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# A STUDY OF CHEMORECEPTOR AND BARORECEPTOR A AND C-FIBRES IN THE CAT CAROTID NERVE

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SUMMARY

1. 149 A-fibres and 52 C-fibres from the cat carotid nerve were studied *in vivo* with single-unit recording techniques. These units subserved chemoreceptor and baroreceptor modalities. In addition, half of the C-fibres were determined to be efferent in origin. The estimated fibre diameter spectrum for chemoreceptor and baroreceptor A-fibres is described.

2. The discharge pattern of chemoreceptor A and C-fibres was characteristically irregular both at rest and during activation. However, about 5 % of the chemoreceptor A-fibre population exhibited a very regular discharge pattern, even at low rates of firing.

3. In comparing A and C-fibres, it was found that chemoreceptor and baroreceptor A-fibres had lower thresholds, shorter response latencies, more rapid acceleration of discharge and higher discharge frequencies than their C-fibre counterparts.

4. During strong chemoreceptor or baroreceptor stimulation, interaction of the 'spontaneous' whole nerve activity with the evoked A and C-fibre compound action potentials provided a method of estimating the relative proportions of chemoreceptors and baroreceptors in the A and C-fibre populations of the carotid nerve. The A-fibre population was found to be comprised of approximately 2/3 chemoreceptors, 1/3 baroreceptors. The reverse was true for the C-fibre population, i.e. 2/3 baroreceptors, 1/3 chemoreceptors.

5. A stepwise C-fibre response is described which may arise from the several C-fibres within a single Schwann cell.

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## INTRODUCTION

The presence of both myelinated (A-type) and unmyelinated (C-type) fibres in the carotid nerve of the cat is well documented (de Castro, 1926, 1951; Eyzaguirre & Uchizono, 1961). The A-fibre population is comprised principally of chemoreceptor afferents from the glomus and baroreceptor afferents from the sinus (cf. Adams, 1958; Heymans & Neil, 1958). In the present investigation, the relative number of chemoreceptor A-fibres in the cat carotid nerve and their response characteristics are explored.

Less well understood is the functional classification of carotid nerve C-fibres. Post-ganglionic sympathetic fibres coursing from the superior cervical ganglion account for many of the C-fibres in the carotid nerve (Eyzaguirre & Lewin, 1961*b*). Eyzaguirre & Uchizono (1961) demonstrated the presence of non-sympathetic C-fibres in the cat carotid nerve, but their functional classification remained undetermined. Douglas & Ritchie (1956) have suggested that electrical stimulation of non-sympathetic C-fibres present in the carotid nerve can elicit depressor reflex effects in the cat, as in the rabbit. However, neither the afferent fibre origin nor the adequate stimulus for this depressor reflex was explored in their experiments. Therefore, in addition to an investigation of chemoreceptor A-fibres, we have studied also the non-sympathetic afferent C-fibre populations of the cat carotid nerve. The results will show that these fibres subserve chemoreceptor and baroreceptor modalities. Preliminary results have been published elsewhere (Fidone, Sato & Eyzaguirre, 1968; Sato, Fidone & Eyzaguirre, 1968).

## METHODS

*Operative procedures.* Fifty-seven adult cats of both sexes were used in the present series of experiments. The animals were anaesthetized with sodium pentobarbital, 40 mg/kg I.P. (Nembutal, Abbott Laboratories). A superficial forelimb vein was catheterized for subsequent administration of the anaesthetic during the course of the experiment. The trachea was cannulated, and the animal was allowed to breathe room air. The neck was opened, and the carotid nerve was dissected free of surrounding structures and cut at its juncture with the glossopharyngeal nerve. The ganglioglomerular nerve(s), coursing from the superior cervical ganglion to provide the sympathetic innervation of the carotid body region, was routinely cut in all experiments. This procedure served two purposes: first, it eliminated the reflex sympathetic action on carotid nerve discharges (Eyzaguirre & Lewin, 1961*b*), and secondly, since many of these sympathetic fibres continue up the carotid nerve, it abolished the background sympathetic activity and thereby served to facilitate the isolation of non-sympathetic unmyelinated fibres from the carotid nerve. The ipsilateral thyroid artery was catheterized for close intra-arterial injections of drugs. The exposed tissues were covered with warm paraffin oil kept at 36° C, and the rectal temperature was maintained at 37–38° C, by means of radiant heat. The blood pressure was recorded from the femoral artery with a pressure transducer (Statham, P23AC) and a rectilinear ink-writer (Texas instruments).

*Isolation and identification of chemoreceptor A and C-fibres.* For subdissection, the carotid nerve was placed on a laryngeal mirror (size 00) and side-lighted with a high intensity fibre optic illuminator. This arrangement proved extremely useful because the incident light was reflected from the mirror up through the nerve preparation, thereby acting as transmitted illumination. Utilizing a variety of micro-dissecting tools, subdissection of the carotid nerve proceeded under a dissecting microscope (50 ×). Owing to the shortness of the nerve (0·5–1·0 cm), the isolation and identification techniques differed for A and C-fibres (Fig. 1). Single-unit C-fibre activity was isolated in very fine filaments of the peripheral cut end of the carotid nerve (Fig. 1*A*). Stimulating electrodes were placed on the nerve, close to the glomus, for

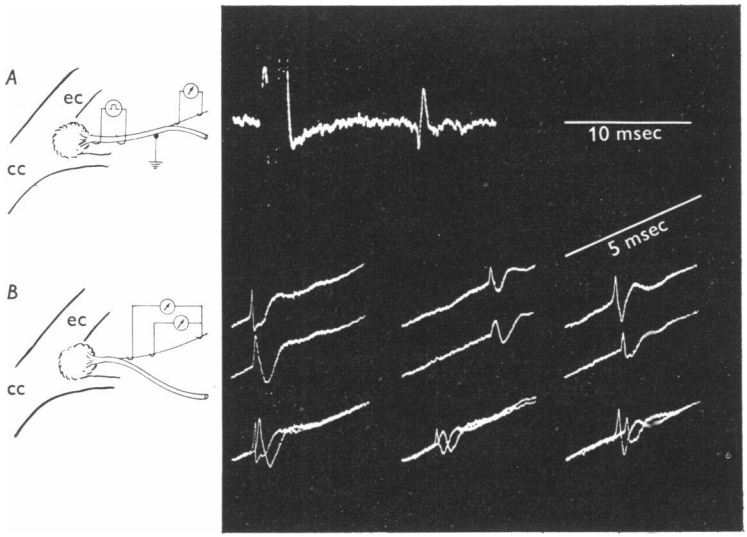


Fig. 1. Identification of carotid nerve A and C-fibres. cc, common carotid; ec, external carotid arteries. *A*, stimulating and recording arrangement for C-fibres. Single evoked C-fibre potential; conduction velocity, 0·7 m/sec. *B*, double recording method for A-fibres. Separate and superimposed oscilloscope sweeps of spontaneous A-fibre activity simultaneously recorded at two points along a single small filament of the carotid nerve. Monopolar electrode separation for all records in *B*, 4 mm. Conduction velocities: (left to right) 30, 23 and 11 m/sec.

conduction velocity measurements. The C-fibre action potentials were led off from the filaments with small platinum bipolar electrodes, amplified (Tektronix Type 122 preamplifier) for display on a cathode ray oscilloscope, and photographed. The evoked C-fibre potentials exhibited the constant shape, latency and all-or-none characteristics of single-unit response. The conduction velocities varied between 0·5 and 2·0 m/sec.

This arrangement proved impractical for measuring A-fibre conduction velocities, because the evoked A-fibre potential was usually lost in the stimulus artifact. For this reason, A-fibre conduction velocities were determined from the spontaneous neural activity by recording fibre conduction time along a measured length of a nerve filament between two monopolar (monotopic) recording electrodes (Fig. 1*B*). The temporal shift between the two recording channels was measured from projected

and enlarged photographs taken at fast oscilloscope sweep speeds (Fidone *et al.* 1968). The subdissection of the carotid nerve proceeded until the activity of a single A-fibre was separately discernible from the background discharges. The criteria for unit activity required that the shape and the temporal shift of the action potentials between recording channels be constant. To avoid the sample bias towards tonically active units inherent in this method of recording, pure $N_2$ was administered to the animal at each successive stage of nerve dissection. This provoked a strong chemoreceptor discharge, making possible the analysis of many otherwise silent units. The conduction velocities so measured varied between 4 and 53 m/sec.

The A and C-fibres identified in the above described manner were subsequently tested for the following chemoreceptor properties: marked sensitivity to high $CO_2$ and low $O_2$, and short latency response to close intra-arterial injections of sodium cyanide (NaCN, 5–20 $\mu$g), acetylcholine (ACh, 5–20 $\mu$g) and saline at pH 2·0 (1·0 ml.). In addition, C-fibres which failed to respond to any of the above stimuli were tested for baroreceptor properties. Raising the carotid pressure by rapid intra-arterial injection of normal saline (5 ml.), or probing the sinus area with a small glass rod proved to be effective stimuli for baroreceptor C-fibres. No detailed study of baroreceptor A-fibres was attempted.

In certain experiments, the activity of the intact carotid nerve was monitored. For this purpose, the whole nerve discharges were led through a counter-timer (Computer Measurements Co.) and integrator (Hewlett Packard). The counter-timer registered all impulses which exceeded an adjustable voltage level set above the base line noise. The action potentials of the smallest units were lost in the base line noise and were not included in the count. The integrated whole nerve discharge is therefore only a qualitative measure of the changes in neural activity. The output of the integrator was plotted on a rectilinear ink-writer (Texas Instruments).

## RESULTS

### *Chemoreceptor A-fibres*

*Response pattern.* A total of 149 units which satisfied the above criteria for chemoreceptor A-fibres was studied. With the animals breathing room air the resting discharge rates of these units ranged from 0 to 15/sec, but most units discharged at 2–5/sec. The response pattern of the unit illustrated in Fig. 2 is representative of that seen amongst carotid body chemoreceptor A-fibres. In Fig. 2*A*, the onset of the responses to close intra-arterial injections of NaCN (10 $\mu$g), ACh (10 $\mu$g) and pH 2·0 saline (1 ml.) are shown together with the effect of administration of pure $N_2$ to the animal. Threshold doses of NaCN and ACh were generally of the order of 1–2 $\mu$g. To verify that the discharge was from a single unit, the response of the chemoreceptor A-fibre to each of the stimuli was recorded on both a horizontal fast sweep and a vertical, slow moving continuous trace. The records in the Figure read from the top downwards in each column; the injections started at the beginning of each vertical trace and were completed within 3–5 sec. The latency from the start of the injection to the abrupt onset of the chemoreceptor A-fibre discharge was between 0·5 and 2·0 sec, and varied with the dose and rate of injection and the

condition of the preparation. The response latency of a given unit to repeated applications of the same stimulus commonly varied a second or more even in the most stable preparations. In addition, repeated injections of the same stimulus yielded considerable variation in response magnitude. However, in comparing responses of similar magnitude, the units

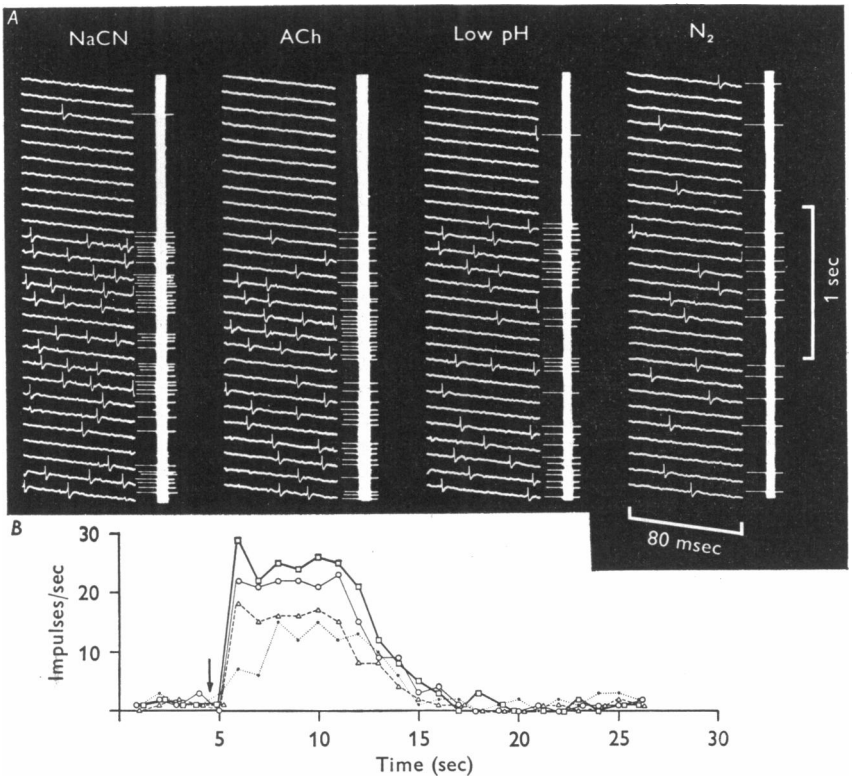


Fig. 2. Chemoreceptor A-fibre, conduction velocity 19 m/sec. *A*, onset of responses to intra-arterial injections of NaCN (10 $\mu$g), ACh (10 $\mu$g), pH 2·0 saline (1 ml.) and to pure $N_2$. All records read from the top downwards, and sweeps read from left to right. Time between sweeps, 20 msec. Injections began at the start of each record (and at arrow in *B*) and continued for 3–5 sec. Pure $N_2$ administered 4 sec before start of record (and at 0 sec in *B*) and continued for 10 sec. *B*, magnitude and time course of responses shown in *A*, measured as the number of impulses in each second. Open circles NaCN, open squares ACh, open triangles low pH, filled circles $N_2$.

showed no consistent difference in response latency to injections of NaCN, ACh or pH 2·0 saline. These observations probably result from the fact that the amount of drug that reached the carotid glomus and excited the chemoreceptor cells fluctuated with the turbulence of the arterial stream. The response to pure $N_2$ inhalation had a latency of the order of

4–10 sec and exhibited maximum discharge frequencies, measured as the number of impulses in 1 sec, between 15 and 30/sec. NaCN and ACh in the doses used were more effective chemoreceptor excitants than pH 2·0 saline, and produced maximum discharge frequencies between 20 and 40/sec (Fig. 2*B*).

The irregular discharge pattern seen in these records is most typical of the chemoreceptor A-fibres encountered during this study. This finding is consistent with the earlier observations of Eyzaguirre & Lewin (1961*a*),

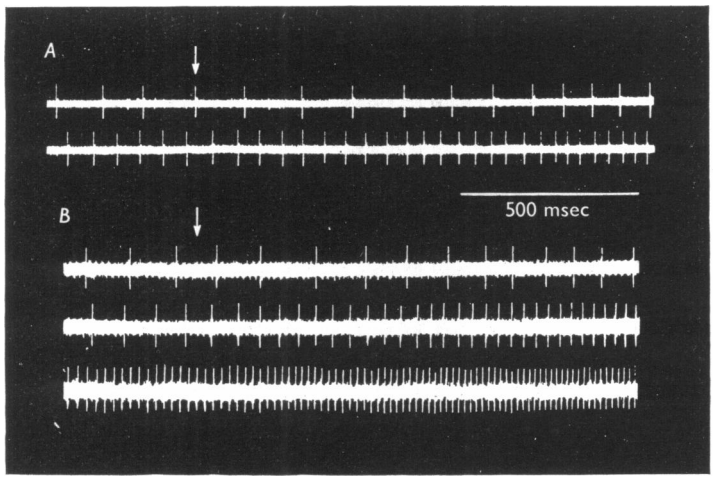


Fig. 3. Marked regularity of discharge of two chemoreceptor A-fibres. Conduction velocities, 9 m/sec (*A*) and 13 m/sec (*B*). Injections of 10 $\mu$g NaCN at arrows; records are continuous in *A* and *B* to time of maximum response of each unit.

who referred to the discharge pattern as 'aperiodic'. The later work of Biscoe & Taylor (1963) showed that at low discharge rates the impulse interval distribution for chemoreceptors is random, but exhibits a tendency towards regularity at frequencies above 10/sec. During the present investigation, however, some units (about 5%) showed a marked tendency towards regularity even at low discharge rates. All had resting discharge rates below 10/sec, and upon stimulation exhibited maximum frequencies of 30–60/sec (Fig. 3*A*, *B*). All were sensitive to $N_2$ inhalation and to intra-arterial injections of small doses of NaCN, ACh and pH 2·0 saline. Pressure applied to the sinus area routinely failed to provoke any increase in discharge. Thus, it was evident that these units were afferent fibres arising from chemoreceptors. It is interesting to note that the conduction velocities of these regularly discharging units were at the lower end of the A-fibre velocity distribution (see later).

*The percentage of chemoreceptor A-fibres in the cat carotid nerve.* The A-fibre population of the carotid nerve consists of chemoreceptor fibres from the glomus and baroreceptor fibres from the carotid sinus wall (Adams, 1958; Heymans & Neil, 1958). Speculation on the relative proportions and size of these fibres has been made on the basis of visual observation of whole nerve activity, but the dominant rhythmic firing of baroreceptors made any quantitative estimate difficult and uncertain (Heymans & Neil, 1958).

This chemoreceptor/baroreceptor ratio was analysed in our experiments by studying the interaction between the 'spontaneous' whole nerve activity and the evoked A-fibre compound action potential. For this purpose, stimulating electrodes were placed on the carotid nerve close to the glomus, and the nerve was stimulated continuously at 1/sec. The spontaneous activity and the evoked A-fibre potential were recorded from the peripheral cut end of the whole nerve. Increasing the chemoreceptor discharge by asphyxia or by intra-arterial injections of NaCN, ACh or acidified saline decreased the amplitude of the evoked A-fibre potential; the larger the increase in discharge, the greater the reduction in evoked potential amplitude. This relies upon the fact that increase in the discharge rate results in a greater number of the A-fibres being refractory to the electrical stimulus (cf. Douglas & Ritchie, 1957).

Figure 4 illustrates the reduction in amplitude of the evoked A-fibre potential following intra-arterial injections of NaCN (*A*) and ACh (*B*). The electrical stimulus was adjusted to be just maximal for A-fibres, and an intense chemoreceptor discharge was initiated by injections of 10–30 $\mu$g NaCN or ACh administered over a period of 10–20 sec. Doubling a selected dosage usually failed to increase the level of chemoreceptor discharge by more than 5 or 10%. These whole nerve responses (middle traces with arrow) were characterized by an initial high frequency discharge which declined slowly during the remainder of the injection, closely resembling the response profile of single A-fibre preparations (cf. Fig. 2*B*). The ends of the injections were marked by an abrupt fall in discharge and were followed by a period of post-activation depression. The changes in the area of the A-fibre potential (filled circles) mirror the changes in the level of chemoreceptor discharge; the maximal decrease and increase in area correspond to the highest and lowest discharge levels, respectively.

The average maximal depression of the A-fibre potential from sixty-eight injections (thirty-eight NaCN, thirty ACh) in eleven animals was 61% of control value. The means for NaCN and ACh were not statistically different because the dose was adjusted in each preparation to yield a maximal chemoreceptor response. It appears from these data that *at least* 61% of the A-fibres present in the carotid nerve are chemoreceptor fibres. The

actual percentage is probably higher since it is unlikely that all chemoreceptor fibres in the nerve would be refractory to the electrical stimulus at the same time, even during the most intense chemoreceptor discharge. In this regard, however, a study of the refractory periods of carotid nerve A-fibres using single maximal conditioning shocks and maximal test

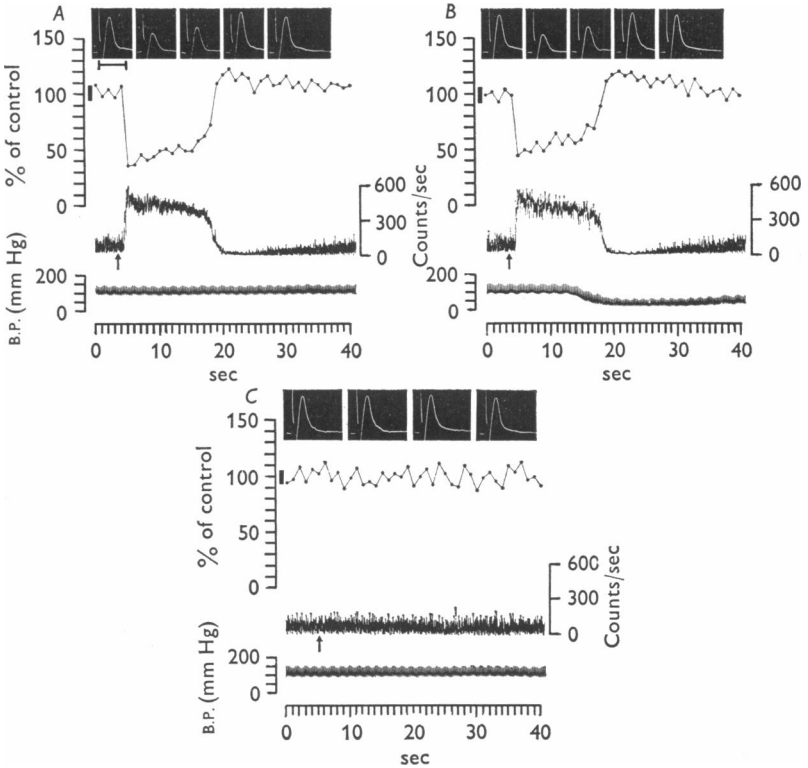


Fig. 4. Interaction of chemoreceptor A-fibre activity with evoked A-fibre compound action potential. From the top downwards in *A*, *B* and *C*: sample records of effect of injection on maximal evoked A-fibre potential, time marker, 2 msec; area of A-fibre potential as percent of control (vertical bar to left, semi-interquartile range from preceding 40 evoked potentials); integrated whole nerve discharge, injection at arrow; blood pressure from femoral artery. *A*, 20 $\mu$g NaCN (1 ml.); *B*, 20 $\mu$g ACh (1 ml.); *C*, normal saline (1 ml.). Preamplifier bandpass: 0·2 c/s–10 kc/s.

shocks has shown an absolute refractory period of 2 msec and a marked hypoexcitability extending to 25–30 msec beyond the conditioning shock. In addition, the above estimate of 61 % is low because the resting chemoreceptor discharge itself depresses slightly the size of the A-potential, as evidenced by an increase in the A-potential during post-activation depression (Fig. 4). Thus, the control level of the A-potential does not represent

a zero base line for chemoreceptor activity. In this respect it is interesting to note that the area of the A-fibre potential undergoes a maximal depression of 30–40 % of control value when a large increase in carotid sinus pressure provokes an intense baroreceptor discharge. In this experiment, contamination from chemoreceptor fibres was avoided by cutting the branch of the carotid nerve to the glomus.

Combining the observed percentages for chemoreceptor and baroreceptor fibres, it appears that this experimental method is able to account for nearly the entire A-fibre population of the carotid nerve. For this reason, it is felt that the value of 61 % for chemoreceptor fibres cannot be significantly in error. Myelinated fibres (A-fibres) in the cat carotid nerve number between 600 and 700 (de Castro, 1951; Eyzaguirre & Uchizono, 1961). Our data would predict, therefore, that the cat carotid nerve contains 350–450 chemoreceptor A-fibres and 200–300 baroreceptor A-fibres.

In regard to the method of A-potential depression, two additional types of experiments were performed. The first is the control experiment shown in Fig. 4*C*. An injection of normal saline, administered at the same rate and duration as the injections in Fig. 4*A* and *B*, failed to alter either the whole nerve resting discharge or the area of the evoked A-fibre potential. From this it can be assumed that any mechanical artifacts consequent to the injection method have no significant effect upon the stability of the stimulating and recording conditions. The second experiment, illustrated in Fig. 5, showed the behaviour of a single chemoreceptor A-fibre during an injection of NaCN. Stimulating electrodes, as before, were positioned on the nerve close to the glomus, and the stimulus intensity was adjusted so that the single A-fibre potential was evoked in approximately all of the stimulus trials applied at 2/sec. The unit exhibited a low threshold to the electrical stimulus, the voltage being only 20 % of that required to evoke a maximal A-fibre potential. The records in the figure read from the bottom upwards in each column, and from left to right the five columns (*A–E*) are continuous. Column *A* shows the control response to nerve stimulation, and the beginning of column *B* marks the start of an intra-arterial injection of 5 $\mu$g NaCN. This dose was sufficient to provoke a maximal response of the single A-fibre, whereas a maximal response from the whole nerve preparation required a dose of 20 $\mu$g NaCN. After a latency of 1 sec from the start of the injection, the electrical stimulus failed to evoke the unit potential in twenty-one of the next twenty-five stimulus trials. During the first 5 sec of the response, the stimulus failed to evoke the A-fibre in any of the trials, and two spontaneously occurring unit potentials may be seen in columns *B* and *C*. As the response began to wane, the stimulus became more effective in evoking the unit potential and finally, in column *E*, returned to control level.

Thus, the decrease in the area of the whole nerve A-fibre potential can be explained from the behaviour of its constituent unitary potentials. Furthermore, the low stimulus intensity and small dose of cyanide required to elicit the marked effect upon the unit of Fig. 5 demonstrates that the depression of the whole nerve A-fibre potential is not an artifact of a higher stimulus intensity and larger dose.

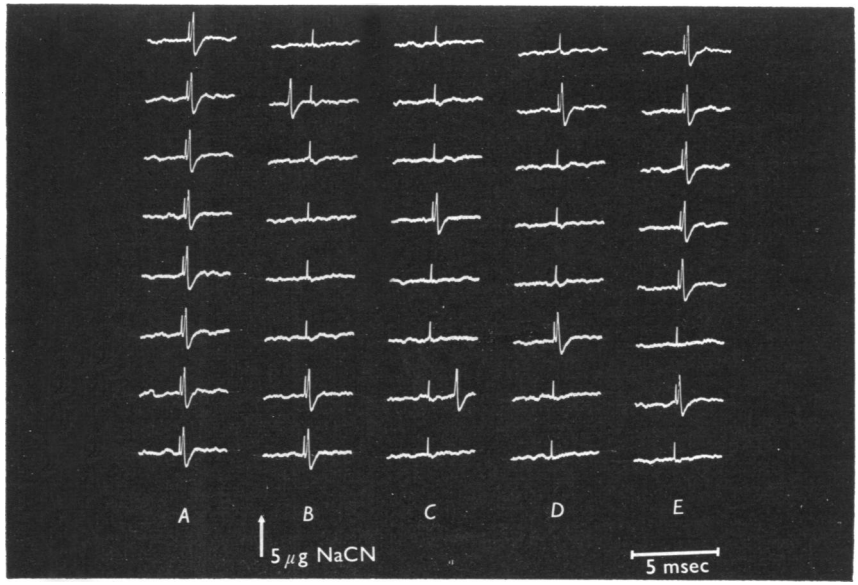


Fig. 5. Single evoked chemoreceptor A-fibre from experiment of Fig. 4, conduction velocity 26 m/sec. Records read from bottom upwards in each column and are continuous from *A* to *E*. Sweep rate, 2/sec. Stimulus intensity just suprathreshold for single A-fibre. *A*, control; *B*, 5 μg NaCN injected at start of column *B* results in failure of evoked A-fibre potential. Note spontaneous A-fibre potentials in *B* and *C*; *C* to *E*, gradual return to control level.

*Diameter spectrum of chemoreceptor A-fibres.* The distribution of the conduction velocities for all chemoreceptor A-fibres studied is shown in Fig. 6*A*. The velocities ranged from 4 to 53 m/sec, with a median of 16 m/sec and a semi-interquartile range from 11 to 21 m/sec. As referred to earlier, the A-fibres which exhibited a highly regular discharge pattern had conduction velocities in the lower half of this distribution, between 7 and 15 m/sec.

The fibre diameter spectrum is also described in Fig. 6*A*, assuming a conversion factor of 5 for the relationship between conduction velocity and fibre diameter (Boyd, 1964, 1965). The bold-lined histogram of Fig. 6*B* is taken from the results of Eyzaguirre & Uchizono (1961), and represents

the fibre diameter distribution for all myelinated fibres (chemoreceptor and baroreceptor A-fibres) from one cat carotid nerve, as measured by phase contrast micrography. With this histogram and our present data, it is possible to estimate the relative fibre diameter distributions for carotid nerve chemoreceptors and baroreceptors. Assuming that 61 % of carotid nerve A-fibres are chemoreceptors (Fig. 4), we may then relate the chemoreceptor fibre diameter distribution of Fig. 6*A* with the fibre diameter

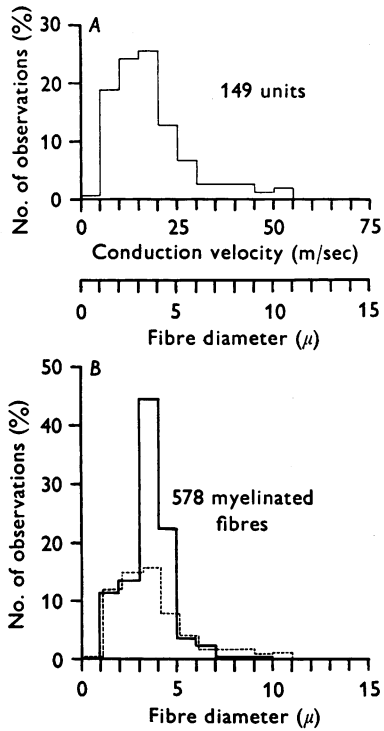


Fig. 6. *A*, percentage distribution of conduction velocities of 149 chemoreceptor A-fibres. Estimated fibre diameter spectrum from conversion factor of 5·0; *B*, bold-lined histogram is fibre diameter spectrum of all myelinated fibres (578) from one cat carotid nerve, taken from Eyzaguirre & Uchizono (1961); dash-lined histogram is estimated chemoreceptor fraction of A-fibre population, drawn by reducing to 61 % all the ordinate values of the histogram in *A*. Bold and dashed lines are offset for purposes of illustration.

distribution for all carotid nerve A-fibres shown in 6*B*. That is, since both histograms are percentage distributions and therefore independent of population size, the chemoreceptor histogram of 6*A* represents the fibre diameter distribution for 61 % of carotid nerve A-fibres. In Fig. 6*B*, the histogram of 6*A* has simply been redrawn to equal 61 % of the area of the

bold-lined histogram. The result, shown by the dashed-lined histogram, represents the chemoreceptor fraction of the carotid nerve A-fibre population. The remainder is presumably comprised principally of baroreceptor fibres. From this, it may be seen that the smallest and largest fibres in the carotid nerve are predominantly of chemoreceptor origin, while the baroreceptors show a pronounced fibre diameter grouping between 3 and 5 $\mu$.

*Classification of carotid nerve C-fibres*

Fifty-two single-unit C-fibre preparations from the carotid nerve were studied during the present investigation. Based on their response to a variety of stimuli, these fibres could be classified into three categories. The first fibre type, of which nine (about 17 %) were obtained, encompasses the standard criteria used for indentification of chemoreceptor A-fibres, i.e. marked sensitivity to high $CO_2$ and low $O_2$, and short latency response to close intra-arterial injections of NaCN, ACh, and saline at pH 2·0. The fifteen fibres (about 29 %) in the second category exhibited the pulse-synchronous discharge and low threshold to mechanical stimuli characteristic of baroreceptors. The third fibre group, the largest of the three (twenty-eight fibres; about 54 %), consisted of those C-fibres which did not respond to any of the chemoreceptor or baroreceptor stimuli described above. As will be seen later, these fibres are largely of sympathetic origin, with perhaps a small contribution from non-sympathetic efferent fibres which run with the carotid nerve. It should be emphasized that each of the C-fibres encountered during this study fell clearly into one of these three fibre-type classifications.

*Chemoreceptor C-fibres*

*Response pattern.* The resting discharge rate of chemoreceptor C-fibres was found to be lower than that of their A-fibre counterparts, being generally between 0 and 2/sec and rarely exceeding 4 or 5/sec. Like the A-fibres, the discharge pattern of chemoreceptor C-fibres was characteristically irregular both at rest and during activation (Fig. 7). Figure 7*A* illustrates the response of a single chemoreceptor C-fibre to close intra-arterial injections of NaCN (15 $\mu$g), ACh (15 $\mu$g), saline at pH 2·0 (2 ml.) and administration of pure $N_2$ to the animal. For each response the upper and lower traces are continuous, and the injections began approximately at the start of each series. In the $N_2$ response, the gas was administered 4 sec before the start of the records and was continued for 10 sec. The graph of Fig. 7*B* describes the magnitude and time course of the responses in 7*A*. The gradual rise to maximum discharge is characteristic of chemoreceptor C-fibre response and is in sharp contrast to the abrupt onset of discharge seen amongst A-fibres (cf. Fig. 2). In addition, the maximal frequency

of C-fibre response rarely exceeded 15–20/sec, whereas maximal A-fibre frequencies were commonly 30–40/sec or more.

As described earlier, the method of intra-arterial injection did not permit quantitatively reproducible responses, and considerable variations in

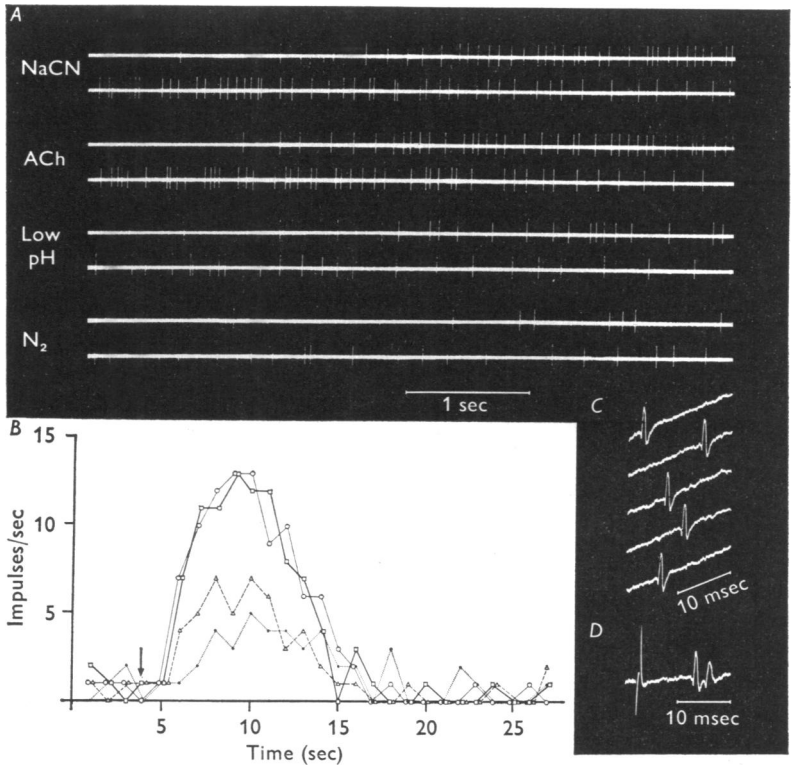


Fig. 7. Chemoreceptor C-fibre, conduction velocity 0·9 m/sec. *A*, responses to intra-arterial injections of NaCN (15 $\mu$g), ACh (15 $\mu$g), pH 2·0 saline (2 ml.) and to pure $N_2$. Upper and lower traces are continuous. Injections began approximately at start of each upper trace (and at arrow in *B*) and continued for 5 sec. $N_2$ administered 4 sec before start of record (and at 0 sec in *B*) and continued for 10 sec; *B*, magnitude and time course of responses shown in *A*, measured as the number of impulses in each second: symbols as in Fig. 2.; *C*, sample records of unit at fast oscilloscope sweep speed; *D*, evoked potentials of two units: the chemoreceptor C-fibre and an efferent C-fibre (see text).

both response latency and magnitude were observed from injection to injection. Thus, a study of the relative thresholds and response latencies of A and C-fibres could not be obtained by comparing an A-fibre response to one injection with a C-fibre response to another injection. For this reason, small filaments were subdissected from the carotid nerve which

contained the activity of both chemoreceptor A and C-fibres. The records of Fig. 8, obtained from such a filament, illustrate the response latencies for two chemoreceptor A-fibres ($A_1$ and $A_2$) and one chemoreceptor C-fibre (C). The resting discharge of all three units was 2–3/sec, but to accurately define the onset of response the units were rendered inactive by allowing the animal to breathe 50 % $O_2$ in $N_2$. The records in each column of 8*A* read from the bottom upwards, and the left column begins with the first unitary discharge ($A_1$) in response to an injection of 10 $\mu$g NaCN. This $A_1$-fibre response occurred slightly less than 1 sec after the start of the

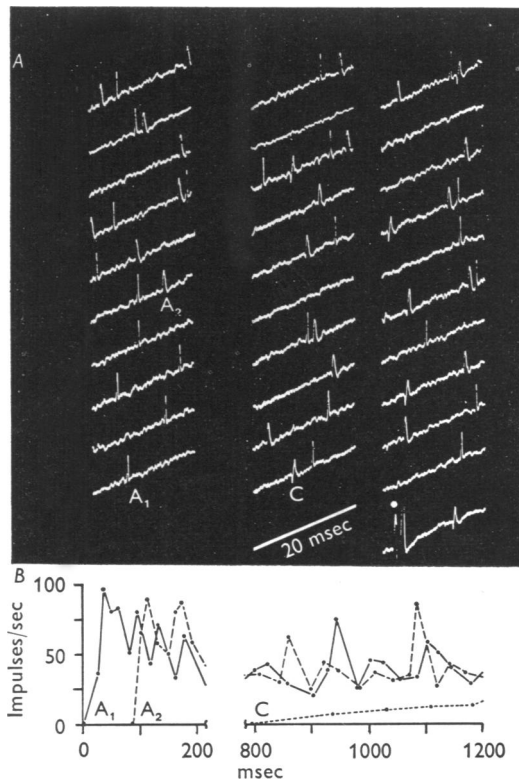


Fig. 8. Relative response latencies of chemoreceptor A and C-fibres. Cat breathing 50 % $O_2$ in $N_2$. Two A-fibres ($A_1$ and $A_2$) and one C-fibre (C) recorded from small filament of carotid nerve. Conduction velocities: $A_1$, 16 m/sec; $A_2$, 12 m/sec; C, 0·7 m/sec (trace with stimulus marked by white dot). *A*, sweeps are continuous and read from bottom upwards in each column. Columns compressed slightly for illustration. NaCN (10 $\mu$g) injected nearly 1 sec before start of left column. Approximately 600 msec elapse between left and middle columns. Middle column begins with first C-fibre response and is continuous with right column; *B*, graph of records in *A*. Ordinates: impulses/sec measured as reciprocal of interval between impulses. Abscissae; time in msec measured from first response of fibre $A_1$.

injection and was followed 85 msec later by the response of fibre $A_2$. Approximately 800 msec after the first A-fibre discharge, the records of the middle column begin with the first C-fibre response. These responses are graphically presented in Fig. 8*B*, which illustrates as well the more gradual onset of the C-fibre discharge compared with that of the two A-fibres.

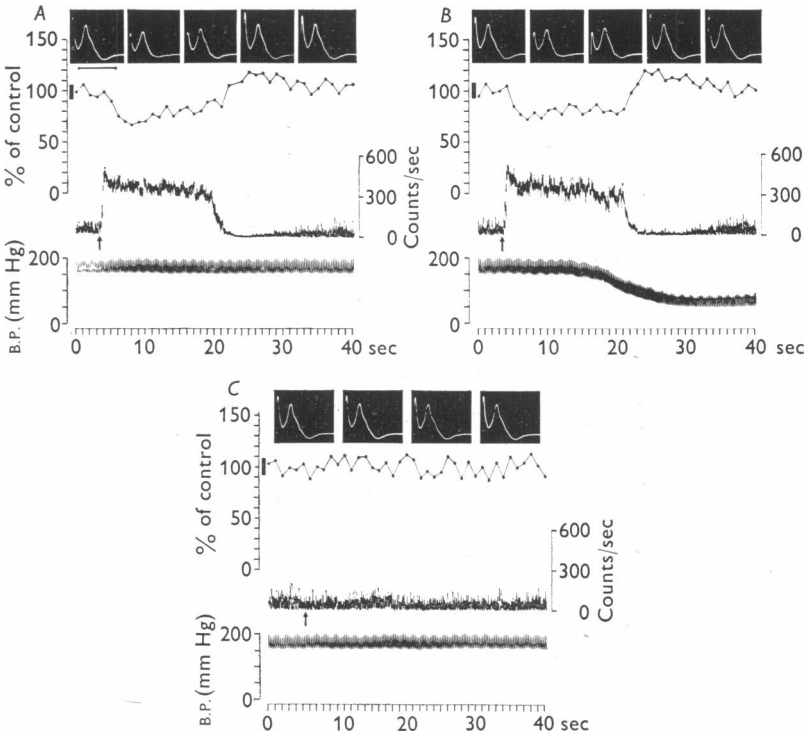


Fig. 9. Interaction of chemoreceptor C-fibre activity with evoked C-fibre compound action potential. Format same as for Fig. 4. *A*, 30 $\mu$g NaCN (1 ml.); *B*, 30 $\mu$g ACh (1 ml.); *C*, normal saline (1 ml.). Time marker for sample records of C-fibre potential, 10 msec.

In all, four filaments were obtained, and the response characteristics of seven chemoreceptor A-fibres and four chemoreceptor C-fibres were compared. In every instance, the A-fibres responded with a shorter latency than the C-fibre. This was true whether NaCN, ACh, acidified saline or $N_2$ was used to excite the units. The difference in response latency between A and C-fibres varied from 300 msec to nearly 2 sec. Although the A-fibre(s) in each filament invariably discharged before the C-fibre, the latency difference was not constant for any given A and C-fibre pair, but varied with repeated injections of the same stimulus. Furthermore,

although the difference in the threshold between A and C-fibres could not be determined quantitatively, it was possible with every filament to find a dose level which excited the A-fibre(s) but not the C-fibre.

*The proportion of chemoreceptor C-fibres in the cat carotid nerve.* The method of evoked-potential depression used to estimate the percentage of chemoreceptor A-fibres (cf. Fig. 4) was also employed to test for the percentage of chemoreceptor C-fibres. Figure 9 illustrates the depression of the C-fibre compound action potential during intra-arterial injections of 30 $\mu$g NaCN (*A*) and 30 $\mu$g ACh (*B*). The depression increased gradually to peak values over a period of several seconds, closely mirroring the frequency curve of unitary C-fibre response (cf. Fig. 7*B*). The percentage

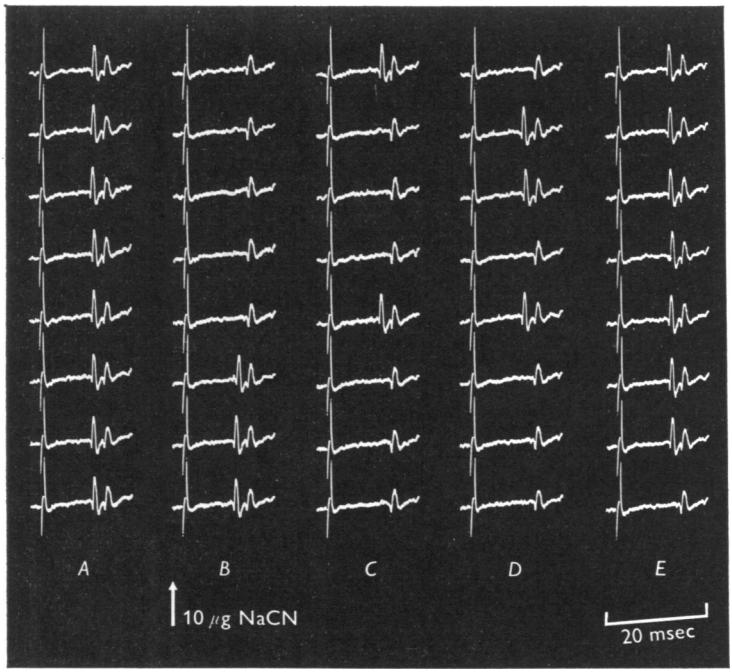


Fig. 10. Evoked potential of chemoreceptor C-fibre (larger, shorter latency unit) from experiment of Fig. 9, conduction velocity 0·9 m/sec. Format same as for Fig. 5. NaCN (10 $\mu$g) injected at start of column *B* results in failure of evoked potential of chemoreceptor C-fibre.

depression of the evoked C-fibre potential varied in sixteen experiments from 7 to 39%, with a mean of 24% from eighty-two injection trials (forty-two NaCN, forty ACh). In the particular experiment shown in Fig. 9, accidental injury to the A-fibres considerably reduced the size of the A-potential (insets). This damage was fortuitous in that it eliminated much of the mixing of the evoked A and C-fibre potentials which was

often the unavoidable consequence of recording only 5–6 mm from the stimulating electrodes. Such overlap makes the area measurements of the evoked potentials unreliable. In a number of experiments, it was possible to apply light pressure to the carotid nerve to block conduction along many of the A-fibres without affecting the size of the C-fibre potential. However, area measurements of C-potential depression still exhibited considerable variation around a slightly lower mean of 20%. In either case, whether 24 or 20% is taken as the value of C-potential depression, the percentage of chemoreceptors in the C-fibre population of the carotid nerve is still comparable with the value of 17% obtained with the method of unitary C-fibre dissection.

As the depression of the single evoked A-fibre potential of Fig. 5 paralleled the depression of the compound A-potential, so too the single evoked C-fibre potential of Fig. 10 reflected the depression of the compound C-potential. In this instance, the evoked responses of two C-fibres were present, both having identical thresholds to the electrical stimulus. The larger, shorter latency unit exhibited the response characteristics of chemoreceptor C-fibres. The other unit failed to respond to any of the chemoreceptor or baroreceptor stimuli described earlier and for this reason was considered to be efferent in origin (see later). Following an injection of 10 $\mu$g NaCN, the electrical stimulus failed to evoke the chemoreceptor C-fibre potential (Fig. 10*B*), but continued to evoke the efferent C-fibre potential. This difference in behaviour as a result of the injection is further proof that the failure of the evoked response of the chemoreceptor fibre is not an artifact of the experimental method.

*Baroreceptor C-fibres*

A total of fifteen of the fifty-two C-fibres dissected from the carotid nerve, or approximately 29%, exhibited the pulse-synchronous discharge and low threshold to mechanical stimuli characteristic of baroreceptors. The unit shown in Fig. 11 discharged at a rate of 2·5–3·0/sec in phase with the carotid pulse (control). Probing the carotid sinus with a small glass rod provoked a short barrage of impulses (pressure). Although the relative discharge characteristics of baroreceptor A and C-fibres have not been extensively investigated, it appears that baroreceptor C-fibres, like chemoreceptor C-fibres, have a lower frequency of discharge and perhaps a higher threshold than their A-fibre counterparts. Under the conditions of our anaesthetized cat preparations, baroreceptor C-fibres seldom discharged more than 1–2 impulses per pulse wave, whereas baroreceptor A-fibres commonly responded with 3–5 impulses or more.

It has been well established that non-medullated fibres are sensitive to ACh (Armett & Ritchie, 1960, 1963). In Fig. 11*A* close intra-arterial

injection of 80 $\mu$g ACh excited the baroreceptor C-fibre, while 20 $\mu$g ACh was ineffective. This latter dose, however, invariably excited any carotid chemoreceptor fibre, whether A or C, and it was consistently found that baroreceptor C-fibres exhibited higher thresholds to injections of ACh than chemoreceptor C-fibres.

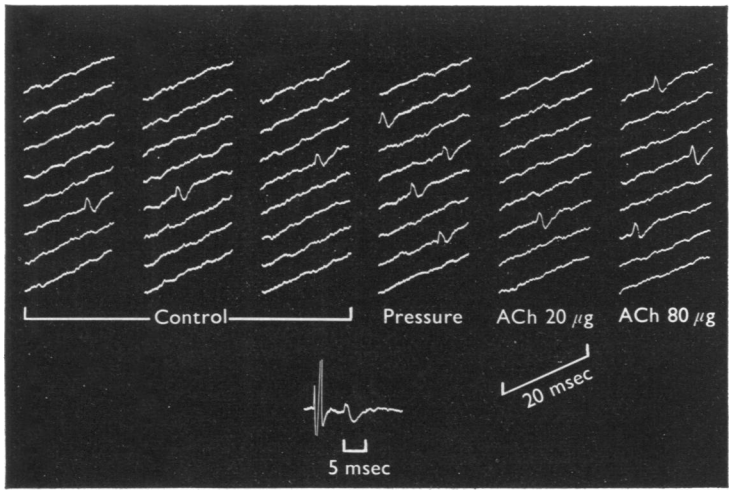


Fig. 11. Baroreceptor C-fibre, conduction velocity 0·53 m/sec. Records read from bottom upwards and 40 msec elapse between the start of each sweep. Columns compressed for illustration. Control: from left to right, the three columns are continuous. Note single impulse discharge every nine sweeps (approximately every 360 msec); pressure, light pressure to carotid sinus area provokes short barrage of impulses; ACh 20 $\mu$g and ACh 80 $\mu$g: equal volumes injected, peak discharges shown.

*Sympathetic and non-sympathetic efferent C-fibres*

This classification was comprised of those C-fibres which did not respond to any of the above-mentioned chemoreceptor or baroreceptor stimuli, even when the stimulus intensity was raised many-fold higher than the usual threshold for known chemoreceptor and baroreceptor C-fibres. Furthermore, the C-fibres in this group, without exception, failed to exhibit any resting discharge. In the present experiments, both the carotid nerve and the ganglio-glomerular nerve(s) were cut, and thus the absence of 'spontaneous' or induced activity suggests that these C-fibres may be efferent in origin. The carotid nerve is known to contain large numbers of sympathetic C-fibres (Eyzaguirre & Uchizono, 1961; Eyzaguirre & Lewin, 1961*b*), and recent evidence suggests that some non-sympathetic C-fibres which may have an inhibitory effect upon carotid body afferent discharge are present in the carotid nerve (Biscoe & Sampson, 1968; Neil & O'Regan,

1969). In all, twenty-eight units or approximately 54% of the C-fibres studied were included in this category.

Figure 12 illustrates a particularly interesting phenomenon encountered while studying this group of C-fibres. In Fig. 12*A*, a single shock to the carotid nerve evoked the characteristic all-or-none response of a unitary C-fibre potential. A slight increase in the stimulus intensity brought in the response of a second C-fibre (Fig. 12*B*). Further increase in stimulus intensity recruited still more C-fibres resulting in the potential configurations

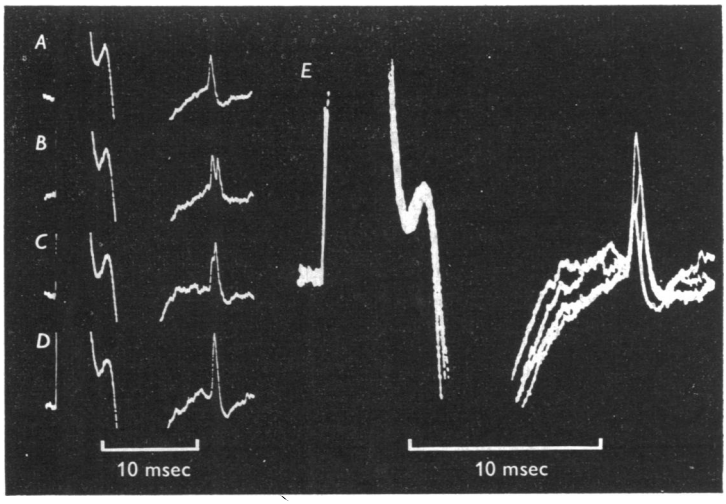


Fig. 12. Stepwise C-fibre response. *A*, evoked response of single C-fibre; *B* to *D*, increasing stimulus intensity recruits several more C-fibres with approximately the same latency; *E*, photographic superimposition of records *A* to *D*. Conduction velocity, 0·65 m/sec.

of Fig. 12*C* and *D*. Increasing the stimulus intensity beyond that of record *D* failed to increase the size of the potential. In raising the stimulus intensity from record *A* to *D*, the recruitment of C-fibres always occurred in this reproducible stepwise fashion, assuming only the four potential configurations shown by the records. Photographic superimposition of records *A* to *D* is shown greatly enlarged in *E*. It may be seen that at least 4 C-fibres contribute to the response, all with nearly identical latencies. This stepwise C-fibre response has been observed upon three other occasions; one involving three fibres, and two involving two fibres each.

Morphologically, C-fibres are surrounded by a Schwann cell membrane and each Schwann cell is invaginated by several C-fibres (Gasser, 1955). It is interesting to speculate that the stepwise C-fibre response observed in our experiments is related to this Schwann cell–C-fibre complex. It may be that with electrical stimulation some or all of the C-fibres within a

single Schwann cell complex can exhibit similar conduction velocities. Although this stepwise C-fibre response has not been observed amongst either chemoreceptor or baroreceptor C-fibres, it may, nonetheless, be present in these fibre groups also, since their sample populations were considerably smaller than this efferent C-fibre group.

## DISCUSSION

A study of chemoreceptor A and C-fibres in the carotid nerve has shown that these fibre groups can be distinguished on the basis of their response characteristics. Chemoreceptor C-fibres exhibited a higher threshold, longer latency of response, more gradual acceleration and lower frequency of discharge than their A-fibre counterparts. In other systems where A and C-fibres subserve the same functional modalities, similar distinctions in discharge properties have been observed. Cutaneous afferent C-fibres, for example, have higher thresholds and lower frequencies of response than their respective A-fibres (Iggo, 1960). Apart from the difference in the refractory period of A and C-fibre axons, which may in part account for the difference in peak discharge rates, the higher threshold, longer latency and slower acceleration of chemoreceptor C-fibre response suggest a sensory terminal differentiation as well. In contrast to our observations on carotid chemoreceptors, chemoreceptor A and C-fibres in the aortic nerve are not distinguishable on the basis of their response to natural stimulation (Paintal, 1967). Furthermore, whereas A-fibres show a lower threshold and shorter latency of response to ACh than C-fibres in the carotid body, the reverse is true of aortic body chemoreceptors. Also, the dose of ACh required to excite the C-fibres of the aortic body was high (30–100 $\mu$g) compared with that for carotid body C-fibres (5–20 $\mu$g); the greater flow in the aortic region may account for this difference. Paintal (1967) concluded that the lower threshold to ACh of aortic body C-fibres compared with A-fibres resulted from the drug's action upon the sensitive spike initiating zone of C-fibre axons, and not from its action on the sensory terminals themselves. However, it would be difficult to reconcile such a schema with the observed action of ACh upon carotid body A and C-fibres. Thus, the two chemoreceptor organs appear to be more dissimilar than has been assumed.

Interaction of whole nerve activity with the evoked compound action potential has provided a method of estimating the percentage of chemoreceptors in the A and C-fibre populations of the carotid nerve. The method was used as an approximation, and its accuracy is admittedly unconfirmed. Apart from the limitations already described (p. 534), the action potentials of small fibres contribute proportionately less to the compound action potential than those of large fibres. The relationship between the

area of the compound action potential and the number of responding fibres is, therefore, not linear. Despite these limitations, the estimated percentage of chemoreceptors in the C-fibre population of the carotid nerve agrees favourably with the value obtained by unit sampling procedures. Furthermore, the sum of the estimated chemoreceptor and baroreceptor A-fibre fractions can account for nearly the entire A-fibre population of the carotid nerve.

With this method it was estimated that the A-fibre population is comprised of approximately 2/3 chemoreceptors and 1/3 baroreceptors. The reverse is true, however, of the C-fibre population, where fifteen of the twenty-four afferent C-fibres were baroreceptors. These baroreceptor C-fibres may have a central reflex action similar to that of A-fibre baroreceptors. Therefore, this dominance of baroreceptor C-fibres strengthens the earlier assumptions of Douglas & Ritchie (1956) that the powerful depressor reflex which they elicited by intense electrical stimulation of the carotid nerve was mediated by unmyelinated afferents.

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