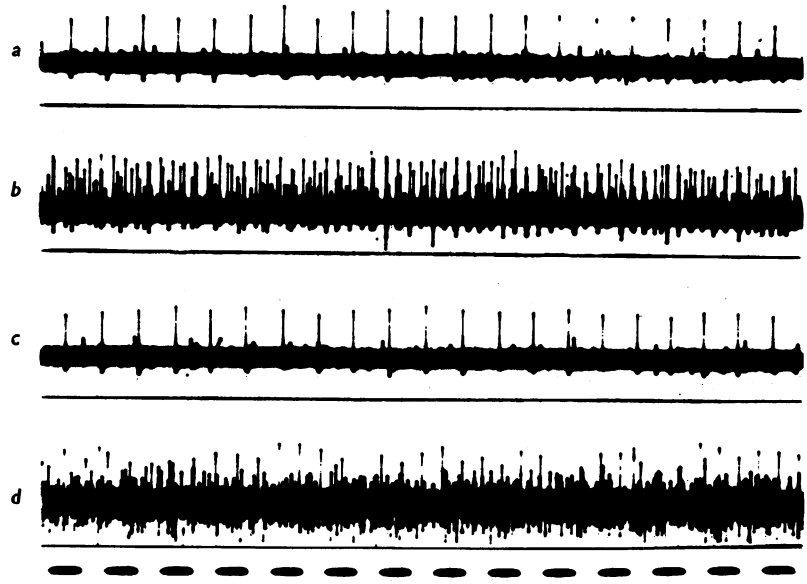

Fig. 3. Sinus nerve activity in an isolated preparation. Perfusion fluid contained atropine 10^{-6} g/ml. Flow 10 ml./min. Intrasinusal pressure as follows: (*a*) 93 mm Hg, (*b*) 215 mm Hg, (*c*) 93 mm Hg, 10 sec after injection of 0·5 ml. of 0·9% NaCl solution, (*d*) 93 mm Hg, 10 sec after injection of 0·5 ml. of a 10^{-4} g/ml. acetylcholine solution, time, $\frac{1}{10}$ sec.

the sinus nerve activity at two different steady pressures. After an injection of 0·5 ml. acetylcholine (10^{-4} g/ml.) the frequency of the largest spike rose from 15 to 27/sec, and there was a massive increase in the number of small and medium spikes (Fig. 3*d*). A control injection of saline was ineffective (Fig. 3*c*). It can be seen that the acetylcholine record differed from that resulting from high pressure in that it consisted mainly of small spikes. This record was typical of most obtained from the whole sinus nerve.

Nicotine. The effects of nicotine were similar to those of acetylcholine, the threshold doses required, i.e. the lowest amounts which would produce a discharge, being about the same for either drug. The discharge of impulses

resulting from an injection of nicotine, like that after acetylcholine, included a majority of small spikes.

Evidence of activity in fibres of pressure-receptor origin. Although it could be shown that chemoreceptors were not responding during anoxia, acetylcholine might still have produced impulses in chemoreceptor fibres by acting on a different part of the sensory pathway from that affected by O_2-lack. The proof that acetylcholine gave rise to impulses in fibres from pressure receptors was given by recording the activity in single fibres of known origin. It was assumed that only one fibre from pressure receptors was active if the spike height was constant, the impulse frequency regular during constant pressure, and an alteration of pressure affected only this frequency. Fig. 4 shows records of activity in a preparation from which a histologically identified complete carotid body

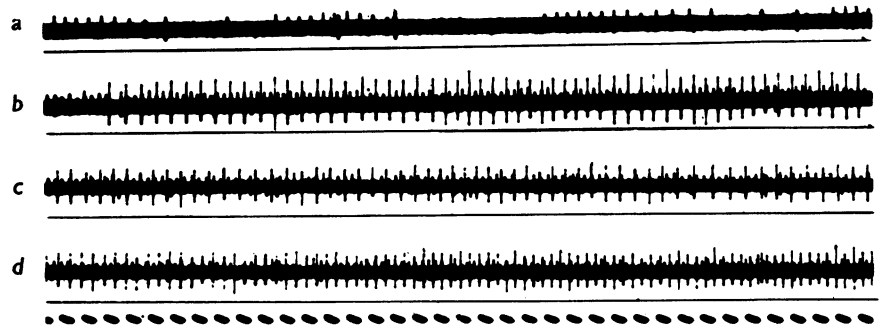

Fig. 4. Sinus nerve activity in isolated preparation (carotid body removed). Flow 15 ml./min. Records begin 9·5 sec after injection. (*a*) Pressure 25 mm Hg, 0·5 ml. saline, (*b*) Pressure 25 mm Hg, 0·5 ml. acetylcholine 10^{-4} g/ml., (*c*) Pressure 111 mm Hg, 1·0 ml. saline, (*d*) Pressure 111 mm Hg, 1·0 ml. acetylcholine 10^{-5} g/ml., time $\frac{1}{10}$ sec. See text for explanation.

had been removed. The threshold pressure of the sensory unit giving rise to the large amplitude potentials was 88 mm Hg. During a steady pressure of 25 mm Hg an injection of 0·5 ml. acetylcholine (10^{-4} g/ml.) evoked a discharge of large spikes, and caused the previously irregular bursts of small spikes to fuse into a continuous discharge, whose frequency was 34/sec (Fig. 4*b*). The maximum frequency of the large spike during the response to acetylcholine, at a pressure 63 mm Hg below its threshold, was 18/sec; this value was similar to that resulting from a steady pressure 23 mm Hg above threshold in the absence of acetylcholine (Fig. 4*c*). The activity when the pressure was 111 mm Hg was not altered by an injection of saline; after an injection of 1·0 ml. acetylcholine (10^{-5} g/ml.) the frequencies of the large and small spikes rose from 17/sec to 26/sec, and 31/sec to 38/sec respectively (Fig. 4*d*). The maximum frequencies of the responses to acetylcholine occurred at approximately the same time after the injection during both subthreshold and suprathreshold pressures. It is certain, therefore, that the activity evoked by acetylcholine was in the same fibres as the response to pressure.

Acetylcholine and nicotine responses have also been obtained from three of six preparations of the common carotid region and the nerve described by Green (1953), which has been shown not to contain fibres from chemoreceptors. It has been possible to mount this preparation only a few times and the acetylcholine responses were not readily obtained with it; this may be attributable to the inaccessibility of the pressure endings in this region.

Even with identical rates of perfusion and closely similar rates of injection the threshold quantities of drugs needed to excite different fibres of the same or different sinuses sometimes varied by more than an hundredfold. The most sensitive receptors were excited by 0·5–1·0 ml. of a 10^{-6} g/ml. solution of acetylcholine, the intrasinusal pressure being as much as 60–70 mm Hg below threshold. However, for many receptors it was necessary for the pressure to be fairly near threshold to obtain convincing responses. In about 15% of the experiments even very large doses of acetylcholine failed to excite any of the fibres producing the large spikes, although a discharge of small spikes was often obtained. There are, however, reasons to believe that some of these may have occurred in chemoreceptor fibres.

Relation between amount of acetylcholine and the response. Examples of the frequency-time relationship of the activity evoked in a single fibre by acetylcholine are seen in Fig. 5, which shows the results of three different injections of acetylcholine in the same preparation. There are two well-defined peaks of activity of which the first, due to the brief pressure pulse caused by the injection, is virtually identical for all three and was synchronous with the injection. It occurred whatever the nature of the solution injected and was separated from the effects of acetylcholine by an interval which depended on the amount injected, the rate of perfusion and the rate of injection; when these were constant responses did not vary greatly. When a fibre was firing at a steady frequency an injection of acetylcholine caused an acceleration of firing whose time course was similar to that of Fig. 5.

The minimum interval between the injection and the beginning of the response depended chiefly on the time taken for the injected material to pass through the dead space. As the dose of acetylcholine was reduced (the injection volume being constant) this interval increased, and there was a clear relation between the amount of acetylcholine injected and the latency (Fig. 6). This presumably is an indication of the time taken for the acetylcholine to rise to a stimulating concentration at the sensory terminal. A similar relation was observed between dose and the time to peak response (Fig. 6).

Over a wide range increasing the amount of acetylcholine injected increased the maximum frequency reached, the duration of the response and the total response integrated with respect to time. Fig. 7 shows that over the range investigated the relation between dose and maximum frequency of response is approximately logarithmic. A similar relation holds for the total number of

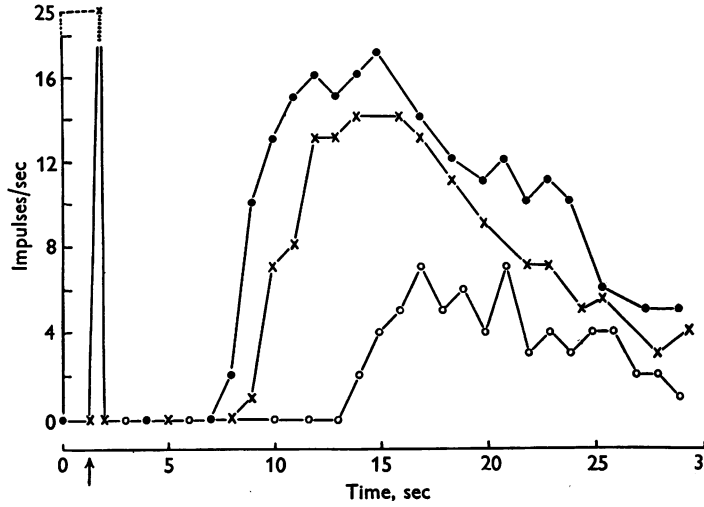

Fig. 5. Single sensory unit. Relation between impulse frequency and time. Injection at arrow, relating to each of the three superimposed graphs. Intrasinusal pressure 36 mm Hg. Flow 15 ml./min. Injection volume 1·0 ml. (●) 100 μg acetylcholine; (×) 30 μg acetylcholine; (○) 3 μg acetylcholine. For explanation see text.

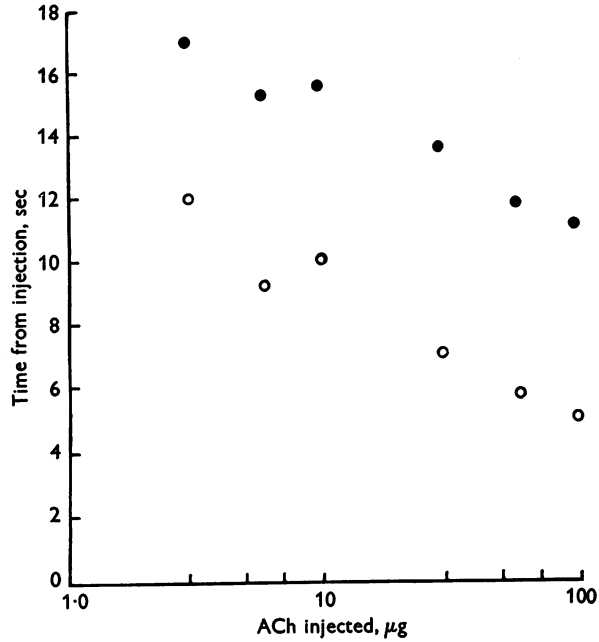

Fig. 6. Relation between dose of acetylcholine and time from injection to beginning (○) and to peak (●) of response. Injection volume 1·0 ml. Intrasinusal pressure 36 mm Hg. Flow 15 ml./min. Acetylcholine dose is plotted on a logarithmic scale.

impulses produced in the first 30 sec after the injection; in this instance it was impracticable to count impulses occurring beyond this time, but it will be seen from Fig. 5 that the bulk of the response was completed in 30 sec. The maximum frequency of impulses evoked by acetylcholine, even when the pressure was below threshold, sometimes equalled the maximum of which the sensory unit was capable during steady pressure.

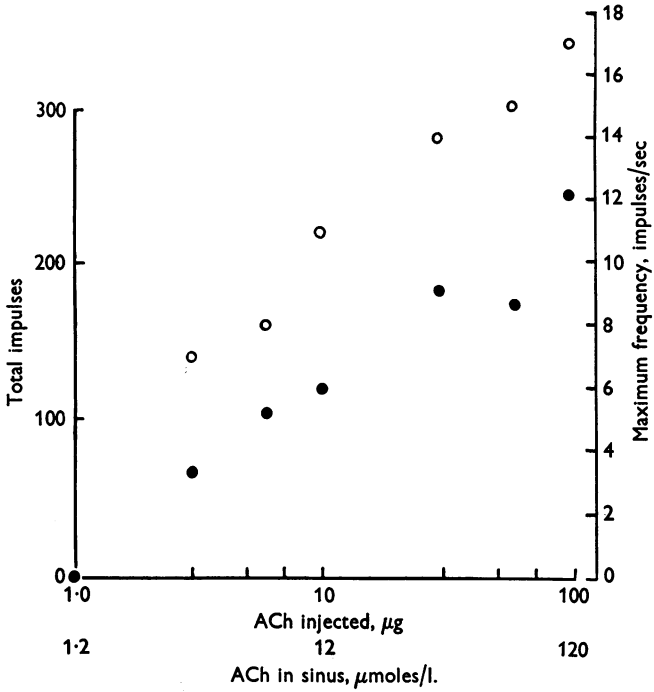

Fig. 7. Relation between dose of acetylcholine and response of single sensory unit. (●), total response in impulses (left-hand ordinate). (○), maximum frequency of response (right-hand ordinate). Injection volume 1·0 ml. Intrasinusal pressure 36 mm Hg. Flow 15 ml./min. Acetylcholine dose is plotted on a logarithmic scale. For explanation of abscissa lower scale see text.

The approximate dilution of injected material when it reached the sinus has been found by simple procedures. The abscissa of Fig. 7 has been scaled both in μg acetylcholine injected, and in μmoles per litre (lower scale); the latter scaling is based on a dilution of 1/3 and represents an upper limit for the concentration reached in the sinus.

Site of action of drugs. The possibility that the effects might be secondary to an action on the smooth muscle was excluded by the use of atropine, which prevents the effects of acetylcholine on smooth muscle elsewhere in the body (Dale, 1914). In the present experiments the responses evoked in a single fibre

by a given dose of acetylcholine was not significantly altered, either in magnitude or in time course, when the perfusion fluid contained atropine in a concentration of 10^{-5} g/ml.

Moreover, the increase of pressure receptor activity resulting from an injection of adrenaline, which acts by increasing the smooth muscle tone (Heymans & Delaunois, 1951; Landgren, 1952*b*, Landgren, Neil & Zotterman, 1952), had a latency as much as five times that of the acetylcholine-evoked response (Fig. 8*a*) the rate of rise was also considerably slower.

Acetylcholine might conceivably act at synapses of the isolated ganglion cells known to occur in this region; the release of adrenaline or noradrenaline from their axon terminals might then increase the tone of the arterial smooth muscle enough to excite the pressure receptors (Landgren, *et al.* 1952). However, as indicated above, the differences in the time courses of the responses to acetylcholine and to adrenaline make this explanation improbable (Fig. 8*a*). The effects of an adrenolytic agent confirmed the impression that sympathetic

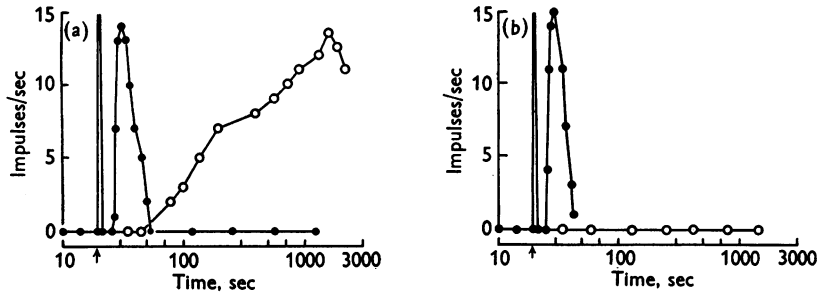

Fig. 8. Time course of response of a single sensory unit to an injection of acetylcholine (●) and adrenaline (○). Injection made at arrow for both drugs; graphs superimposed. Intrasinusal pressure 10 mm Hg below threshold. Flow 15 ml./min. Injection volume 1·0 ml. (*a*) acetylcholine 30 μg; adrenaline 1·0 mg; normal perfusion fluid. (*b*) acetylcholine 10 μg; adrenaline 1·0 mg; perfusion fluid contained 2×10^{-6} g/ml. dihydroergotamine. Time is plotted on a logarithmic scale.

stimulation was not responsible for the excitant action of acetylcholine. The response that followed an injection of adrenaline was completely abolished when the perfusion fluid contained dihydroergotamine, a substance known to block the effects of sympathetic stimulation elsewhere; the response to acetylcholine was not diminished (Fig. 8*b*). It is concluded, therefore, that the actions of acetylcholine and of nicotine were directly on the terminal sensory pathway.

Effects of blocking drugs

Hexamethonium. An injection of 0·5–1·2 ml. of hexamethonium 10^{-3} g/ml. prevented excitation by a succeeding injection of acetylcholine or nicotine. The minimum concentration of hexamethonium in the perfusion fluid necessary to block completely the effects of a threshold dose of acetylcholine or nicotine

varied from 10^{-7} to 10^{-5} g/ml. In most experiments this same concentration of hexamethonium also blocked the effects of a dose of acetylcholine 10 times larger than the threshold. An example of this is seen in Fig. 9, made from a preparation containing a single active fibre. In many experiments even an acetylcholine dose 100 times the threshold was blocked by this minimum effective hexamethonium concentration; when the latter was increased ten times an injection of 1 % acetylcholine solution often failed to excite.

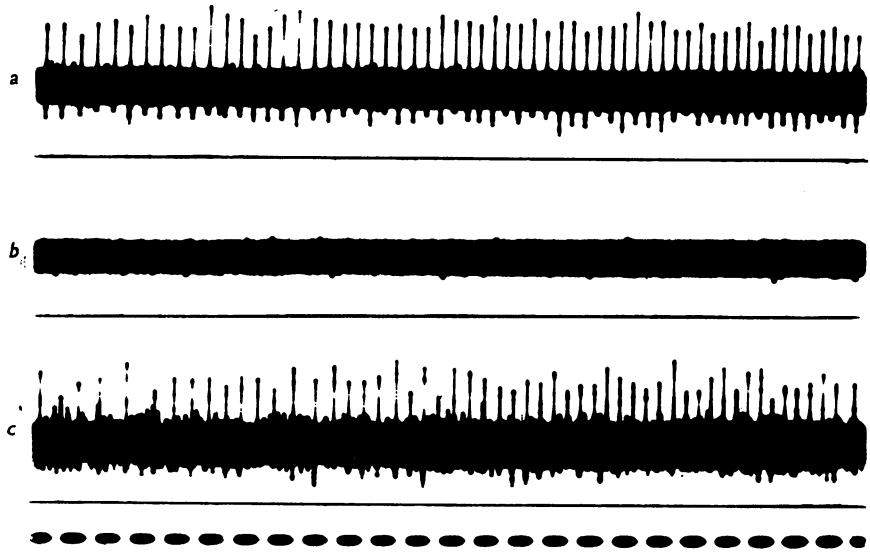

Fig. 9. Impulses from single sensory unit. Intrasinusal pressure in (*a*) (*b*) and (*c*) 60 mm Hg. In (*b*) perfusion fluid contained hexamethonium 10^{-6} g/ml. Flow 10 ml./min. (*a*) 9 sec after injection of 1·0 ml. 10^{-4} g/ml. acetylcholine solution; (*b*) 9 sec after injection of 1·0 ml. 10^{-3}g/ml. acetylcholine solution; (*c*) 9 sec after injection of 1·0 ml. 10^{-4} g/ml. acetylcholine solution, after recovery from hexamethonium (see text for explanation), time, $\frac{1}{10}$ sec.

The effects of hexamethonium were extremely difficult to reverse. In the majority of experiments it was necessary to perfuse for at least 30–40 min and often as long as $1\frac{1}{2}$ or more hours with a hexamethonium-free solution before the response to acetylcholine returned. The block did, however, appear to be fully reversible, though the long times involved make a quantitative comparison between the response to a given dose of acetylcholine before hexamethonium and after recovery difficult. Fig. 9*c* is a record of impulses evoked by acetylcholine in a preparation which had been perfused with hexamethonium-free solution for $1\frac{3}{4}$ hr after the block illustrated in *b*. There was an interval of nearly 4 hr between records *a* and *c* in Fig. 9, (*c*) being made some $6\frac{1}{4}$ hr after perfusion was begun.

Tubocurarine. The potency of this blocking agent was similar to that of hexamethonium. An injection of 1 ml. of tubocurarine (10^{-3} g/ml.) abolished the effects of a succeeding injection of acetylcholine at least ten times the threshold dose.

Acetylcholine. In a few experiments a response to a second injection of acetylcholine could not be obtained during a period lasting 1–4 min after the first response. The effect was more obvious when the initial acetylcholine dose was well above threshold and the rate of perfusion low. However, in many experiments even very large doses did not block the effects of a subsequent injection of acetylcholine. The potency of acetylcholine as a blocking agent was thus in no way comparable to that of hexamethonium or tubocurarine in the conditions of the present experiment.

Response to pressure during depression or potentiation of acetylcholine response

The phenomena so far described might occur if the initiation of impulses at a pressure receptor involved chemical transmission such as occurs at autonomic synapses or skeletal neuromuscular junctions. An implication of this hypothesis would be that the part of the sensory pathway stimulated by injected acetylcholine would be identical with that normally excited by acetylcholine, and it would be expected that drugs which greatly modify the effects of externally applied acetylcholine should likewise modify the normal activity of the receptors. The response to pressure has therefore been investigated at a time when the effects of acetylcholine were (*a*) prevented by blocking agents, and (*b*) potentiated by eserine.

Blocking drugs. When the effects of acetylcholine or nicotine were abolished by either hexamethonium, tubocurarine or acetylcholine, the receptors still responded normally to pressure. This is indicated strikingly when the pressure-frequency graphs of a single sensory unit in the presence and in the absence of hexamethonium are compared; the variation between such graphs has never been greater than that usually found between graphs made from the same preparation at different times. This applied even when high concentrations of hexamethonium, e.g. 1%, were present; an injection of 1% acetylcholine solution was always ineffective in the presence of 1% hexamethonium, but it will be seen from Fig. 10 that the responses of a single sensory unit to steady pressures were not altered significantly from the responses obtained in a normal solution or a solution made equally hypertonic by the addition of glucose. There was a tendency for the threshold pressure to be lowered by very high concentrations of hexamethonium, but not by the usual blocking levels (10^{-7}–10^{-4} g/ml.).

Fig. 11 shows the relation between the duration of a maintained pressure and the frequency of impulses from two sensory units in the same preparation. The

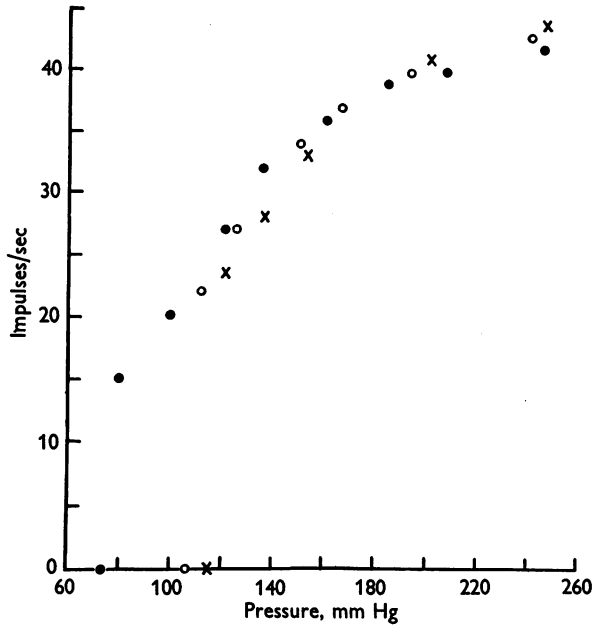

Fig. 10. Relation between pressure and impulse frequency of a single sensory unit. (○), normal perfusion fluid, (●), perfusion fluid contained 1% hexamethonium; (×), perfusion fluid contained extra glucose (see text for explanation).

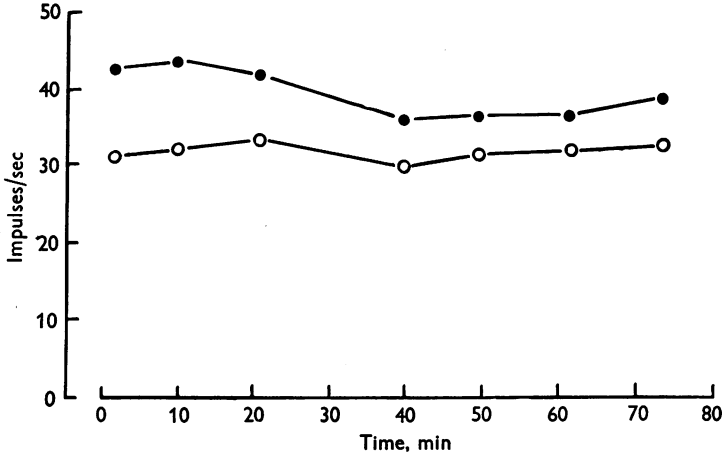

Fig. 11. Relation between duration of a constant pressure and impulse frequency from two sensory units in the same preparation. Perfusion fluid contained 1% hexamethonium. First reading was taken 1½ min after pressure became constant (see text for explanation).

recording was begun after the frequency had reached a constant value and was continued for 1¼ hr; the preparation had already been perfused with 1% hexamethonium for 1 hr. The firing rates at 1½ min and 74 min after the pressure became constant were 42·5 and 38·0/sec respectively for the one sensory unit and 31·0 and 32·0/sec for the other. These frequencies do not differ significantly from those observed at a corresponding pressure before hexamethonium was present. The response of the receptors to steady pressure in the presence of blocking levels of tubocurarine or acetylcholine were qualitatively

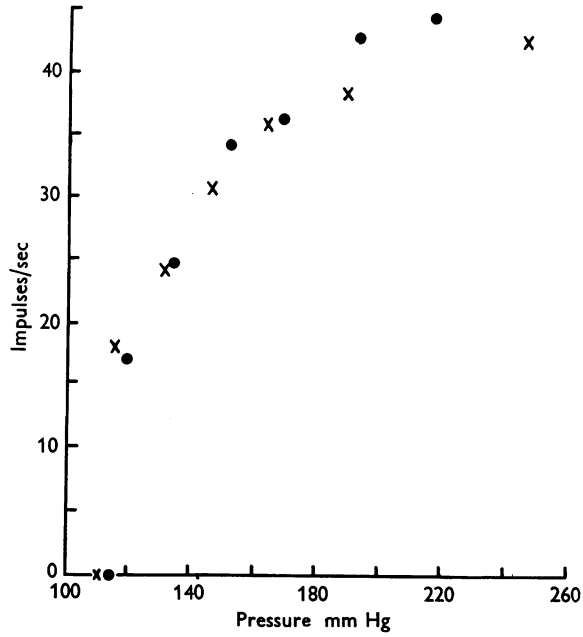

Fig. 12. Relation between pressure and impulse frequency from a single sensory unit. (×), perfusion fluid contained eserine 10^{-5} g/ml.; (●), normal perfusion fluid.

unchanged; moreover, the activity of receptors during a sudden rise of pressure appeared to be unaffected by blocking agents (quantitative measurements were not made).

Atropine. Although the response to acetylcholine was unaffected by atropine the opportunity was taken to investigate the response to pressure in the presence of this drug. It was found that concentrations of atropine which block the muscarine-like effects of acetylcholine elsewhere (10^{-7}–10^{-5} g/ml.) did not detectably alter the pressure-frequency graphs of single sensory units.

Eserine. The presence of this anticholinesterase in the perfusion fluid potentiated the effects of an acetylcholine injection but not those of nicotine.

The most marked effect was on the threshold dose, which was often diminished in the presence of eserine 10^{-5} g/ml. to a tenth or twentieth of that previously required to excite. This concentration of eserine, however, did not appreciably alter the response of the receptors to pressure (Fig. 12). A further indication of the failure of eserine to influence the response to pressure is that an injection of eserine (10^{-3} g/ml.), made during a pressure only slightly below threshold did not induce a response as acetylcholine had done previously, nor did such an injection affect the frequency of impulses in a fibre already discharging at a low rate.

DISCUSSION

These experiments have shown that the injection of acetylcholine or nicotine into the fluid perfusing the isolated carotid sinus causes impulses to appear in fibres from pressure receptors, even when the intrasinusal pressure is as much as 70 mm Hg below the threshold for the steady discharge. The effect is not due to stimulation of the smooth muscle in the sinus wall, because: (*a*) it is unaffected by atropine; (*b*) nicotine, in doses comparable to those used in the present experiments, has no significant effect on arterial smooth muscle; (*c*) the response evoked by adrenaline, which does act by increasing smooth muscle tone, has a considerably greater latency and much slower rate of rise than the response to acetylcholine; and (*d*) acetylcholine dilates systemic arteries, and would be expected therefore to relax the sinus wall and thereby diminish pressure receptor activity, as has been found for the dilator substance $NaNO_2$ (Landgren *et al.* 1952; Landgren, 1952*b*). Nor is the acetylcholine effective because it stimulates local sympathetic synapses, resulting in the release of adrenaline or noradrenaline from the post-ganglionic terminals, with subsequent smooth muscle effects, because (*a*) the responses to acetylcholine and adrenaline are so different, and (*b*) the response to acetylcholine was undiminished when that to adrenaline was blocked by an adrenolytic agent.

It is concluded, therefore, that acetylcholine and nicotine excite impulses in fibres from pressure receptors by a direct action on a peripheral part of the sensory pathway.

It appeared that not all the mass response in a whole sinus nerve could be attributed to pressure receptors. Even when conventional tests had indicated that chemoreceptors were not functioning, the response evoked by acetylcholine or nicotine included many more small spikes than were seen in the response to steady pressures greater than 240 mm Hg; the small spikes usually appeared before and tended to outlast the large ones following an acetylcholine injection, and often less acetylcholine was required to produce them. Furthermore, a discharge of small spikes could often be 'added' by an acetylcholine injection to the steady response to constant pressures of about 250 mm Hg, when it is improbable that there were any pressure receptors not already firing (Landgren, 1952*a*). It is unlikely that these impulses arose in post-ganglionic fibres

from the ganglion cells known to occur in the region. The possibility that the extra impulses occurred in chemoreceptor fibres cannot be discounted. A chemoreceptor-sensitivity to acetylcholine and nicotine is well known, and it seems likely that acetylcholine, and O_2-lack act at different sites (Hollinshead & Sawyer, 1945). It cannot be supposed that the sensitivity to acetylcholine will be abolished when the chemoreceptors no longer respond to O_2-lack.

The amounts of acetylcholine often needed to excite the pressure receptors seem higher than those used to stimulate other sites sensitive to acetylcholine. Here, however, the situation is not comparable to those experiments in which the injection was 'close arterial'. In the present experiments the injected substance must have been considerably more diluted when it arrived at the receptors and the rate of rise of concentration there must have been slower. Moreover, the proportion of the injected acetylcholine destroyed in the tissues before exciting would be greater. Even so in most experiments an injection of 3×10^{-5} g of acetylcholine was quite effective, and as explained earlier a generous estimate of the peak concentration of acetylcholine achieved in the sinus after this injection would be 10^{-5} g/ml., or 37 μmole/l. On this basis many pressure endings have responded to intrasinusal concentrations of acetylcholine of less than 5 μmole/l., in the absence of an anticholinesterase, and to only a tenth or twentieth of this when eserine was present. These concentrations compare favourably with those used by Fatt (1950) to depolarize frog muscle end-plates by immersion in an acetylcholine solution. The relative sensitivity to acetylcholine of the pressure receptors giving rise to large and small spikes has not been assessed, although experiments involving a very few active fibres have shown that both types are excited by acetylcholine.

The mechanism by which acetylcholine produces a discharge at the sensory endings is not known, but it does not appear to be related to the rate of recovery after an impulse or the occurrence of after-potentials, because activity was induced in a unit previously quiescent. It would seem that the relationship between the membrane potential and the critical potential was disturbed. This could occur either from a change in the critical level, as in a calcium-free solution, or by a depolarization. The latter seems the more likely in view of the known actions of acetylcholine elsewhere, and the evidence obtained by Jarrett (1955) from receptors in frog's skin. In either case the process must be regarded as excitation of the sensory ending by acetylcholine. Katz (1950) has shown that the frequency of impulses from the muscle spindle is proportional to the local depolarization; Fatt (1950) found that the depolarization of the end-plate is proportional to the logarithm of the acetylcholine concentration in the working range. It is of interest, therefore, that the frequency of impulses from single pressure receptors was linearly related to the logarithm of the acetylcholine dose, a result supporting the hypothesis that acetylcholine acts by depolarizing the terminal membrane. The fact that acetylcholine sometimes

blocked the effects of subsequent injections of acetylcholine might suggest a depolarization block, which would appear incompatible with the observation that pressure still excited impulses. However, acetylcholine has been shown to block at the motor end-plate even after repolarization (Thesleff, 1955). Such a block might occur both at the pressure receptors and the mechanoreceptors studied by Brown & Gray (1948). The evidence suggests that summation occurs between the effects of mechanical and chemical stimuli applied together; a dose of acetylcholine which was ineffective when the intrasinusal pressure was low often excited when the pressure was brought near to the threshold for the particular sensory unit, and at a given pressure the frequency was increased by acetylcholine. The former may have depended in part on a greater circulation of fluid in the sinus wall resulting from the increased pressure, but it seems more likely that these results are due to the summation of effects at the sensory endings.

The range of sensory endings which respond to acetylcholine or nicotine is now known to be considerable. As well as the sinus pressure receptors and carotid body chemoreceptors are receptors responding to touch (Brown & Gray, 1948; Douglas & Gray, 1953); stretch (Wiersma *et al.* 1953); temperature change (Dodt *et al.* 1953); and probably also nociceptive stimuli (Skouby,1951; Armstrong *et al.* 1953). The pharmacological properties of some of these sensory endings have given rise to the suggestion that some form of cholinergic synapse may exist at sensory receptors (Landgren, Liljestrand & Zotterman, 1954; Hellauer, 1950). This suggestion relies mainly upon comparisons between the responses of sensory endings and those of autonomic ganglia. Examination of the pharmacological differences between the two sites indicates, however, that the evidence opposes such an interpretation, at least as applied to certain sensory endings (Brown & Gray, 1948; Douglas & Gray, 1953; Douglas, 1952). The present results from the sinus pressure receptors support the conclusions of the latter authors. The constant impulse frequency of single sensory units during steady pressure was not altered when excitation by acetylcholine or nicotine was prevented by hexamethonium or tubocurarine, even when the concentration of hexamethonium in the perfusion fluid was raised to 1% and maintained thus for hours. The responses during sudden pressure jumps appeared also to be unaffected by blocking agents. At autonomic ganglia and motor end-plates the abolition of the response to externally applied acetylcholine is always accompanied by severe impairment or complete cessation of function. Furthermore, eserine, in concentrations that potentiated the effects of injected acetylcholine, had no detectable effect on the response to pressure. Similar or smaller concentrations of eserine at sites where acetylcholine is known to have a physiological role invariably produce profound modification of function. It must be concluded that chemical transmission, analogous to that occurring at autonomic synapses and skeletal neuromuscular junctions, is

not involved in the initiation of the response of the pressure receptors to mechanical stimulation.

Broadly speaking there are two parts of the sensory pathway where the excitation by acetylcholine might occur; the nerve fibre throughout its length and the sensory ending. The evidence, as reviewed by Brown & Gray (1948), indicates that acetylcholine does not excite either peripheral nerve fibres or preganglionic terminals, which are morphologically similar to sensory terminals. It seems therefore that the sensory ending is specialized both with regard to the specific stimulus and to acetylcholine. There is good evidence from certain mechanoreceptors that the mechanical stimulus sets up a non-propagated potential at the sensory terminals (Katz, 1950; Alvarez-Buylla & de Arellano, 1953; Gray & Sato, 1953). Such receptor potentials may occur at all sensory terminals during normal activity and the acetylcholine sensitivity may be related to those areas across which receptor potentials are generated and which are probably specialized to this end. Such an hypothesis would account for the diversity of sensory receptors which appear to be sensitive to acetylcholine. Whether or not the mechanism acted on by acetylcholine is related to that 'triggered' by the specific sensory stimulus remains to be seen. The demonstration by Bülbring (1954) that smooth muscle is depolarized by both acetylcholine and mechanical stimulation is of interest in this connexion.

SUMMARY

1. A method is described whereby the behaviour of pressure receptors in an isolated and perfused carotid sinus of a cat can be studied under controlled conditions.

2. Under these conditions the receptors have remained active for more than 11 hr. Their response to pressure was the same as those in blood-perfused carotid sinuses *in situ*.

3. The impulse frequency of single sensory units became constant within $1\frac{1}{2}$ min of producing a constant intrasinusal pressure and remained unchanged or altered only slightly for as long as the pressure was maintained; this sometimes exceeded 1 hr. The receptors have been studied during this state of equilibrium only.

4. Reduction of temperature increased the threshold pressure and flattened the pressure-frequency curve of a single sensory unit. At room temperature most receptors did not respond to steady pressure. These effects were reversible.

5. An injection of 0·5–1·0 ml. of 10^{-6} g/ml. or stronger solution of acetylcholine or nicotine initiated a discharge of impulses in fibres from pressure receptors and increased the impulse frequency in fibres already active.

The total number of impulses induced in a single fibre and the maximum frequency of this response were linearly related to the logarithm of the dose of

acetylcholine; the latency of the response and the time to maximum response were inversely related to the logarithm of the dose of acetylcholine.

6. The results of various controls indicate that acetylcholine excites impulses by a direct action on the peripheral sensory pathway.

7. The excitation by acetylcholine or nicotine was blocked when tubocurarine or hexamethonium was present in concentrations of 10^{-7}–10^{-5} g/ml. The minimum effective dose of acetylcholine was considerably reduced in the presence of eserine 10^{-5} g/ml.

8. When acetylcholine excitation was abolished or potentiated by these drugs the response of single sensory units to steady pressure, as indicated by pressure-frequency graphs and the stability of the impulse frequency, was not altered. This applied even in the presence of 1% hexamethonium.

9. The evidence indicates that chemical transmission, analogous to that occurring at autonomic synapses and skeletal neuromuscular junctions, is not involved in the initiation of the response of the pressure receptors to mechanical stimulation. It seems that the sensory terminal is specialized both with regard to the specific stimulus and to acetylcholine excitation. The possible significance of this is discussed.

I am indebted to Dr J. A. B. Gray for his stimulating discussion of both the work and the manuscript. Prof. G. L. Brown, F.R.S., has a been source of helpful advice throughout.

The histological identification of carotid body tissue was made by Mr A. Howe, to whom I am most grateful. Miss B. Williams provided invaluable technical assistance.

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