J. Physiol. (1964), **175**, *pp.* 321–357
With 18 *text-figures*
Printed in Great Britain

ACTIVITY OF SINGLE EFFERENT FIBRES IN THE CERVICAL VAGUS NERVE OF THE DOG, WITH SPECIAL REFERENCE TO POSSIBLE CARDIO-INHIBITORY FIBRES

BY D. L. JEWETT*

From the University Laboratory of Physiology, Oxford, and the Cardiovascular Research Institute, University of California Medical School, San Francisco, California, U.S.A.

(*Received* 24 *June* 1963)

In spite of the great elegance and accuracy of single-fibre recording techniques in the analysis of afferent nerve impulses, the techniques have been applied to efferent vagal discharge only in recent years (Green & Neil, 1955; Vinogradova, 1955; Andrew, 1956; Widdicombe, 1961*a*, *b*, 1962; Eyzaguirre & Taylor, 1963). None of these studies has analysed the variety of fibres that can be found in the cervical vagus by nerve-splitting techniques.

This study was motivated by an interest in the pattern of activity of single cardio-inhibitory fibres. Although it may be desirable to record from such fibres in the intrathoracic cardiac nerves, the technical difficulties of recording are overcome more easily by preparing single fibres from the cervical vagus nerve, as in this study. This method, however, increases the number of different modalities of efferent fibres that are likely to be met. This paper deals with all the different 'types' of fibre found during the study. Roman numerals have been used to designate the 'types'.

There have been no published studies of single-fibre cardio-inhibitory activity in the dog. In the cat, Okada, Okamoto & Nisida (1961*a*, *b*) have recorded steady firing and pulse-modulated activity in single fibres of the cardiac branches of the vagus nerve. Other recordings from vagal cardiac nerves have been of multifibre preparations (Rijlant, 1936*a*, *b*; Marguth, Raule & Schaefer, 1951; Green, 1959; Weidinger, Hetzel & Schaefer, 1962).

A preliminary report of part of this work has been published (Jewett, 1962).

METHODS

The experimental animals were seventy mongrel dogs, of either sex, weighing between 5·5 and 18·5 kg. The usual anaesthetic was morphine sulphate (1 mg/kg body wt. subcutaneously) followed after an hour by chloralose (Roche, Kuhlmann, or Rossiger, 100 mg/kg

* Present address: Department of Physiology, University of California Medical Center, San Francisco, California 94122, U.S.A.

body wt. intravenously) as a warm 1 or 2% solution in 0·9% or 0·75% NaCl (wt./vol.) Supplemental doses were given as needed to prevent spontaneous movements or shivering chloralose up to 35 mg/kg/hr and morphine up to 0·125 mg/kg/hr. It was found necessary to empty the urinary bladder by suprapubic puncture every 4–5 hr to reduce shivering. Other anaesthetics used were (1) a mixture of chloralose, 25 mg/ml., and urethane, 250 mg/ml. (2 ml./kg body wt. intravenously), with or without preceding morphine; (2) pentobarbitone sodium (30 mg/kg body wt., intravenously; one animal).

With the animal supine the trachea was cannulated towards the lungs and blocked by a plug towards the larynx. The carotid sheath was removed from the artery and vagus nerve without preservation of any baroreceptor areas which might lie along the artery (Boss & Green, 1956). When necessary, one or both superior thyroid arteries were cannulated with fine polyethylene tubing so that injections could be made into the common carotid artery. Usually the pre-tracheal musculature on the right side was excised to allow more space for the dissecting platform. A pool of liquid paraffin (sp.gr. 0·850) was enclosed by supported skin flaps, and although it was usually unheated, it remained within 2° C below the animal's rectal temperature, which was usually 36–38° C.

Beneath the surface of the paraffin the very thick vagal sheath was removed for a length of about 1·5 cm about midway between the larynx and the sternum by gently tearing small strands of sheath between jeweller's forceps or by cutting with iridectomy scissors, under a 10 × binocular microscope. The cleaned nerve was partially cut with iridectomy scissors and the group of fibres thus cut was teased about 1 cm cephalad. The group was divided by sharpened needles or bits of razor blade, until strands which showed single unit activity were obtained. The bulk of the vagus was usually intact so that reflex changes in heart rate could still occur. It seemed somewhat easier to find cardio-inhibitory fibres on the dorso-medial aspect of the nerve and in the right nerve as compared with the left. Preliminary identification of Type I fibres by means of the loud-speaker system was made relatively easy by the regular absence of activity during inspiration.

For recordings from the right cardiac branches, the chest was opened in the right 4th inter-space and the animal maintained on positive pressure ventilation with a Starling 'Ideal' pump, the stroke volume of which was set so that the animal made moderate respiratory movements. The right upper lobe of the lung was removed, the azygos vein divided between ligatures, and the superior vena cava reflected gently until a cardiac nerve branch was found, which was then cut and freed cephalad from surrounding tissue. The end of the central segment was placed in a small liquid paraffin pool in a trough containing a black plastic dissecting platform. The sheath was removed and the fibres divided with bits of razor blades. The right stellate ganglion was ligated, or the sympathetic cardiac nerves were cut and recorded from. In these experiments, no liquid paraffin pool was made in the neck.

Recording electrodes were AgCl-coated Ag wire, or Pt, of 0·01 or 0·025 in. diameter, positioned by Prior manipulators. Since the experiments were carried out in three different laboratories, a variety of equipment was used. Up to four separate channels could be recorded, either on 35 mm film photographing a dual-beam Tektronix 551 oscilloscope with two two-channel plug-in amplifiers, or on 70 mm paper photographing two dual-beam oscilloscopes. Electrode potentials were amplified initially by battery powered a.c. differential amplifiers, Grass P5R, Tektronix 122 or Ediswan. Usually one side of the differential input was grounded near the point of recording. Filters attenuated the signal to half value at 1 kc and to more than half value at 50 c/s. A loudspeaker and audio amplifier monitored the action potential channel.

As many as three pressure channels could be recorded simultaneously, by use of either Southern instruments capacitance manometers or Statham resistance manometers (the manometer resistance was one limb of a bridge balance which was energized by the square wave calibrator of the oscilloscope and amplified by an a.c. amplifier; this system, designed by S. Elliott and used in the first six experiments, gives the pressure reading as the distance

between the resulting two traces). Aortic arch blood pressure was obtained by feeding a long nylon or polyethylene catheter up a femoral artery; the catheter was filled with heparin (10 i.u./ml.) in 0·9 % NaCl solution. Since blood pressure recordings were used only for timing and approximate blood pressure, the frequency characteristics of the system were not measured or controlled. Respiration was recorded in one of several ways: (1) intra-oesophageal (intra-thoracic) pressure changes (air or water-filled catheter, heavily damped); (2) chest wall movements causing pressure changes in a pneumograph; (3) action potentials from a root or the whole trunk of the phrenic nerve; (4) diaphragmatic electromyograms recorded with a unipolar concentric needle electrode inserted through a high abdominal mid-line incision. Intra-tracheal pressure indicated lung excursions when a respiratory pump was used, or when lung inflations were performed. When the electrocardiogram was recorded, subcutaneous needles were inserted in the forelimbs.

Additional anaesthetics and the following drugs were injected through a femoral vein catheter: adrenaline chloride, noradrenaline bitartrate, succinylcholine chloride. Amyl nitrite was given by holding a crushed 5-minim vial to the tracheal cannula or the respiratory pump inlet. KCN and nicotine hydrogen tartrate were injected into the retrograde superior thyroid artery catheter; the volume injected varied from 0·25 to 1·5 ml. (followed by 0·25–0·5 ml. of 0·9 % NaCl to flush the catheter). The responses were most consistent when the dose was given in the smaller volume.

Lung inflations were carried out either by blowing into a tube attached to the tracheal cannula or by the use of a large calibrated syringe attached to the tube. When gas mixtures were used, the tubing was flushed repeatedly before the test inflation. The lungs were denervated by cutting the right vagus at the level of the azygos vein and the left at the level of the aortic arch (see Mizeres, 1957).

Mechanical stimulation of the laryngeal mucosa for 1–3 sec was accomplished by passing a small plastic catheter through a larger catheter, which in turn passed through the plug which blocked the trachea towards the larynx.

Isolated carotid-sinus preparations were formed by tying off, under a 10 × binocular microscope, all arteries distal to the sinus and cannulating the common carotid artery proximal to the sinus. Pressure was applied by a small syringe operated manually or by a pressurized bottle. Intrasinal pressure was measured through a catheter inserted in the external carotid artery. No attempt was made to preserve the venous drainage of the carotid bodies or to oxygenate the perfusing fluid (0·75 % NaCl). Baroreceptor activity was still present for several hours as was shown by slowing of the heart rate and a fall in blood pressure when pressure in the sinus was increased.

Rapid infusions (20 ml. in 4–5 sec) of normal saline, dextran, or heparinized autologous blood were given through a polyethylene catheter threaded down the right jugular vein as far as the superior vena cava. A thermometer which was read immediately following the infusion recorded the temperature of the fluid just before it entered the catheter; the temperature was always at or slightly higher than the rectal temperature of the animal.

Activity in small multifibre groups was recorded either when it seemed impracticable to divide further a bundle of fibres or when what appeared at the time of recording to be a single fibre was found upon examination of the records to have been two or three fibres of similar spike heights. A record was considered to show single fibre activity when the spike heights did not vary by more than the noise level and the impulses were not irregular in pattern (as occurs when activity in two fibres is recorded simultaneously). Control records were always taken immediately preceding any tests. When the firing rates of single fibres were examined, the number of impulses occurring within the full second containing the most impulses was noted; this method was adopted because the firing rates varied considerably with respiration, to such an extent that averages taken over longer periods would be influenced unduly by changes in respiration.

Conduction velocities were computed on the basis of the time from the start of the stimulus

artifact to the start of the impulse and the measurement of the *in situ* conduction distance only when the shape of the electrically activated impulse was clearly the same as that of the spontaneous impulses when viewed on a fast oscilloscope sweep.

RESULTS

Activity of Type I fibres

Type I fibres are thought to be cardio-inhibitory primarily because changes in their activity were opposite from those of the heart rate when the heart rate was altered reflexly. This inverse relation was consistent and was present even at unexpected times; e.g. on rare occasions when the heart rate did not change upon release of occluded common carotid arteries, upon stimulation of chemoreceptors, with lung inflations, or with hyperventilation, the fibre activity did not change. Only in the following circumstances did the inverse relation between heart rate and Type I activity not occur: with high heart rates the fibre activity might be decreased during inspiration without measureable change in the heart rate; in four fibres, following chemoreceptor stimulation in animals breathing spontaneously, there was a small change in either heart rate or fibre activity without change in the other.

It was not possible to study all the fibres under all the experimental conditions; however, of fifty-two single fibres, twenty (four of them in the cardiac branches) each had all of the following characteristics taken to define Type I activity (Table 1): pulse modulation and changes in activity inverse to heart rate changes during respiration and after injection of adrenaline, inhalation of amyl nitrite, and release of occluded common carotid arteries. Fibres were included in the Type I classification if they had several features of the group, exhibited no inconsistent responses, and showed variation in activity appropriate to changes in heart rate. The findings from the twenty multifibre preparations recorded supported those from single fibres; they will be described only where necessary.

Since no difference was observed between the activities of Type I fibres on the right (eighteen multi- and forty-three single-fibre preparations) and left (two multi- and five single-fibre preparations) sides, the results of both sides are reported together.

Modulation of activity with respiration. Under morphine-chloralose anaesthesia, dogs often show a conspicuous sinus arrhythmia; in these experiments the heart rate increased during the inspiratory movement of the animal, and, at the same time, the activity of Type I fibres was either completely absent (Figs. 1, 7, 8) or decreased (two fibres). Similarly, the activity of all fibres in thirteen of twenty multifibre preparations was completely absent during inspiration. Heart rate and fibre activity variations were always associated with the inspiratory efforts of the animal,

whether the chest was closed or open, whether the lungs were inflated by positive pressure with the chest opened or closed, after both cervical vagi had been sectioned below the point of recording, and after the animal had been completely paralysed by succinylcholine.

TABLE 1. Summary of results of tests defining Type I activity

	Cervical vagus*	Vagal cardiac branches*	Cervical vagus; isolated carotid sinus*
Total number of single fibres	48	4	6
Respiratory inhibition:			
Conspicuous	46/48	3/4	3/6
Slight but present	2/48	—	2/6
None	—	1/4	1/6
Response to adrenaline:			
Increased activity	38/38	4/4	2/3
Unchanged activity	—	—	1/3
Response to amyl nitrite:			
Decreased activity	35/36	4/4	4/4
No change in activity (only small B.P. change)	1/36	—	—
Tested both adrenaline and amyl nitrite	29/48	4/4	3/6
Response to release of occluded common carotid arteries			
Ipsilateral or bilateral release:			
Increased activity	26/32	3/4 (?1/4)	—
No change in activity or heart rate	6/32	—	—
Contralateral release:			
Increased activity	10/12	1/1	1/2
No change in activity (heart slowed)	2/12	—	1/2
Modulation of activity with pulse:			
Conspicuous (change of rate during cardiac cycle)	36/48	4/4	—
Slight but present (usually by graph only)	9/48	—	4/6
None	3/48	—	2/6
Fibres meeting all criteria of Type I	16	4	3 (excl. pulse modulation)

* In this and the following tables, in the fractions: numerator = number of single fibres which possess the given quality; denominator = number of single fibres examined for that quality.

During adrenaline hypertension, Type I fibre activity was increased and the period of silence was shortened (Figs. 1, 7) even when the strength of inspiration, as measured by intra-oesophageal pressure or diaphragmatic electromyogram, appeared the same as in the control records. When both strength of inspiration and fibre activity were increased by chemoreceptor stimulation, the length of the silent period was shorter than in the control record. During intense Type I activity following 30–45 sec of asphyxia, there was very little decrease of activity during inspiration (Fig. 9). Amyl nitrite lengthened the period of silence (Fig. 1); however, the strength of inspiration was increased.

Responses to injections of adrenaline. When 10–50 μg of adrenaline were injected intravenously, the blood pressure rose (often to 250/150 mm Hg), the heart rate fell markedly (often below 50 beats/min, with several ventricular ectopic beats) and each of the thirty-eight single Type I fibres so studied showed increased activity (Figs. 1, 7). The activity began to increase when the blood pressure had risen by as little as 10 mm Hg; the

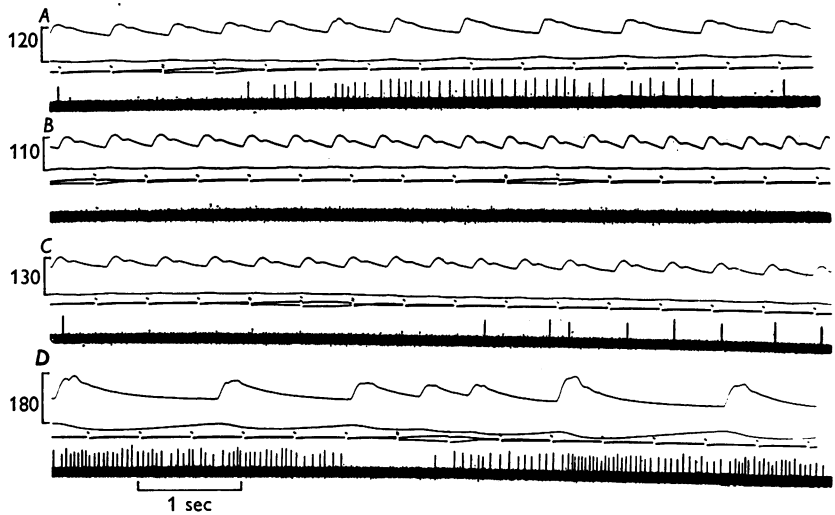

Fig. 1. Activity of a single Type I fibre recorded from the right cervical vagus. In each record, from top down: aortic arch blood pressure (mm Hg); blood pressure base line; chest wall movement by pneumograph (expansion of chest causes formation of double line) with time marker 2/sec; single Type I fibre (retouched). *A*: control. *B*: after amyl nitrite inhalation. *C*: during recovery from amyl nitrite. *D*: after 10 μg adrenaline, I.V. Note: inhibition of activity of various lengths during inspiration (*A*, *C*, *D*); tachycardia and absent activity following amyl nitrite (*B*); bradycardia and increased activity following adrenaline (*D*); increased firing rate in systole compared with diastole (*A*, *D*); eight of the nine impulses in *C* occur during systole.

recruitment of additional fibres was not uncommon. The firing rates of Type I fibres before and after adrenaline are shown in Fig. 2. The shortest inter-impulse time for a single Type I fibre, 10 msec (equal to a rate of 100/sec), was observed following an injection of adrenaline.

During adrenaline hypertension the heart rate was faster during inspiration than in control records (Fig. 1); this may be due partly to a direct cardiac excitation by the injected adrenaline, since the same doses increased the heart rate after atropine or bilateral vagal section.

One fibre recorded after isolation of both carotid sinuses showed decreased activity after injection of adrenaline following division of both vagi, although adrenaline had increased the activity before the division.

Five single fibres were tested with 10–50 μg of noradrenaline, and in each case the effects on heart rate, blood pressure, and fibre activity were similar to those due to the same amounts of adrenaline in the same preparation.

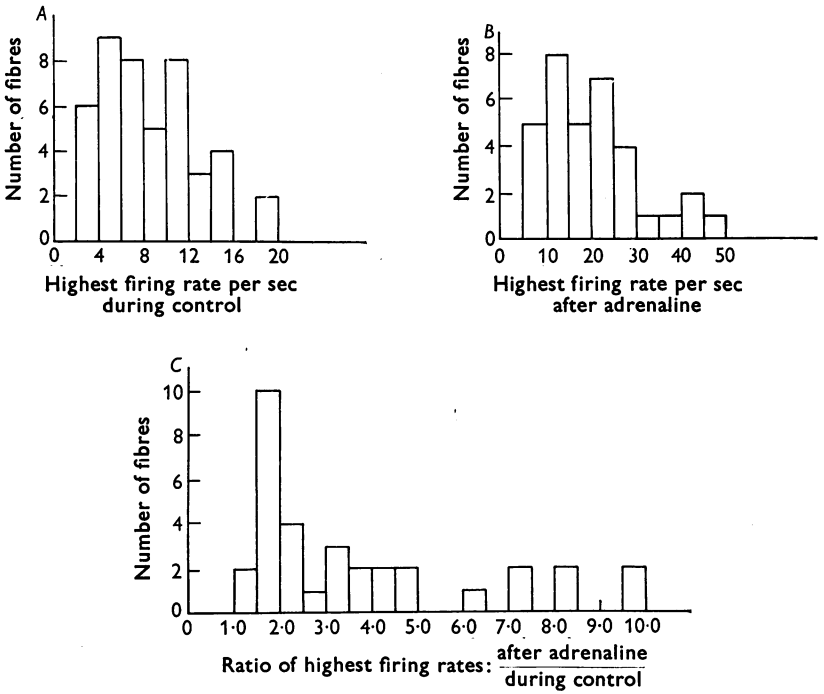

Fig. 2. *A*: Frequency distribution of highest firing rate (grouped in intervals of two) during control period of forty-five Type I single fibres in the cervical vagi (mean = 8/sec). *B*: Frequency distribution of highest firing rate (grouped in intervals of five) after adrenaline of 34 Type I single fibres in the cervical vagi (mean = 20/sec). *C*: Frequency distribution of increase in firing rate relative to control firing rate of thirty-three Type I single fibres recorded from the cervical vagi.

Responses to amyl nitrite inhalation. Amyl nitrite was usually administered until a noticeable effect occurred in the firing rate of the fibre, the systolic pressure being then about 100 mm Hg (Figs. 1, 7); the heart rate was increased and Type I fibres were completely silent (21 fibres) or showed decreased activity (fourteen fibres). One fibre showed no change in activity, but there was little blood pressure change. As the effect of the amyl nitrite wore off gradually over 3–5 min, fibre activity gradually returned to control values (Fig. 1).

Responses to occlusion and release of common carotid arteries. Occlusion of both common carotid arteries caused hypertension, increase in heart

rate, and decrease in Type I activity. Upon release of one (the other still occluded) or both the arteries, the following occurred (in temporal order): an increase in fibre activity, a slowing of the heart, and a fall in blood pressure (Fig. 3). It was not unusual for the fibre activity to return to control values before the heart rate, and both were usually at control levels

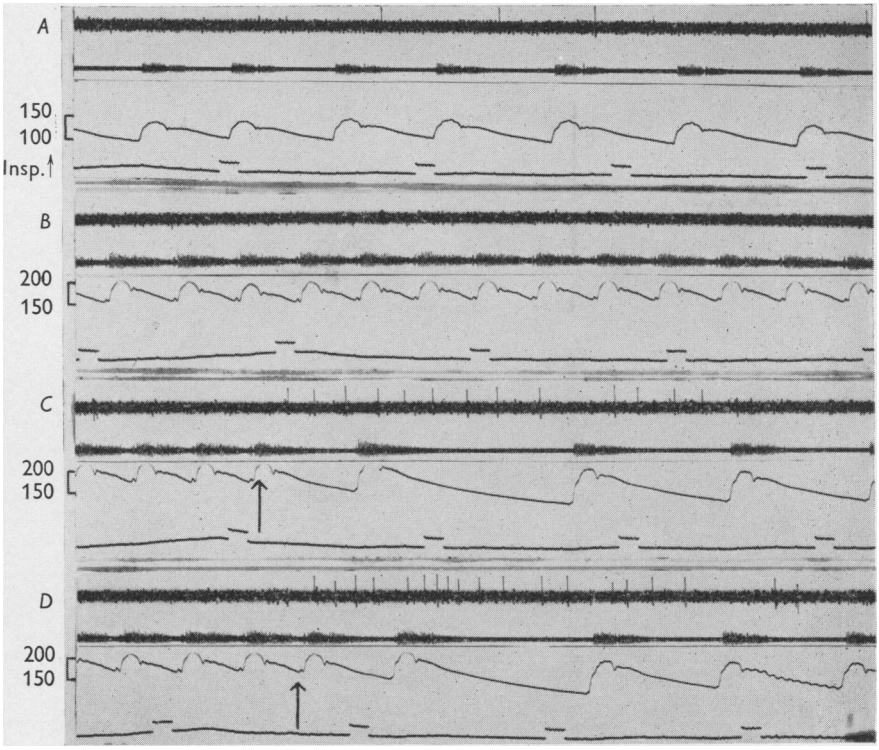

Fig. 3. Effect of bilateral common carotid artery occlusion and ipsilateral or contralateral release on a Type I single fibre. In each record, from top down: single Type I fibre recorded from the left cervical vagus; intact, left depressor nerve; aortic arch blood pressure; intra-oesophageal pressure, inspiration upwards, with time marker 1/sec. *A*: control, *B*: both common carotid arteries occluded, *C*: right (contralateral) common carotid released at arrow, *D*: left (ipsilateral) common carotid released at arrow. Note: complete inhibition of Type I activity and simultaneous tachycardia in *B*; burst of activity and slowing of the heart in *C* and *D*; burst of activity in depressor nerve following dicrotic notch.

following the next inspiration. The carotid arteries were always released at the start of expiration after it was found that the effect on the discharge and heart rate was greatest at that time, when the fibre discharge was at its highest in control runs (Fig. 1); release of the arteries during inspiration resulted in no immediate change in heart rate or fibre activity.

Such 'carotid occlusion tests' were applied to thirty-two single fibres, twenty-six of which showed increased activity upon bilateral or ipsilateral release; the remaining six fibres showed no change in activity, but in these cases there was no change in heart rate either. Of the twelve fibres tested for response to release of the contralateral artery, ten showed increased activity (Fig. 3), while two showed no change, although the heart rate slowed. It is thought that contralateral release increases reflexly the activity of Type I fibres by increased baroreceptor activity from the contralateral sinus rather than by increasing the ipsilateral carotid sinus pressure by collateral artery backflow (Chungcharoen, Daly, Neil & Schweitzer, 1952; Mazzella & Migliaro, 1953) for the following reasons: (1) one single fibre showed increased activity upon contralateral but not upon ipsilateral release; (2) the response from the contralateral side was often similar to that from the ipsilateral side (Fig. 3); (3) with the ipsilateral carotid sinus isolated and maintained at a constant pressure, release of the contralateral occluded common carotid artery caused a burst of activity in one fibre.

Since no attempt was made to release the arteries at the same height of aortic blood pressure or after the same period of occlusion, it was not possible to compare quantitatively the influence of each carotid sinus upon Type I activity (see Wang & Borison, 1947*b*; Scott & Reed, 1955).

Modulation of activity related to the pulse. Of the forty-eight single fibres, forty-five showed a modulation in activity related to the pulse, although it was not always conspicuous, nor was it present at all times. The pulse modulation could be seen in at least one of three ways: (1) there were discrete bursts of activity with each pulse; (2) when single fibre activity was high, there was a faster firing rate in the early part of the cardiac cycle (about 100 msec after the start of the aortic arch pulse wave) compared with the rest of the cycle (Figs. 1*A*, *D*, 7*B*); (3) when single fibre activity was low, the pulse modulation could be demonstrated by histograms of the number of impulses occurring at various times in the cardiac cycle (Figs. 4, 5), thus showing that impulses were more likely to occur in the early part of the cardiac cycle. All but seven of the single fibres showed a peak in at least one of the graphs from the records, although most were not as dramatic as those shown in fig. 4. The peaks in activity for a given fibre often occurred at nearly the same time if the conditions in the animal (blood pressure, respiration, level of anaesthesia, etc.) were similar. With administration of amyl nitrite or adrenaline the times of the peaks sometimes changed (Fig. 5*A*), but without consistent pattern.

All but a few of the peaks occurred between 60 and 240 msec from the start of the aortic arch pulse wave, and in those records analysed in 20 msec intervals a peak was never found at less than 80 msec from the

start of the aortic arch pulse wave. Statistical analysis of the pooled graphs of thirty-one consecutive Type I fibres showed that the difference between impulse counts in the interval 60–239 msec and the interval 0–59 msec was statistically extremely significant (Table 2). At heart rates above 200 beats/min the difference in numbers of impulses in the two intervals was not significant; of the seven fibres not showing a peak in their graphs, three were from dogs with heart rates above 200 beats/min. The number of impulses in the interval 60–239 msec differed significantly from those in the following interval only at heart rates below 167 beats/min (Table 2).

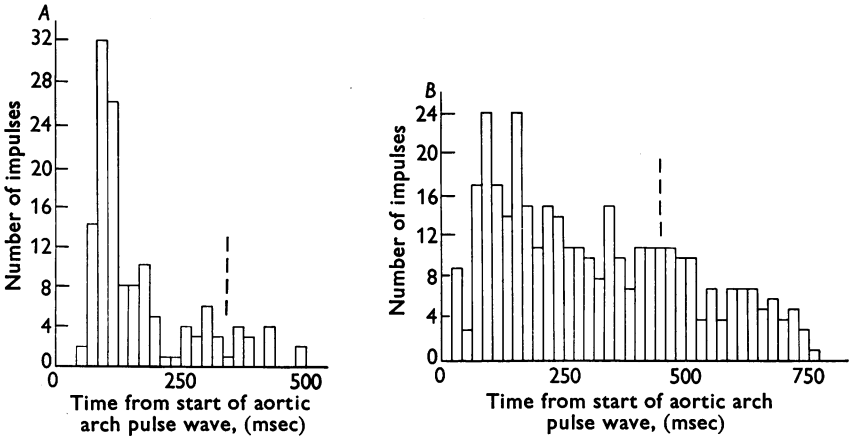

Fig. 4. Temporal distribution of nerve impulses relative to the start of the aortic arch pulse wave. *A*: Single Type I fibre of Fig. 1*C* during recovery from amyl nitrite (223 consecutive cardiac cycles, 105 of which contained nerve impulses). *B*: same fibre as *A*, during control period (Fig. 1*A*; 98 consecutive cardiac cycles, 78 of which contained nerve impulses). The vertical interrupted lines indicate the length of the shortest cardiac cycle containing a nerve impulse; hence, any decrease in numbers of impulses to the right of this line is due partly to the reduced number of cardiac cycles counted. Note: the second smaller peaks, in *A* at 295 msec, in *B* at 300 msec.

About half the graphs with peaks also showed a second smaller peak at about 180–300 msec after the first peak (Figs. 4*A*, *B*, 5*A*, *B*). Of the several possible explanations for these second peaks, three will be mentioned: (1) chance alone, which as a cause was not tested statistically because of the large variability and small size of the second peaks; (2) the increased activity in baroreceptor afferent nerves coinciding with the dicrotic notch (Figs. 3, 18; Bronk & Stella, 1932), which often occurs about 170 msec after the start of the systolic upstroke; (3) a period of lessened excitability in the efferent neurone immediately following the firing of an impulse. Evidence against this last explanation was obtained from two

fibres whose activity was so low that it was possible to graph together impulses from many cardiac cycles containing only a single nerve impulse. Since these graphs showed a second peak (Fig. 5*B*), no preceding impulse was needed to develop the second peak in these instances.

Relationship of cardiac cycle length and antecedent fibre activity. (Results of this and subsequent sections are summarized in Table 3.) If Type I activity is cardio-inhibitory, and if the activity of a single fibre represents the firing of all cardio-inhibitory neurones, then the length of the cardiac cycle should be related to the antecedent Type I activity. Figure 6 shows,

TABLE 2

Length of shortest cardiac cycle containing a nerve impulse, msec	Equivalent heart rate cycles/min	Number of impulses in intervals in cardiac cycle (*A*, *B*, *C*, *D*) Time from start of aortic arch pulse wave (msec) 0–59	60–239	240–359	240–299	χ^2 values Between *A*, *B*	Between *B*, *C* or *B*, *D*
⩾ 360	< 167	581	3047	1500	—	**156**	**83·6**
300 to 359	167 to 200	177	802	—	240	**25·1**	2·05
⩽ 299	> 200	67	260	—	—	3·66	—
Total		825	4109	—	—	**180**	—

Shown here are the numbers of impulses occurring in various intervals (data pooled together from thirty-one consecutive single Type I fibres) in groups according to (for each fibre) the length of the shortest cycle containing a nerve impulse. Interval *B*, in all cases, when compared with another interval contains more impulses than would occur if the impulses were evenly distributed in time, allowing for the different interval lengths; the probabilities that the distributions are due to chance can be determined from the χ^2 values in the right-hand columns. The χ^2 values in heavy type are statistically significant ($P \ll 0{\cdot}001$); $P = 0{\cdot}05$ when $\chi^2 = 3{\cdot}84$; $P = 0{\cdot}01$ when $\chi^2 = 6{\cdot}64$; $P = 0{\cdot}001$ when $\chi^2 = 10{\cdot}8$.

for the fibre of Fig. 1*A*, that the number of impulses in the 2 sec ending 0·3 sec before the start of the aortic arch pulse wave gives a fair indication of the length of the cardiac cycle ended by that pulse wave. (For this fibre, graphs based upon the number of impulses in the 1 or 3 sec period ending at the same time were more widely dispersed.) Of the four other single fibres analysed by this laborious method, two gave similar graphs (one fibre showed least dispersion when the interval 3·3–0·3 sec was used); one rapidly firing fibre gave a widely dispersed graph; one slowly firing fibre (4/sec) showed no relationship whatever.

The time of 0·3 sec used above was derived from experiments similar to those of Brown & Eccles (1934); in four dogs a single shock, applied to the peripheral segment of a cut cervical vagus nerve just below the level

at which efferent activity was recorded, slowed the heart only after a latent period of from 240 to 300 msec, measured to the R wave of the e.c.g. Since the R wave occurred about 60 msec before the start of the aortic arch pulse wave, a single shock to the cervical vagus could not lengthen a given cardiac cycle unless it occurred at least 0·3 sec before the start of the aortic arch pulse wave ending the cycle, the value used in counting impulses as described above. A single shock could slow the heart for several seconds, which justifies counting impulses in the 2–3 sec interval before the end of the cycle.

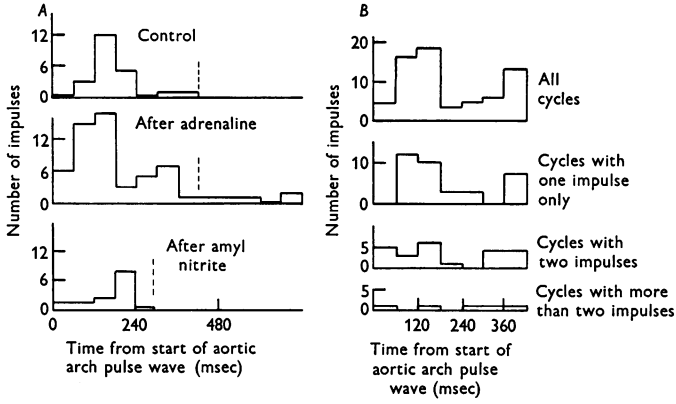

Fig. 5. *A*: Temporal distribution of nerve impulses of the same single Type I fibre relative to the start of the aortic arch pulse wave during a control period, after 50 μg adrenaline, and after amyl nitrite. The interrupted lines have the same significance as in Fig. 4. Note small second peak at 300 msec in adrenaline graph; the number of cardiac cycles counted is different for each graph. *B*: Temporal distribution of nerve impulses of a single Type I fibre relative to the start of the aortic arch pulse wave, graphing all cardiac cycles, then graphing only those cycles containing a single impulse, then graphing only those cycles containing two impulses, etc. Note: second peak at 360 msec is still present when only cycles with one impulse/cycle are counted.

Recordings from the cardiac branches. Successful recordings (four single and six multifibre preparations) from the right cardiac branches of the vagus nerve were obtained in six animals. The activity of these fibres (Fig. 7) was like that of Type I fibres in the cervical vagus; each of the fibres met all the criteria of Type I fibres except as follows: there was no respiratory modulation in an animal that was making no spontaneous respiratory movements; one multifibre preparation showed no pulse modulation; one multifibre preparation was not tested with amyl nitrite.

Conduction velocities were obtained for two of the single fibres in the cardiac branches. One had a conduction velocity of 8·0 m/sec over a 10 cm distance (rectal temp. = 36° C). The other fibre (Fig. 7) had a conduction velocity of 6·2 m/sec over 7·5 cm (rectal temp. = 36·5° C); when the point

of stimulation was moved 3·75 mm craniad, the mean conduction velocity became 7·1 m/sec so that the calculated conduction velocity between the two points of stimulation was 9·5 m/sec.

On three occasions sympathetic multifibre activity in a cardiac branch from the stellate ganglion was recorded simultaneously with Type I activity. The pulse-modulated sympathetic activity varied in direction opposite to that of the Type I activity: increased during diaphragmatic contraction, decreased during adrenaline hypertension, and increased during amyl nitrite hypotension.

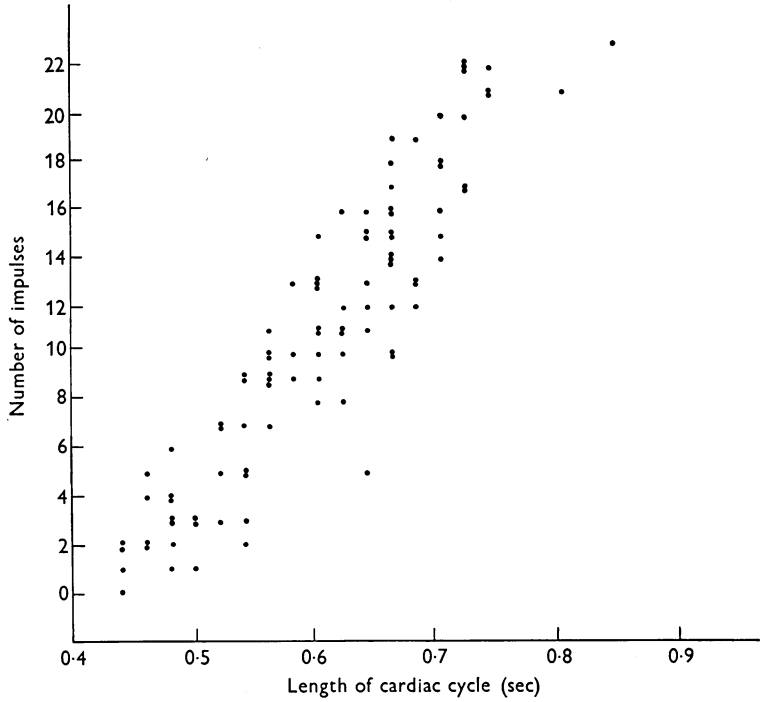

Fig. 6. Relation of the length of a cardiac cycle and the number of impulses in the interval 2·3–0·3 sec before the start of the aortic arch pulse wave ending a given cardiac cycle, during spontaneous respiration with sinus arrhythmia. Taken from the control record of Fig. 1 (95 consecutive cardiac cycles).

Effects of open pneumothorax. Before attempts were made to record from the cardiac branches, a technique which necessitates opening the chest, the effect of open pneumothorax upon Type I activity was determined. This was thought especially important since Tang, Maire & Amassian (1957) have suggested that positive-pressure ventilation and an open pneumothorax reverse the relationship of sympathetic activity and respiration. In two animals, four cervical single fibres showed no qualitative difference

in their activities before and after the chest was opened or re-closed (pneumothorax reduced). Seven other single fibres (four in the cardiac branches), studied only with the chest open, showed no qualitative difference in activity compared with fibres recorded with closed chests.

Effects of changes in ventilation. Activity of Type I fibres was very sensitive to the level of artificial ventilation, whether the chest was open (Fig. 8) or closed. Of thirteen single fibres tested, ten showed the following changes: decreased pump tidal volume caused an increase in activity and

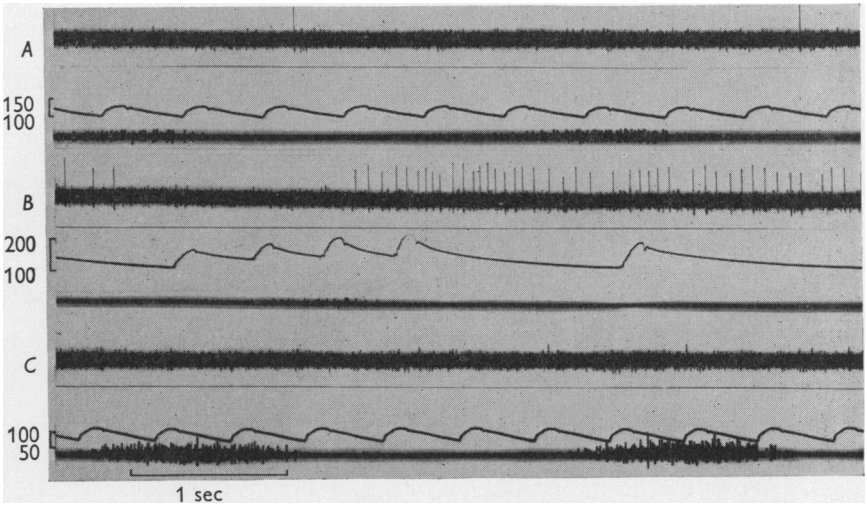

Fig. 7. Responses of a single Type I fibre recorded from a cardiac branch of the right vagus nerve of a 9 kg dog. In each record, from top down: single fibre; aortic arch blood pressure (mm Hg); diaphragmatic electromyogram (retouched). The respiratory pump was set at 19 strokes/min, 175 ml./stroke. *A*: control, *B*: after 50 μg adrenaline, *C*: after amyl nitrite.

respiratory efforts and slowing of the heart; increased pump tidal volume resulted in a decrease or absence of Type I activity and of respiratory efforts, and an increase in heart rate. One fibre showed increased activity with increased pump rate as the heart rate decreased. Two fibres showed no change when the heart rate was unaffected by brief periods of increased pump volume.

Four additional single fibres (described in the next section) were studied in animals temporarily paralysed by succinylcholine; each of these fibres showed increased activity when the artificial respiration was discontinued with the lungs deflated, the heart rate slowing markedly.

Effects of lung inflations. Since Anrep, Pascual & Rössler (1936*a*) and Daly & Scott (1963) have offered good evidence that inflation of the lungs can reflexly increase heart rate, various methods were attempted to demon-

strate clearly an effect of inflation on Type I discharge. The following methods were unsuccessful: (1) brief inflations (2–5 sec) by blowing into the tracheal cannula had no effect upon heart rate or Type I activity; (2) when the chest was closed, sustained inflations (up to 30 sec) by a syringe (up to 20 cm H_2O intra-tracheal pressure) increased heart rate and lowered Type I activity, but the blood pressure was markedly lowered.

TABLE 3. Summary of other tests applied to Type I single fibres

	Cervical vagus	Cardiac branches
Response to changes in artificial ventilation:		
Decreased activity with hyperventilation	10/13	1/1
No change in activity or heart rate	2/13	—
Increased activity with hyperventilation (heart slowed)	1/13	—
Response to lung inflation:		
No change in activity or heart rate (brief inflation)	4/11	1/2
Activity decreased, but B.P. depressed (chest closed)	3/11	—
Activity decreased, no B.P. change (chest open)	4/11	1/2
Response to intra-carotid arterial injection of chemoreceptor stimulants:		
Increased activity (heart slowed) (spontaneous respiration)	11/21	2/3
No change in activity or heart rate (spontaneous respiration)	7/21	1/3
No change in activity, slight heart slowing) (spontaneous respiration)	3/21	—
Increased activity, heart slowing, vagi sectioned or animal paralysed	3/3	—
Division of vagi (activity still present in all cases):		
Opposite vagus only	3/3	—
Both vagi (below point of recording)	6/6	—
Infusions into superior vena cava:		
No heart rate or fibre change	2/2	—
Mechanical stimulation of laryngeal mucosa—no change in activity	3/3	—
Relationship of cardiac cycle length and antecedent impulses:		
Clear	3/5	—
Unclear or none	2/5	—

With each of four single fibres the following method gave a clear result repeatedly: the chest was opened to lessen blood-pressure changes due to the inflation, and the lungs were inflated with air from a syringe (up to 20 cm H_2O intra-tracheal pressure) after the fibre activity had been increased either by injection of adrenaline or by a 30–45 sec period of apnoea with lungs deflated (animal paralysed with succinylcholine). Under these conditions the lung inflation decreased the discharge (Fig. 9), beginning about 60 msec after the start of the inflation and lasting about 3–4 sec. Only one of the four fibres showed sufficient decrease in activity

with inflation to be noticeable over the loudspeaker when the discharge had not been increased by apnoea or adrenaline. Section of the thoracic vagi (see Methods) abolished the decrease in activity with lung inflation for one fibre. For another, although recruitment of other fibres prevented a single fibre analysis after division of the thoracic vagi, the heart rate was no longer increased by the lung inflation, as it had been before division. Two fibres were tested with inflations of 100 % N_2 and one of these fibres was also tested with inflations of a mixture of 8 % CO_2 and 92 % N_2; the decreases in activity were similar, whichever gas mixture was used.

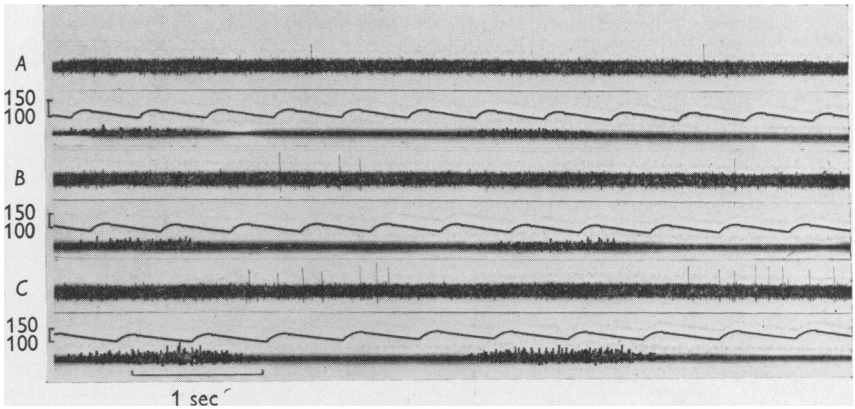

Fig. 8. Effect of changes in ventilation on Type I activity recorded from a cardiac branch of the right vagus nerve of a 9 kg dog. In each record, from top down: single fibre; aortic arch blood pressure (mm Hg); diaphragmatic electromyogram (retouched). This is the same fibre as in Fig. 7. The respiratory pump rate was 19 strokes/min and time was allowed for the activity to adjust to the new level of ventilation. *A*: 175 ml./stroke. *B*: 125 ml./stroke. *C*: 75 ml./stroke.

Effects of chemoreceptor stimulation by drugs. Retrograde injection into the superior thyroid artery of either 50–200 μg KCN or (infrequently) 10–20 μg nicotine caused one or two deep, rapid breaths, and the following effects (none lasting longer than the respiratory stimulation) on the twenty-one single fibres tested: heart rate slowing and increased activity in eleven fibres (Fig. 10), a slight slowing of the heart and no change in three fibres; and no change in either heart rate or activity in seven fibres. In each of three single and two multifibre preparations for which either the animal was paralysed or the cervical vagi were cut, there was increased fibre activity and slowing of the heart following injection, although this did not occur before these interventions. For example, the fibre of Fig. 10, before paralysis (not shown) doubled its firing rate following injection of KCN, although there was no difference in heart rate compared with before the injection, whereas after paralysis the heart rate slowed as the fibre in-

creased its activity (Fig. 10). The firing during inspiration shown in Fig. 10 was very rare; however, the inspiratory inhibition of Type I fibres was always present. Tachycardia was never observed during the brief chemoreceptor stimulation.

Effects of section of the cervical vagosympathetic trunk. Activity in six single fibres was present after section of both vagosympathetic trunks below the point of recording, indicating that these fibres are not sympathetic fibres which ascend the cervical vagosympathetic trunk before

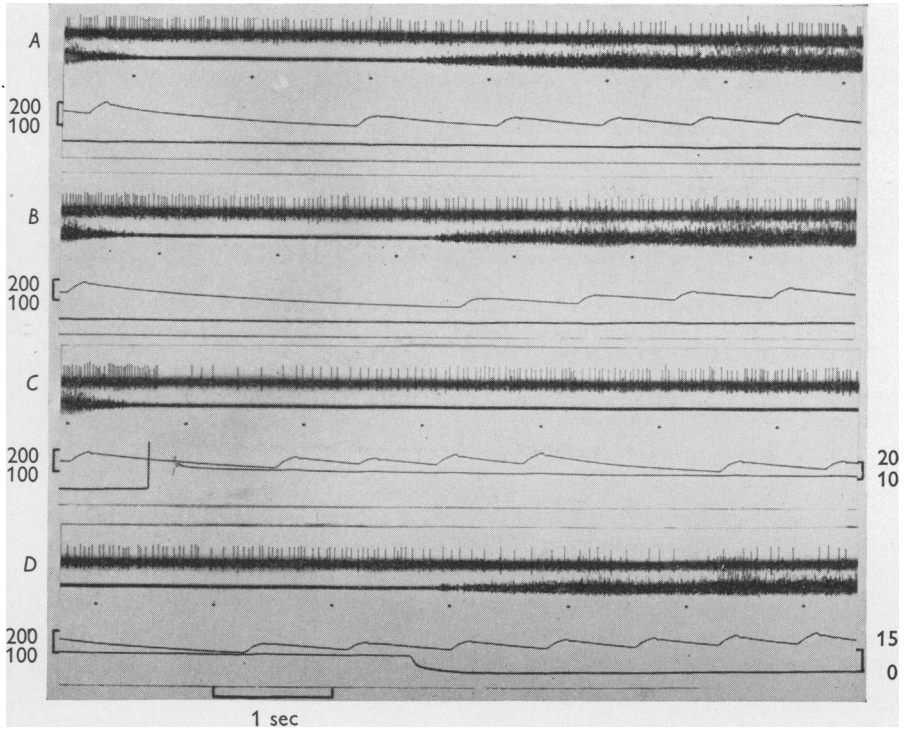

Fig. 9. Effect of lung inflation upon Type I activity during asphyxia. In each record, from top down: single Type I fibre recorded from the cervical vagus; whole phrenic root activity; time marker 1/sec; aortic arch blood pressure (mm Hg); intra-tracheal pressure (cm H_2O), inflation upwards. This is a continuous record. The anaesthetized animal was temporarily paralysed by succinylcholine. The vagi were intact; there was a bilateral open pneumothorax. The respiratory pump was stopped with lungs deflated, 15 sec before the start of the record. In *C*, the onset of a 300 ml. lung inflation is indicated by the retouched upsweep of the intra-tracheal pressure record. Note: the length of phrenic activity is unusual; inhibition of Type I activity during inspiration lessens as asphyxia deepens; inhibition of phrenic activity during inflation; heart rate follows change in Type I activity; the onset of inhibition is so rapid that it easily occurs during the time the aortic valves are closed.

turning downward to innervate the thoracic viscera (Daly & Mount, 1951; Waites, 1957*a*, *b*). Activity in these fibres was still decreased during the inspiration of the animal, indicating that such a decrease is not due solely to a reflex originating in organs innervated by the vagus below the point of section. The activity of five single fibres was recorded before and after section of one or both cervical vagi. Immediately following section, three showed decrease in or absence of activity; sometimes the activity returned

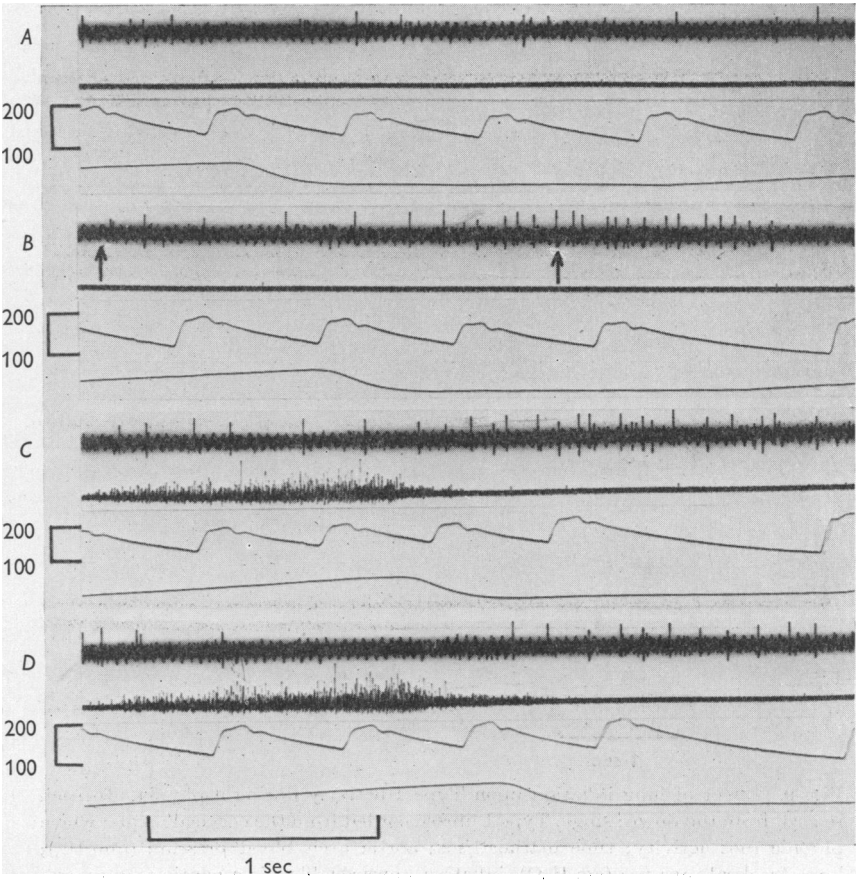

Fig. 10. Effect of intra-carotid arterial KCN on Type I activity in a dog temporarily paralysed by succinylcholine. In each record, from top down: single Type I fibre recorded from the right cervical vagus; phrenic root activity; aortic arch blood pressure; intra-tracheal pressure, inflation upwards. This is a continuous record. The animal was hyperventilated to apnoea. At the first arrow (in *B*), 50 μg KCN injected retrograde into right superior thyroid artery. At second arrow, catheter is flushed. Note: inhibition of increased activity during inspiration; heart rate changes with change in fibre activity (this was not true for this fibre when animal was not paralysed).

slowly; sometimes injections of adrenaline were needed to elicit the activity. The two other fibres showed increased activity following vagal section.

Effects of supplemental doses of anaesthetics. When animals required additional anaesthetic the respiratory and heart rates were higher and Type I activity was less than earlier in the experiment; further doses of anaesthetic at such times reversed these changes. Additional doses of chloralose above that required for anaesthesia did not affect noticeably the discharge or heart rate. Urethane was found useful in reducing spontaneous movements and shivering, but changes in heart rate, blood pressure and fibre activity were less conspicuous when it was used, especially if morphine was not given. The rather large volume of fluid given to the animal to maintain anaesthesia should be noted (see Methods).

Effects of stimulation of the laryngeal mucosa. Since Widdicombe (1964) has used mechanical stimulation of the inferior laryngeal mucus membrane by a plastic catheter as a stimulus causing reflex excitation of some vagal efferent fibres, this stimulus was used in the study of three Type I fibres. No changes in heart rate or fibre activity were found. These results were observed under chloralose-urethane (as used by Widdicombe) because when morphine–chloralose was the anaesthetic the animals did not cough in response to the stimulus.

Effect of superior vena caval infusions. In the cardiac branches of the cat, Okada *et al.* (1961*b*) have recorded single fibres whose activity was increased by infusions. Two Type I fibres were tested with repeated trials of normal saline, dextran, or heparinized blood rapidly infused (20 ml. in 4–5 sec) into the superior vena cava. There was no change in heart rate or the activity of the fibres. The heart rate before infusion was always less than 120/min (morphine-chloralose).

Isolated carotid sinus preparations. In five dogs, six single fibres from the right cervical vagus were studied when one or both carotid sinuses were isolated from the circulation as blind sacs; these fibres were all characterized by an increase in firing rate as the pressure in the isolated carotid sinus was increased, and the recruitment of additional fibres was not uncommon (Fig. 11). While in most respect these fibres were like Type I fibres, none of these fibres has been included in any of the results thus far presented because several were atypical in some respects (Table 1). Pulse modulation was often slight or absent, possibly due to removal of pulsatile afferent discharge from carotid sinus baroreceptors.

Activity of Type II fibres

These fibres showed increased activity during hypotension caused by inhalation of amyl nitrite and decreased activity during hypertension due to intravenous injection of adrenaline (Fig. 12). There was no clear respira-

tory modulation of the activity, but a pulse modulation was sometimes present. The observations made upon these fibres are summarized in Table 4. In addition, hyperventilation increased the activity of the only fibre so tested.

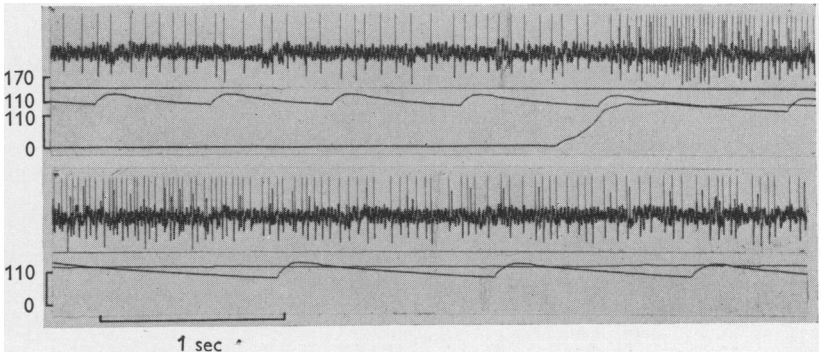

Fig. 11. Effect of increased pressure in an isolated carotid sinus upon activity similar to Type I. In each record, from top down: fibre activity recorded from the right cervical vagus; aortic arch blood pressure (mm Hg); isolated right carotid sinus pressure (mm Hg). This is a continuous record. Pressure in isolated carotid sinus was suddenly raised. Note: slight pulse modulation; immediate increase of firing rate, recruitment, and heart slowing; there was an adaptation of rate of discharge even though the pressure was held constant.

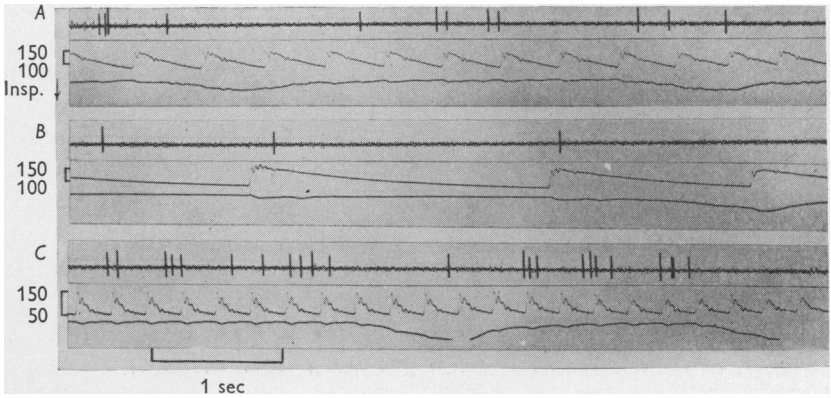

Fig. 12. Effect of adrenaline and amyl nitrite on Type II activity. In each record, from top down: two single fibres of Type II from the right cervical vagus (spikes retouched); aortic arch blood pressure (mm Hg); intra-oesophageal pressure, inspiration downwards. *A*: control. *B*: after 100 μg adrenaline, I.V. *C*: after amyl nitrite.

Activity of Type III fibres

The activity of fibres of this type was extremely characteristic and easy to identify. A rapid, short burst of impulses always coincided with the inspiratory effort of the animal; the activity was increased when the inspiratory effort was increased, e.g. during inspiration against an occluded trachea (as in Fig. 13) or after intra-carotid arterial injection of KCN (Fig. 14). Eight type III fibres were found in the right recurrent laryngeal

TABLE 4. Summary of observations on single fibre types II through VII in the cervical vagus

	Type II	Type III	Type IV	Type V	Type VI	Type VII
Total number...	7	21	3	3	1	24
Respiratory modulation:						
None	7/7	—	—	—	—	18/24
Increased during inspiration	—	21/21	—	—	—	—
Increased during inspiration if B.P. increases; otherwise unchanged	—	—	—	—	1/1	—
Usually silent during inspiration, but fires at times	—	—	1/3	3/3	—	—
Slight decrease in inspiration	—	—	—	—	—	3/24
Slight increase in inspiration	—	—	—	—	—	3/24
Always silent during inspiration	—	—	2/3	—	—	—
Response to adrenaline:						
Activity increased	1/6	—	—	3/3	1/1	2/13
Activity unchanged	—	2/2	—	—	—	10/13
Activity decreased	5/6	—	2/2	—	—	1/13
Response to amyl nitrite:						
Activity increased	5/5	—	—	—	—	1/8
Activity unchanged	—	4/5	1/2	—	—	5/8
Activity decreased (or remains silent)	—	1/5	—	2/2	1/1	2/8
Activity decreased, then increased	—	—	1/2	—	—	—
Response to release of carotid occlusion:						
Activity increased	—	—	—	3/3	1/1	1/6
Activity unchanged	—	2/2	2/2	—	—	4/6
Activity decreased	—	—	—	—	—	1/6
Response to increased pressure in isolated carotid sinus:						
Activity unchanged	—	2/2	—	—	—	2/3
Activity decreased	—	—	—	—	—	1/3
Pulse modulation:						
Obvious (change of rate during cardiac cycle)	2/7	—	—	3/3	1/1	1/24
Slight (by graph)	3/7	—	—	—	—	2/24
None	2/7	21/21	3/3	—	—	21/24
Response to intra-carotid arterial KCN injection:						
Activity increased	—	2/2	—	2/2	—	—
Activity unchanged	—	—	—	—	1/1	6/6

nerve (Fig. 13). Type III activity sometimes appeared and disappeared inexplicably; rarely a fibre was silent during some inspirations but showed the characteristic pattern during others. Rarely a Type III fibre fired continuously or was influenced for some seconds by occlusion of the tracheal cannula (Fig. 13). Figure 14 shows a simultaneous recording of a Type III and a Type IV fibre; the fibres fire reciprocally, never at the same time

even when the respiratory pattern is changed by an intra-carotid arterial injection of KCN. (Figure 14 should be compared with Fig. 6 in Green & Neil, 1955.)

Type III fibres generally had large action potentials and were the most easily found of all the types. They often ran in the same sector of the nerve as Type I fibres so that simultaneous discharges in Type I and Type III fibres were sometimes recorded. The discharge of Type III fibres was usually unaffected by the tests used to characterize Type I fibres (Table 4), although these tests were not frequently applied.

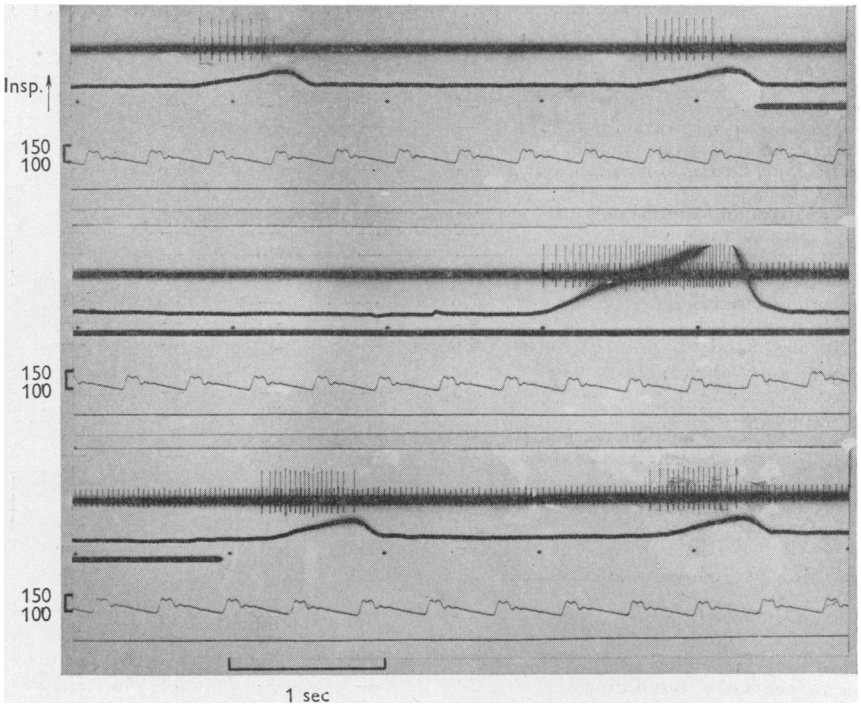

Fig. 13. Type III activity in the recurrent laryngeal nerve. In each record, from top down: two Type III single fibres in the central segment of the cut right recurrent laryngeal nerve; intra-oesophageal pressure, inspiration upwards; time marker 1/sec; signal marker; aortic-arch blood pressure (mm Hg); intra-tracheal pressure (low sensitivity). This is a continuous record. During the signal the tracheal cannula was occluded. Note: the response of the smaller unit is unusual.

Activity of Type IV fibres in the cervical vagus nerve

Type IV activity was characterized by the following: quite regular rate of firing, decreased activity before and during inspiration, fastest firing in early expiration and no pulse modulation (Figs. 14, 15); the activity was liable to vary in intensity or to appear and disappear inexplicably. Type IV

activity alternated with Type III activity (Fig. 14). Although Type IV fibres, like Type I fibres, showed decreased activity during inspiration, they differed in other ways; Fig. 15 shows that activity in a Type IV fibre does not change with release of occluded carotid arteries, is slightly decreased in activity by injection of adrenaline, and has an even firing rate without pulse modulation, whereas the Type I fibres react typically. One 'atypical' Type IV single fibre started firing suddenly and then behaved quite like the two Type IV fibres already illustrated; however, often its discharge was not completely absent until mid-inspiration, and when the dog was artificially ventilated, the fibre sometimes fired during phrenic activity, being in these ways similar to fibres described in the next section.

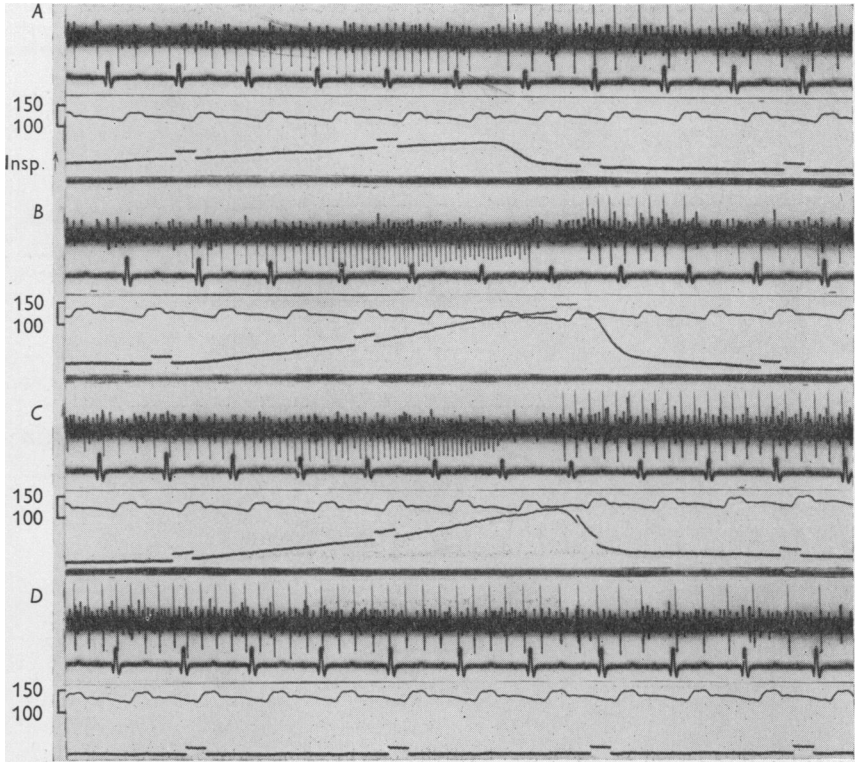

Fig. 14. Simultaneous recording of a single Type III fibre and a single Type IV fibre. In each record, from top down: fibre activity from right cervical vagus, Type III below the line only, Type IV both above and below the line; electrocardiogram, lead I; aortic arch blood pressure (mm Hg); intra-oesophageal pressure, inspiration upwards, with time marker 1/sec. Both vagi are cut below the point of recording. *A*: spontaneous respiration; all Type III activity is shown, but Type IV activity continues at about same rate for 4·5 sec more, ceasing 3·5 sec before the start of next Type III activity. *B, C, D*: continuous record; 3 sec before start, 50 μg KCN injected retrograde into right superior thyroid artery.

Activity of Type IV fibres in the recurrent laryngeal nerve

Since so few Type IV fibres were found in the cervical vagus, they were sought in the recurrent laryngeal nerves of three dogs under morphine-chloralose anaesthesia. The eleven single fibres found which were silent during inspiration were not uniform in their behaviour.

The activity of five fibres (found in two dogs) decreased slightly during adrenaline hypertension. Two of the fibres were not always silent during inspiration; their behaviour was very like that of the 'atypical' Type IV

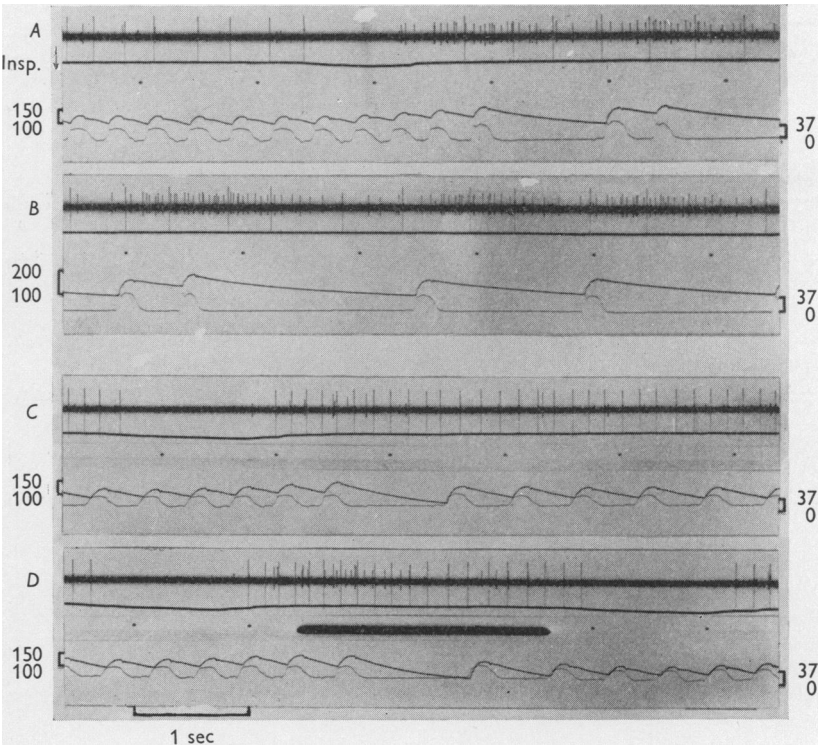

Fig. 15. Effect upon Types IV and I activity of adrenaline and release of occluded carotid arteries. In each record, from top down; simultaneous recording of a single Type IV fibre (large spikes above and below the line) and multifibre Type I activity (smaller spikes); intraoesophageal pressure, inspiration downwards; time marker 1/sec; aortic arch blood pressure (mm Hg); right ventricular pressure (mm Hg). *A*, *B*: continuous record; 20 μg adrenaline I.V. 4 sec before start. *C*, *D*: continuous record; both common carotid arteries occluded before the start and released at start of signal. Note: with adrenaline, increased Type I activity, as Type IV fibre fires more slowly; with release of occluded carotids, increased Type I activity with no change in Type IV activity.

fibre found in the cervical vagus. Two other fibres behaved like the 'typical' Type IV fibres.

Three fibres (found in two dogs) showed no change in activity following injection of adrenaline; all continued to fire until mid-inspiration and all delayed commencement of firing until about 1 sec after the start of expiration. The one fibre tested with intra-carotid arterial injection of KCN responded by firing in both inspiration and expiration, reminiscent of Type V fibres.

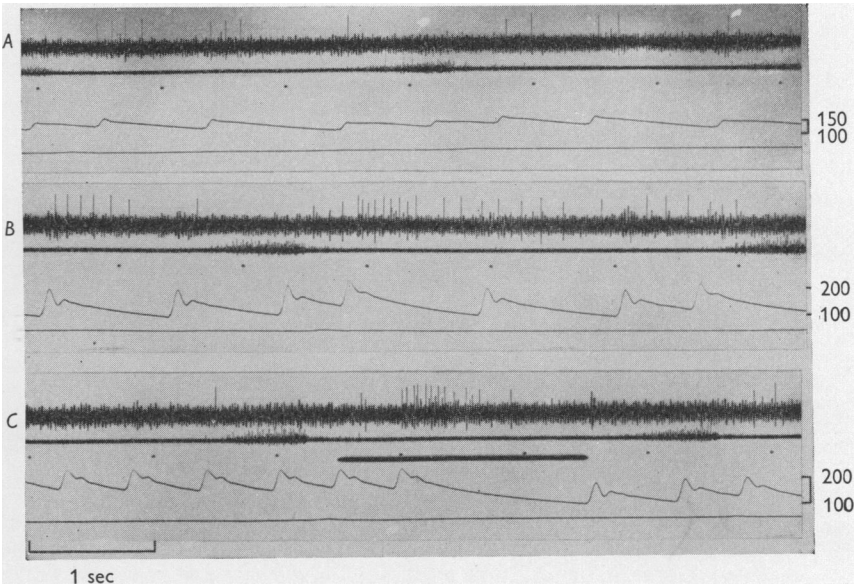

Fig. 16. Effect of adrenaline and release of occluded carotids on one recurrent laryngeal Type IV fibre (similar to Type I). In each record, from top down: single fibre from right recurrent laryngeal nerve; whole right phrenic root; time marker 1/sec; signal marker; aortic arch blood pressure (mm Hg); intra-tracheal pressure (low sensitivity). *A*: control (blood-pressure cannula probably partly clotted). *B*: after 20 μg adrenaline I.V. *C*: occluded common carotid arteries released at start of signal. Note: rather even firing rate, little changed by adrenaline.

Three fibres (found in one animal only) slightly increased their activity after injection of adrenaline (12–15, 13–17, and 5–10 impulses/sec respectively; compare with Fig. 2). Each fibre discharge had a pulse modulation, and each increased upon release of carotid artery occlusion. One fibre could not be distinguished by its responses from Type I fibres on the basis of the features of Type I fibres summarized previously (Fig. 16); this fibre had a widely dispersed graph relating number of impulses to cardiac cycle length, and it seemed to have a more regular discharge than many Type I

fibres. The other two fibres were unlike Type I fibres in that one continued to fire until mid-inspiration and did not fire rapidly in early expiration; the other did not start firing until well after the start of expiration.

Activity of Type V fibres

The activity of the three Type V fibres was similar to that of Type I fibres (Table 4) with the following exceptions: two of the fibres were silent during the control records, being activated by injections of adrenaline or

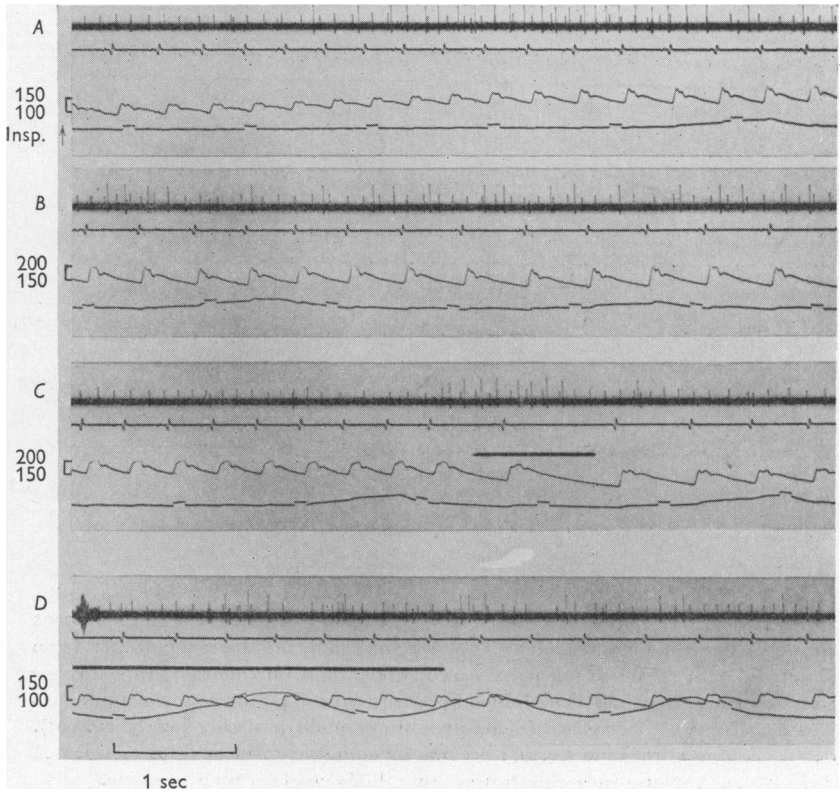

Fig. 17. A single Type V fibre and Type VII fibres as affected by adrenaline, release of occluded carotid arteries, and intra-carotid arterial KCN. In each record, from top down: Type V single fibre (large spikes) and multifibre Type VII activity (small spikes) recorded from the right cervical vagus; electrocardiogram; signal marker; aortic arch blood pressure (mm Hg); intraoesophageal pressure, inspiration upwards, with time marker 1/sec. The left vagus has been cut in the neck. *A*, *B*: continuous record; 40 μg adrenaline, I.V., injected 3 sec before start (the large fibre was silent). *C*: release of occluded common carotid arteries just before signal (large fibre was silent). *D*: starting before start of record and during signal, 100 μg KCN injected retrograde into left superior thyroid artery (large fibre was silent). Note: Type V fibre fires more during inspiration than expiration after KCN; even firing rate of Type V fibre.

KCN and by release of occluded carotid arteries (Fig. 17). The other fibre had a control firing rate of less than 2/sec; such a slow rate was never found in Type I fibres during spontaneous respiration (Fig. 2). At times, usually after intra-carotid arterial injection of KCN, the activity was present during inspiration (Fig. 17). The type V fibres showed a relatively regular firing rate, like the recurrent laryngeal Type IV and some Type I fibres.

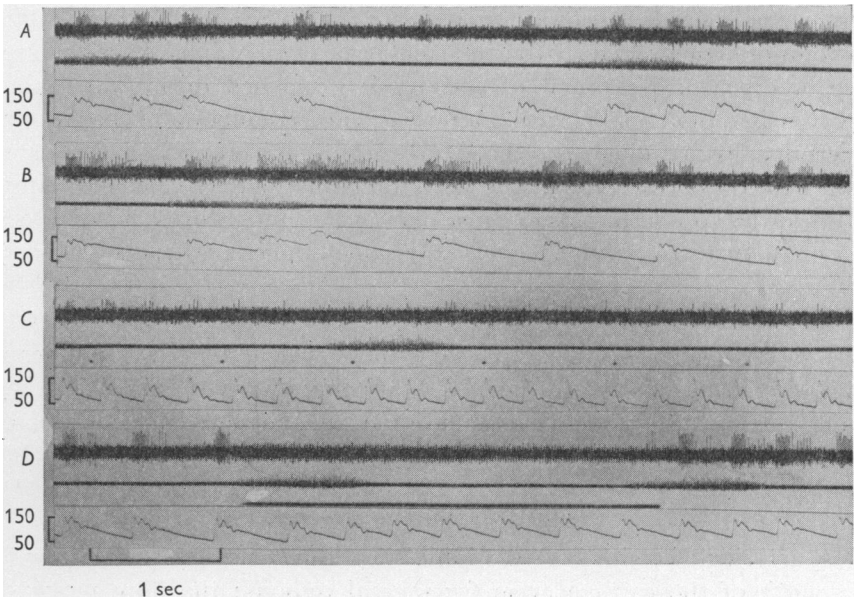

Fig. 18. Type VI activity as affected by adrenaline, amyl nitrite, and occlusion of the ipsilateral common carotid artery. In each record, from top down: single Type VI fibre recorded in the right cervical vagus of a dog, central segment; whole phrenic root activity; time marker 1/sec or signal marker; aortic arch blood pressure (mm Hg). *A*: control. *B*: 15 μg adrenaline given 8 sec before start. *C*: after amyl nitrite. *D*: during signal, right common carotid artery occluded above the superior thyroid artery by 'bulldog' clip.

Activity of Type VI fibre

Only one Type VI fibre was encountered (Fig. 18; Table 4); it behaved like a fibre from a carotid sinus baroreceptor, firing a burst of impulses with each pulse and being silenced immediately by occlusion (above the superior thyroid artery) of the common carotid artery of the same side (Fig. 18), whereas it was unaffected by occlusion or release of the opposite carotid. The activity followed blood pressure, increasing during spontaneous inspiration and adrenaline hypertension, decreasing during inflation of the lungs (chest closed; blood pressure fell) and amyl nitrite hypotension.

Since this fibre seems to be a carotid sinus baroreceptor fibre, it is of interest to compare this activity with efferent Type I activity. In what follows, the Type VI (presumed baroreceptor) activity will be described, and the Type I activity will be indicated in parentheses: The activity is increased during the blood pressure rise accompanying inspiration (activity decreased). The highest firing rate is about 130/sec (40–50/sec, rarely 100/sec), about 40 msec (80–240 msec) after the start of the aortic arch pulse wave. There is a second slower burst of activity about 180 msec after the point of highest activity (second smaller peak on graphs, 180–300 msec after first peak). The burst of activity is prolonged during adrenaline hypertension, but there is within a few seconds a reduction in the amount of activity, even though the blood pressure remains elevated (the same applies to Type I activity). The activity is markedly reduced by amyl nitrite, often to one impulse per cardiac cycle, but returns as the blood pressure returns to previous levels (the same applies to Type I activity).

Activity of Type VII fibres

The fibres placed in Type VII were those which showed insignificant changes in firing relative to the variables being observed (Table 4). They usually fired steadily without modulation during changes in respiration or blood pressure (e.g. small fibre, Fig. 17).

DISCUSSION

The activity of a single cardio-inhibitory fibre may not correlate with the length of the cardiac cycle for several reasons: the fibre may not represent the 'population' of cardio-inhibitory fibres, especially if the rate of single unit firing is not proportional to the total number of impulses owing to differing thresholds and recruitment; temporal and spatial summation can occur at two synapses distal to the point of recording; sympathetic activity is not accounted for; the heart rate may be influenced by direct pressure effects, circulating hormones, drugs and anaesthetics; axon reflexes may occur. In spite of this, Type I activity correlates inversely with the heart rate remarkably well throughout all the tests and observations described in this paper. In addition, the Type I activity shows many features of vagal cardio-inhibition *in the dog*: inhibition of activity by a direct central mechanism (Anrep, Pascual & Rössler, 1936*b*; Schlicher, Peiper, Krug & Bohme, 1959; Koepchen, Wagner & Lux, 1961); inhibition by a lung inflation reflex (Anrep *et al.* 1936*a*; Daly & Scott, 1963); excitation by pressure increases in the carotid sinus (Wang & Borison, 1947*a*; Reed & Scott, 1955), including the contralateral carotid sinus (Scott & Reed, 1955; Wang & Borison, 1947*b*); increased activity from stimulation of carotid chemo-receptors when ventilation is controlled

(Daly & Scott, 1958); the frequency of impulses allows prediction of the heart rate (Warner & Cox, 1962). Type I activity also correlates with that of multifibre recordings in the cardiac branches of the dog, which, in the only previous study, had both pulse and respiratory modulations (Rijland, 1936*a*, *b*). Finally, Type I activity was found in the vagal cardiac branches.

If all Type I fibres are cardio-inhibitory, then it is difficult to explain the finding of one fibre in the recurrent laryngeal nerve which responded to the definitive tests like Type I fibres. The function of this fibre is unknown, but it may be significant that Widdicombe (1964) has found one fibre (no. DL 55) with similar behaviour entering a dog's lung, but differing in that it was sometimes silent during control records and had a delayed response to injections of adrenaline. This fibre (recorded by Widdicombe) and Type IV fibres seem to fire more regularly than do the majority (about 80%) of Type I fibres, but differences in level of anaesthesia, circulatory conditions, ventilation, etc., have not been excluded as causes of this difference. Not all Type I fibres need slow the heart; some may act on vascular beds or the force of the heart beat. The Type V fibres and the fibre in the recurrent laryngeal nerve, together with the atypical fibres found with the isolated carotid sinus preparations in this work, may represent a group of fibres which are not cardio-inhibitory, some of which are at present included in the Type I classification for want of a test which will clearly distinguish them. Although one cannot be sure that any particular single fibre is cardio-inhibitory, in the rest of this discussion I will assume that at least some of the Type I fibres have this function, since their behaviour correlates well with what is known about vagal chronotropic control of the heart, but does not correlate with other demonstrated non-cardiac functions of the vagus nerve, such as control of bronchial smooth muscle or the pulmonary vascular bed, etc.

The inhibition of Type I activity during inspiration was probably a combination of a central action and a reflex, as has been shown by Anrep *et al.* (1936*a*, *b*) for vagal inhibition of the heart, since (1) Type I activity was inhibited when phrenic discharge occurred in paralysed dogs, and (2) inflation of the lungs could be shown to inhibit Type I activity. The central inhibition of cardio-inhibitory activity has been studied by Koepchen *et al.* (1961), whose findings on the time course of the inhibition correlate well with that of Type I fibres during inspiration. The tachycardia and inhibition of Type I activity due to inflations of the lungs correspond quite well with the findings of Anrep *et al.* (1936*a*). They used innervated dog heart-lung preparations under morphine-chloralose and found that cardiac acceleration occurred within one or two heart beats from the start of a lung inflation (up to 20 cm H_2O) but was often superceded by a 'secondary slowing' 5–6 sec after the peak acceleration. Type I

fibres were inhibited in about 60 msec from the start of an inflation, and the inhibition lasted for 3–4 sec. In order to demonstrate the effect of lung inflation upon three of the four fibres so studied, I found it necessary to increase the activity by asphyxia or injection of adrenaline; other authors have also had to use extreme conditions in order to show the effect of inflation on heart rate. Anrep *et al.* (1936*a*) hyperventilated their animals to apnoea and then increased the perfusion pressure to the head until they obtained a slow heart rate; Daly & Scott (1958, 1963) have shown decreased bradycardia due to lung movement, but during intense carotid chemoreceptor stimulation which would otherwise give a slow heart rate; Heymans, Bouckaert & Samaan (1934) have shown that hyperventilation reduces the bradycardia of carotid sinus nerve stimulation. In all four types of experiment the effects are abolished by division of the vagi below the cardiac branches.

Intra-carotid arterial injection of KCN caused a tachypnoea, indicating that chemoreceptors were being stimulated. At times a transient bradycardia occurred. In about half the experiments no change in heart rate occurred when the animals were breathing spontaneously. After the cervical vagi were cut below the point of recording or the animal was paralysed and artificially ventilated, then a decrease of heart rate and an increase in Type I activity occurred. These findings are consistent with the findings of previous workers (see Daly & Scott, 1963; Korner, 1959; Heymans & Neil, 1958).

When pulse modulation of Type I activity occurs during adrenaline hypertension, the highest firing rate occurs about 100 msec after the start of the aortic arch pulse wave. Similarly, when pulse modulation is shown by a graphical technique, then the largest number of impulses occurs no sooner than 80 msec after the start of the aortic arch pulse wave. If we assume that the pulse modulation is due to arterial baroreceptor activity, then there is sufficient time for the impulses to traverse the reflex arc from either the carotid sinus or the aortic arch; the calculation is based upon the following: since the conduction distances are similar, the first carotid sinus baroreceptor impulses might be expected to arrive at the brain at about the same time as those of the Type VI fibre recorded on the central segment of the cut mid-cervical vagus, i.e. about 40 msec after the start of the aortic arch pulse; aortic arch baroreceptor impulses initiated at the start of the pulse wave would arrive at the brain in 17 msec, assuming that the fibres conduct at12 m/sec, the slowest baroreceptor conduction velocity reported by Paintal (1953) in the cat, over a 20 cm distance; conduction time from the brain to the point of recording would take 16 msec, assuming a conduction velocity of 6·2 m/sec (the slower velocity measured in my experiments) over a 10 cm distance. Thus, if no central delay or temporal

summation time is added, conduction from the aortic arch might take 33 msec and from the carotid sinus 56 msec, both considerably less than the 80 msec lag between the start of the aortic arch pulse wave and the increase in discharge of Type I fibres.

The timing of the increase in discharge of Type I fibres relative to the start of the aortic arch pulse wave suggests that baroreceptor impulses from one pulse wave can influence the heart before the next beat at heart rates less than 160 beats/min; the calculation is as follows: addition of 80 msec (the reflex time from the start of the aortic arch pulse wave to the point of recording), 240 msec (the minimum time before the R wave of the e.c.g. for single shocks to the vagus near the point of recording to delay the next beat) and 60 msec (the interval between the R wave and the start of the aortic arch pulse wave) gives a total minimum time of 380 msec, which is equivalent to 160 beats/min. Thus, the heart rate, at values during which vagal activity might be expected to be present, can probably be adjusted on a 'beat-to-beat' basis. It seems less likely that the pulse modulation of sympathetic activity (Bronk, Ferguson, Margaria & Solandt, 1936; Govaerts, 1936*a*, *b*; Okada *et al.* 1961*b*; Downing & Siegal, 1963; this work) also acts to modify the heart on a 'beat-to-beat' basis, since the speeding of the heart due to sympathetic stimulation takes several seconds to occur (Samaan, 1935; Bronk *et al.* 1936; Warner & Cox, 1962), even though sympathetic reflex times are quite rapid (180–400 msec by various methods; Bronk *et al.* 1936; Tang, Maire & Amassian, 1954; Weidinger *et al.* 1962).

The conduction velocities of two single Type I fibres in the vagal cardiac branches were 8·0 and 6·2 m/sec. In the cat, cardio-inhibitory fibres have been reported to conduct at rates between 7·5 and 30 m/sec (Brown & Eccles, 1934; Heinbecker & Bishop, 1935; Middleton, Middleton & Grundfest, 1950). One Type I fibre had a higher conduction velocity in the proximal vagus than in the distal vagus, which has been reported for some single fibres in the vagus nerve (Iggo, 1958; Paintal, 1962). This suggests that the fibres may become smaller as they descend, which might explain the difficulties in demonstrating histologically that there are myelinated efferent fibres in the vagal cardiac branches (Heinbecker, 1931; Heinbecker & O'Leary, 1933; Daly & Evans, 1953; Evans & Murray, 1954; Mizeres, 1955, 1957; Agostoni, Chinnock, Daly & Murray, 1957).

Type I fibres are probably not the same as those reported by Okada *et al.* (1961*b*) since two Type I fibres were not affected by superior vena caval infusions; furthermore, there is a possibility that the fibres reported by these authors are not cardio-inhibitory (see Jewett, 1964).

Type II activity was not investigated extensively. This activity is similar to that in fibres efferent to the lungs and trachea of dogs and cats

and considered to be bronchoconstrictor (Vinogradova, 1955; Widdicombe, 1961*a*, *b*, 1962, 1964). The conflicting reports in the literature concerning the effect of baroreceptors upon bronchomotor reflexes have been reviewed (Widdicombe, 1961*a*, 1963). No obvious respiratory modulation of Type II activity was found, but pulse modulation was present. Widdicombe (1961*a*, 1964) has found that respiratory modulation of bronchoconstrictor fibre activity is variable; no pulse modulation was found by Widdicombe initially (1961*a*), but later studies with a graphical analysis have shown a frequent pulse modulation (1964). Since there are no important differences between activity in Type II fibres in the cervical vagus nerves and 'bronchoconstrictor' fibres entering the lungs and trachea, Type II fibres are considered to be bronchoconstrictor. However, Type II activity has the same qualitative responses to administration of adrenaline and amyl nitrite as sympathetic fibres; thus, these could conceivably be sympathetic fibres which ascend the cervical vagosympathetic trunk before descending to innervate the thoracic viscera (Daly & Mount, 1951; Waites, 1957*a*, *b*).

Since Type III activity was found in the recurrent laryngeal nerve as well as in the cervical vagus, and since the activity is so similar to that recorded by Green & Neil (1955) and Eyzaguirre & Taylor (1963) in the cat, there seems little doubt that these fibres innervate the abductors of the vocal cords.

The type IV fibres recorded from the cervical vagus probably innervated the abductors of the vocal cords since their activity alternated accurately with Type III activity and resembled the activity reported by Green & Neil (1955) to innervate the abductor muscles in the cat. The activity of two of the Type IV fibres was found to decrease slightly as the lungs were rhythmically inflated; Green & Neil (1955) found that lung inflation increased the activity of fibres active in expiration, but this was not confirmed by Eyzaguirre & Taylor (1963). Only three Type IV fibres were recorded from the cervical vagus in my entire study. Eyzaguirre & Taylor (1963) have reported difficulty in recording laryngeal expiratory activity; Green & Neil (1955) found that there was greater electromyographic activity in the abductor muscles than in the adductor muscles in the cat during quiet breathing. The Type IV fibres found in the recurrent laryngeal nerve varied not only in their responses to injections of adrenaline but also in the timing of their activity relative to inspiration. Since the recurrent laryngeal nerve supplies all the intrinsic musculature of the larynx, such differences might be expected in fibres going to different muscles. Entirely unexpected was the finding of one fibre which could not be distinguished from Type I fibres on the basis of the usual tests (see earlier in this discussion).

Type V activity was very similar to Type I activity except that the fibres were silent (or fired very slowly) during the control records (like some Group III fibres in Widdicombe, 1964) and that at times the activity was not silent during inspiration (like some recurrent laryngeal Type IV fibres). Type V fibres may be 'high threshold' Type I fibres, or they may represent an additional fibre type which may be included in the Type I classification. At least this much can be said: the characteristics of Type V fibres which distinguish them from Type I fibres are the characteristics which make them similar to fibres found in non-cardiac branches of the vagus.

Fibres similar to Type VI have been found descending the aortic nerve of the cat by Holmes (1954), Bianconi & Raschi (1959), and Jewett (1964). Possibly the Type VI fibre was also descending amongst the baroreceptors of the right 'aortic' nerve since there was (as is frequent) no separate aortic nerve in the mid-cervical region in this animal. Green (1959) has reported similar activity in a cardiac branch of a cat. The function of these fibres is unknown.

Little can be said about Type VII fibre activity except that the variables significant to their activity were not recorded in this study.

SUMMARY

1. Single fibre activity was recorded on the central segments of small cut strands separated from the cervical vagus of dogs under morphine-chloralose or morphine-chloralose-urethane anaesthesia. Respiratory efforts and aortic-arch blood pressure were recorded simultaneously with the nerve activity. Several 'types' of activity were found; roman numerals were used to designate the 'types'.

2. Type I was considered to be, for the most part, cardio-inhibitory in function because under most conditions changes in the activity were inverse to changes in the heart rate, because the number of single impulses in a preceding 2–3 sec interval was related to the length of a cardiac cycle, and because Type I activity could be found in the vagal cardiac branches. That some Type I fibres are not cardio-inhibitory was suggested by the activity of some similar, but atypical fibres found in the cervical vagus, and the recurrent laryngeal nerve. Forty-eight Type I single fibres were studied in the cervical vagi.

3. Variations in heart rate and Type I activity were most frequently observed during adrenaline hypertension, during amyl nitrite hypotension, following release of occluded carotid arteries of either side, during the respiratory cycle, and following administration of supplemental doses of anaesthetic. The activity was inhibited during the inspiratory efforts of the animal whether the chest was opened or closed, after section of the

vagi below the point of recording, and after complete paralysis with the lungs motionless; the inhibition was opposed by procedures tending to increase Type I activity.

4. Type I activity showed a pulse modulation by either a higher firing rate or a preponderance of impulses early in the cardiac cycle. The shortest reflex time of 80 msec would permit control of the heart rate by baroreceptor reflexes on a 'beat-to-beat' basis.

5. Hyperventilation markedly reduced Type I discharge, probably by both increased lung inflation activity and a decrease in chemoreceptor discharge. Inflation of the lungs with air, 100 % N_2, or 8 % CO_2 + 92 % N_2 caused an inhibition of Type I activity (increased by asphyxia or injection of adrenaline) within 60 msec, lasting 3–4 sec, which was abolished by pulmonary denervation. Intracarotid arterial injection of KCN slowed the heart and increased Type I discharge most consistently when the vagi had been divided or the animal paralysed.

6. Activity similar to Type I activity showed increased discharge when pressure was increased in an isolated carotid sinus.

7. The conduction velocities of two single Type I fibres in the vagal cardiac branches were found to be 8·0 and 6·2 m/sec; one fibre had a higher conduction velocity in the proximal than in the distal vagus nerve.

8. Type II activity decreased during adrenaline hypertension, increased during amyl nitrite hypotension and at times showed a pulse modulation; it is thought to be bronchoconstrictor in function.

9. Type III activity showed short, rapid bursts with inspiration, was also recorded from the recurrent laryngeal nerve, and probably innervates the abductor muscles of the vocal cords.

10. Type IV activity fired during expiration and probably innervates the adductor muscles of the vocal cords; however recordings from the recurrent laryngeal nerve revealed fibres which differed in their responses to adrenaline hypertension and inspiration. One fibre was similar to Type I fibres.

11. Type V activity was similar to Type I activity and also to activity found in non-cardiac branches of the vagus.

12. The single Type VI fibre seemed to be from a carotid sinus baroreceptor.

13. Type VII fibres showed no significant changes in activity relative to respiration or blood pressure.

This work was begun with the encouragement and in the facilities of Professors J. H. Comroe, Jr., and L. L. Bennett at the University of California School of Medicine, San Francisco, U.S.A. The majority of the work was completed with the helpful advice and criticism of Dr J. G. Widdicombe (who also aided in the recordings from the cardiac nerves) in his laboratories at St Bartholomew's Hospital Medical School (with the kind permission

of Professor M. de Burgh Daly) and at Oxford University Laboratory of Physiology (with the kind permission of Professor Sir Lindor Brown). Mr L. Gale provided technical assistance and Mr A. Austin the photographic reproductions.

This investigation was supported in part by United States Public Health Service Graduate Training Grant HTS-5251 and Fellowship HF-13,842, National Heart Institute. This work was part of a thesis presented for the degree of D.Phil. at Oxford University.

REFERENCES

AGOSTONI, E., CHINNOCK, J. E., DALY, M. DE B. & MURRAY, J. G. (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat. *J. Physiol.* **135**, 182–205.

ANDREW, B. L. (1956). A functional analysis of the myelinated fibres of the superior laryngeal nerve of the rat. *J. Physiol.* **133**, 420–432.

ANREP, G. V., PASCUAL, W. & RÖSSLER, R. (1936*a*). Respiratory variations of the heart rate: I. The reflex mechanisms of the respiratory arrhythmia. *Proc. Roy. Soc.* B, **119**, 191–217.

ANREP, G. V., PASCUAL, W. & RÖSSLER, R. (1936*b*). Respiratory variations of the heart rate: II. The central mechanisms of the respiratory arrhythmia and the inter-relations between the central and the reflex mechanisms. *Proc. Roy. Soc.* B, **119**, 218–230.

BIANCONI, R. & RASCHI, F. (1959). Impulsi pressocettivi antidromici nel vago cervicale del gatto. *Boll. Soc. ital. Biol. sper.* **35**, 1913–1917.

BOSS, J. & GREEN, J. H. (1956). The histology of the common carotid baroceptor areas of the cat. *Circ. Res.* **4**, 12–17.

BRONK, D. W., FERGUSON, L. K., MARGARIA, R. & SOLANDT, D. Y. (1936). The activity of the cardiac sympathetic centers. *Amer. J. Physiol.* **117**, 237–249.

BRONK, D. W. & STELLA, G. (1932). Afferent impulses in the carotid sinus nerve. I. The relation of the discharge from single end organs to arterial blood pressure. *J. cell. comp. Physiol.* **1**, 113–130.

BROWN, G. L. & ECCLES, J. C. (1934). The action of a single vagal volley on the rhythm of the heart beat. *J. Physiol.* **82**, 211–241.

CHUNGCHAROEN, D., DALY, M. DE B., NEIL, E. & SCHWEITZER, A. (1952). The effect of carotid occlusion upon the intra-sinusal pressure with special reference to vascular communication between the carotid and vertebral circulations in the dog, cat and rabbit. *J. Physiol.* **117**, 56–76.

DALY, M. DE B. & EVANS, D. H. L. (1953). Functional and histological changes in the vagus nerve of the cat after degenerative section at various levels. *J. Physiol.* **120**, 579–595.

DALY, M. DE B. & MOUNT, L. E. (1951). The origin, course, and nature of broncho-motor fibres in the cervical sympathetic nerve of the cat. *J. Physiol.* **113**, 43–62.

DALY, M. DE B. & SCOTT, M. J. (1958). The effects of stimulation of the carotid body chemoreceptors on heart rate in the dog. *J. Physiol.* **144**, 148–166.

DALY, M. DE B. & SCOTT, M. J. (1963). The effects of changes in respiration on the cardiovascular responses to stimulation of the carotid body chemoreceptors. In *The Regulation of Human Respiration.* Ed. CUNNINGHAM, D. J. C. & LLOYD, B. B. Oxford: Blackwell.

DOWNING, S. E. & SIEGAL, J. H. (1963). Baroreceptor and chemoreceptor influences on sympathetic discharge to the heart. *Amer. J. Physiol.* **204**, 471–479.

EVANS, D. H. L. & MURRAY, J. G. (1954). Histological and functional studies on the fibre composition of the vagus nerve of the rabbit. *J. Anat., Lond.*, **88**, 320–337.

EYZAGUIRRE, C. & TAYLOR, J. R. (1963). Respiratory discharge of some vagal motoneurons. *J. Neurophysiol.* **26**, 61–78.

GOVAERTS, J. (1936*a*). Etude oscillographique de l'activite electrique des nerfs cardiaques en connexion avec le nevrare. *C.R. Soc. Biol., Paris*, **122**, 449.

GOVAERTS, J. (1936*b*). Contribution à l'étude de l'innervation sympathique du cœur. *Arch. intern. Med. exp.* **11**, 629–707.

GREEN, J. H. (1959). Cardiac vagal efferent activity in the cat. *J. Physiol.* **149**, 47–49*P*.

GREEN, J. H. & NEIL, E. (1955). The respiratory function of the laryngeal muscles. *J. Physiol.* **129**, 134–141.

HEINBECKER, P. (1931). The effect of fibers of specific types in the vagus and sympathetic nerves on the sinus and atrium of the turtle and frog heart. *Amer. J. Physiol.* **98**, 220–229.

HEINBECKER, P. & BISHOP, G. (1935). Studies on the extrinsic and intrinsic nerve mechanisms of the heart. *Amer. J. Physiol.* **114**, 212–223.

HEINBECKER, P. & O'LEARY, J. (1933). The mammalian vagus nerve—a functional and histologic study. *Amer. J. Physiol.* **106**, 623–646.

HEYMANS, C., BOUCKAERT, J. J. & SAMAAN, A. (1934). Influences des variations de la teneur du sang en oxygène et en CO_2 sur l'excitabilité réflexe et directe des éléments centraux et périphériques des nerfs cardio-régulateurs. *Arch. intern. Pharmacodyn.* **48**, 457–487.

HEYMANS, C. & NEIL, E. (1958). *Reflexogenic Areas of the Cardiovascular System.* Boston: Little, Brown and Co.

HOLMES, R. (1954). Carotid sinus baroceptor afferent fibres in the aortic nerve of the cat. *J. Physiol.* **126**, 40–41*P*.

IGGO, A. (1958). The electrophysiologic identification of single nerve fibres with particular reference to the slowest-conducting vagal afferent fibres in the cat. *J. Physiol.* **142**, 110–126.

JEWETT, D. L. (1962). Activity of single vagal efferent cardiac fibres in the dog. *J. Physiol.* **163**, 33–35*P*.

JEWETT, D. L. (1964). Direct nervous pathways between the cervical vagus and aortic or superior laryngeal nerves traversed by baroreceptor, 'pulmonary stretch', and other fibres in the cat. *J. Physiol.* **175**, 358–371.

KOEPCHEN, H. P., WAGNER, P. H. & LUX, H. D. (1961). Über die Zusammenhänge zwischen zentraler Erregbarkeit, reflektorischem Tonus und Atemrhythmus bei der nervösen Steuerung der Herzfrequenz. *Pflüg. Arch. ges. Physiol.* **273**, 443–465.

KORNER, P. I. (1959). Circulatory adaptations in hypoxia. *Physiol. Rev.* **39**, 687–730.

MARGUTH, H., RAULE, W. & SCHAEFER, H. (1951). Atkionströme in zentrifugalen Herznerven. *Pflüg. Arch. ges. Physiol.* **254**, 224–245.

MAZZELLA, H. & MIGLIARO, E. F. (1953). Hemodynamics of the carotid sinus and its repercussions on the pressor reflex. *Amer. J. Physiol.* **175**, 383–385.

MIDDLETON, S., MIDDLETON, H. H. & GRUNDFEST, H. (1950). Spike potentials and cardiac effects of mammalian vagus nerve. *Amer. J. Physiol.* **163**, 545–552.

MIZERES, N. J. (1955). Isolation of the cardio-inhibitory branches of the right vagus nerve in the dog. *Anat. Rec.* **123**, 437–445.

MIZERES, N. J. (1957). The course of the left cardio-inhibitory fibers in the dog. *Anat. Rec.* **127**, 109–115.

OKADA, H., OKAMOTO, K. & NISIDA, I. (1961*a*). The activity of the cardioregulatory and abdominal sympathetic nerves during swallowing. *Jap. J. Physiol.* **11**, 44–53.

OKADA, H., OKAMOTO, K. & NISIDA, I. (1961*b*). Activity of the cardioregulatory and abdominal sympathetic nerves of the cat in the Bainbridge reflex. *Jap. J. Physiol.* **11**, 520–529.

PAINTAL, A. S. (1953). The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. *J. Physiol.* **121**, 341–359.

PAINTAL, A. S. (1962). Determination of intrathoracic conduction time in cardiovascular afferent fibres of the vagus nerve. *J. Physiol.* **163**, 222–238.

REED, E. A. & SCOTT, J. C. (1955). Factors influencing the carotid sinus cardioinhibitory reflex. *Amer. J. Physiol.* **181**, 21–26.

RIJLANT, P. (1936*a*). Le contrôle extrinsèque de la fréquence du battement cardiaque. *C.R. Soc. Biol., Paris*, **123**, 99–101.

RIJLANT, P. (1936*b*). L'arythmie cardiaque respiratoire. *C.R. Soc. Biol., Paris*, **123**, 997–1001.

SAMAAN, A. (1935). The antagonistic cardiac nerves and heart rate. *J. Physiol.* **83**, 332–340.

SCHLICHER, L., PEIPER, U., KRUG, H. & BOHME, H. (1959). Zur Genese der respiratorischen Arrhythmie. *Z. KreislForsch.* **48**, 835–843.

SCOTT, J. C. & REED, E. A. (1955). Comparison of unilateral with bilateral stimulation of carotid sinus cardioinhibitory reflex. *Amer. J. Physiol.* **181**, 27–29.

TANG, P. C., MAIRE, F. W. & AMASSIAN, V. E. (1954). Factors influencing vasomotor center activity. *Amer. J. Physiol.* **179**, 679.

TANG, P. C., MAIRE, F. W. & AMASSIAN, V. E. (1957). Respiratory influences on the vasomotor center. *Amer. J. Physiol.* **191**, 218–224.

VINOGRADOVA, M. I. (1955). Efferent impulses in the pulmonary nerves. *Ezhegodnik: Inst. eksper. med. Akad. med. Nauk SSR* 113. Cited by WIDDICOMBE, J. G. (1961) in *Arch. exp. Path. Pharmak.* **241**, 415–432.

WAITES, G. M. H. (1957*a*). Recurrent cardio-accelerator fibres in the right cervical sympathetic nerve of the sheep. *J. Physiol.* **135**, 58–65.

WAITES, G. M. H. (1957*b*). The course of the efferent cardiac nerves of the sheep. *J. Physiol.* **139**, 417–433.

WANG, S. C. & BORISON, H. L. (1947*a*). An analysis of the carotid sinus cardiovascular reflex mechanism. *Amer. J. Physiol.* **150**, 712–721.

WANG, S. C. & BORISON, H. L. (1947*b*). Decussation of the pathways in the carotid sinus cardiovascular reflex: an example of the principle of convergence. *Amer. J. Physiol.* **150**, 722–728.

WARNER, H. R. & COX, A. (1962). A mathematical model of heart rate control by sympathetic and vagus efferent information. *J. appl. Physiol.* **17**, 349–355.

WEIDINGER, H., HETZEL, R. & SCHAEFER, H. (1962). Acktionsströme in zentrifugalen vagalen Herznerven und deren Bedeutung für den Kreislauf. *Pflüg. Arch. ges. Physiol.* **276**, 262–279.

WIDDICOMBE, J. C. (1961*a*). Action potentials in vagal efferent nerve fibres to the lungs of the cat. *Arch. exp. Path. Pharmak.* **241**, 415–432.

WIDDICOMBE, J. G. (1961*b*). Impulses in efferent nerve fibres to the trachea. *Fed. Proc.* **20**, (no. I, pt. I), 429*d*.

WIDDICOMBE, J. G. (1962). Action potentials in efferent nerve fibres to the trachea. *J. Physiol.* **162**, 66–67*P*.

WIDDICOMBE, J. G. (1963). Regulation of tracheobronchial smooth muscle. *Physiol. Rev.* **43**, 1–37.

WIDDICOMBE, J. G. (1964). Action potentials in parasympathetic and sympathetic efferent fibres to the trachea and lungs of dogs and cats. *J. Physiol.* (In the Press).

Note added in proof: My findings on Type III and Type IV fibres are generally supported by the findings of this recent paper:

BIANCONI, R. & RASCHI, F. (1964). Respiratory control of motoneurones of the recurrent laryngeal nerve and hypocapnic apnoea. *Arch. ital. Biol.* **102**, 56–73.