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THE EFFECT OF SYMPATHETIC STIMULATION ON CAROTID NERVE ACTIVITY

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An effect of sympathetic activity on carotid-body chemoreceptor discharge might be expected, since the carotid body receives an abundant nerve supply from the superior cervical ganglion (de Castro, 1926). Moreover, Floyd & Neil (1952) have found a small but definite increase in carotid-nerve activity when the sympathetic branches supplying the carotid body were stimulated. Hereafter, the nerve(s) linking the superior cervical ganglion with the carotid body will be referred to as the ganglio-glomerular nerve(s). This term is not entirely adequate, since many of the nerve fibres join the carotid nerve close to the glomus; they continue in such a nerve to join the glossopharyngeal nerve (see Eyzaguirre & Uchizono, 1961). Floyd & Neil interpreted this effect as increased chemoreceptor discharge. A significant problem remains, however, namely whether or not sympathetic effects, if present, are due to a direct action upon the sensory receptors or to construction of local vessels. In other sensory receptors such as tactile units of the frog's skin (Loewenstein, 1956) and mammalian spindles (Hunt, 1960) sympathetic actions may be exerted by liberation of adrenaline or noradrenaline from the sympathetic terminals, acting directly on neighbouring nerve endings (for an opposite view see Eldred, Schnitzlein & Buchwald, 1960). In the case of the carotid-body chemoreceptors sympathetic effects which have been observed might well be indirect, since such studies have been performed in the whole animal where a reduction of glomerular blood flow is known to occur during stimulation of the cervical sympathetic trunk (Daly, Lambertsen & Schweitzer, 1954).

In the present study the possibility of a direct sympathetic action on chemoreceptors was investigated *in vitro*, where vascular effects are absent, and the results obtained in the excised preparation were compared with similar observations in the whole animal. Results presented below indicate that stimulation of fibres emerging from the superior cervical ganglion

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in vivo produces only a small increase in chemoreceptor discharge apparently due to vasoconstriction. No evidence of a direct sympathetic effect either on the sensory nerve endings or on glomerular cells has been found. However, a number of non-myelinated (C) fibres of sympathetic origin elicit potentials in the carotid nerve upon stimulation and their response may be confused with normally occurring sensory discharges. A preliminary report has already been published (Eyzaguirre, Uchizono & Lewin, 1961).

METHODS

Adult cats were anaesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories) 40 mg/kg I.P. One series of experiments was performed in the animal while another series was done *in vitro*. For experiments performed *in vivo* the animal was allowed to breathe the room air. Its rectal temperature was kept at 37–38° C. The neck was opened in order to expose the superior cervical ganglion, its preganglionic fibres and its glomerular connexions. The exposed surface was covered with warm mineral oil. Stimulating electrodes were placed either on the preganglionic trunk or on the ganglio-glomerular nerve. Nerve action potentials recorded from the carotid nerve were fed to an amplifier, which in turn was connected to a double-beam oscilloscope and to a counter-printer system. With this system the potentials could be displayed, photographed and their frequency per second recorded once every 2 sec (cf. Eyzaguirre & Lewin, 1961*a*). In some animals recording of carotid nerve impulses was accomplished 2–6 days after aseptic glossopharyngeal section in the neck performed under sodium pentobarbital anaesthesia. For *in vitro* experiments the carotid body and its own nerve plus a stretch of carotid artery were excised together with their sympathetic connexions, i.e. the superior cervical ganglion, a length of the preganglionic trunk and the ganglio-glomerular nerve. The excised tissues were placed in a Petri dish containing Locke's solution for further dissection and removal of unwanted connective tissues. Care was taken to decapsulate the ganglion soon after excision in order to facilitate diffusion of substances. The tissues were then mounted in a transparent chamber containing Locke's solution (pH 7·4) at about 36° C, previously mixed with glucose 1·0 g/l. The saline was bubbled with a mixture of 94 % O_2 and 6 % CO_2 and covered by a layer of mineral oil. When substances were applied locally the whole preparation was placed in oil and the desired portion was lowered into a small box containing the substance dissolved in Locke's solution (cf. Eyzaguirre, 1960). Stimulating electrodes could be placed either on the sympathetic preganglionic fibres, on the ganglio-glomerular nerve or on the carotid nerve. Recording of action potentials from the carotid or ganglio-glomerular nerves was accomplished in a manner similar to that already described for experiments *in vivo* (see also Eyzaguirre & Lewin, 1961*b*).

The membrane potential of glomerular cells was measured with intracellular micropipettes filled with 3 M-KCl, having an a.c. resistance of from 10·5 to 70 MΩ. The glomerular capsule proved too tough for easy penetration of the micro-electrode, consequently the glomus was immersed in a solution of chymotrypsin (1 mg/ml.) in Locke's solution and digestion was allowed to proceed for 30–60 min. After this procedure the sensory discharges did not change and penetration of the glomerular cells was readily accomplished. Since membrane potentials were low the tip potential of each electrode was measured (cf. del Castillo & Katz, 1955). Tip potentials ranged from less than 5 to 45 mV but they did not appreciably alter the recorded membrane potential values.

RESULTS

Changes in carotid-nerve impulses during sympathetic stimulation

Stimulation of preganglionic fibres. In the cat, repetitive preganglionic stimulation at 5–10/sec produced an increase in the frequency of carotid-nerve impulses. Once stimulation was discontinued the frequency declined rapidly to base-line levels (Fig. 1A). In order to determine whether or not this effect was acting upon chemo- or pressoreceptor, the carotid nerve was split into a number of small filaments; care was taken to record activity from chemoreceptors to the exclusion of pressoreceptor discharges. Such an experiment is presented in Fig. 1B, where it may be seen that sympathetic effects on this small filament are quite similar to those obtained from the whole nerve. Splitting the nerve revealed that preganglionic stimulation produced, besides the normally occurring sensory discharges, a polyphasic potential which appeared after a constant latency (Fig. 2A). The waves of this complex were small enough in the intact nerve to be interspersed with the background discharge, thus adding to the counts of sensory impulses.

The contribution of sympathetic fibres to carotid-nerve activity was further analysed by recording from carotid nerves in which afferent fibres had been caused to degenerate by section of the nerve centrally from 2 to 6 days before the experiment. These nerves showed little or no background discharge. Sympathetic stimulation evoked a compound action potential in the carotid nerve, which appeared after a constant latency. These potentials were clearly recognized and photographed from the oscilloscope screen (Fig. 2B and C); they corresponded to activity derived from a fairly large number of C fibres (see later). The C-fibre potentials thus evoked were relatively rapid and were readily recorded by the electronic counter, as shown in Fig. 1C. At rest the counter picked up some of the amplifier noise. Between arrows the sympathetic trunk was stimulated at 10/sec and the sudden appearance of the sympathetic potentials was evident by the rapid increase in the counts. These potentials disappeared abruptly once the stimulator was turned off. Experiments performed *in vitro* yielded essentially similar results, although the potentials of sympathetic origin were more conspicuous, owing to less background discharge (see below).

In conclusion it may be stated that the appreciable increase in counts obtained from the carotid nerve during preganglionic sympathetic stimulation is largely produced by the appearance of polyphasic potentials of sympathetic origin. This would also appear in records obtained from the oscilloscope screen and it may lead one to assume, erroneously, that chemoreceptor discharges have increased.

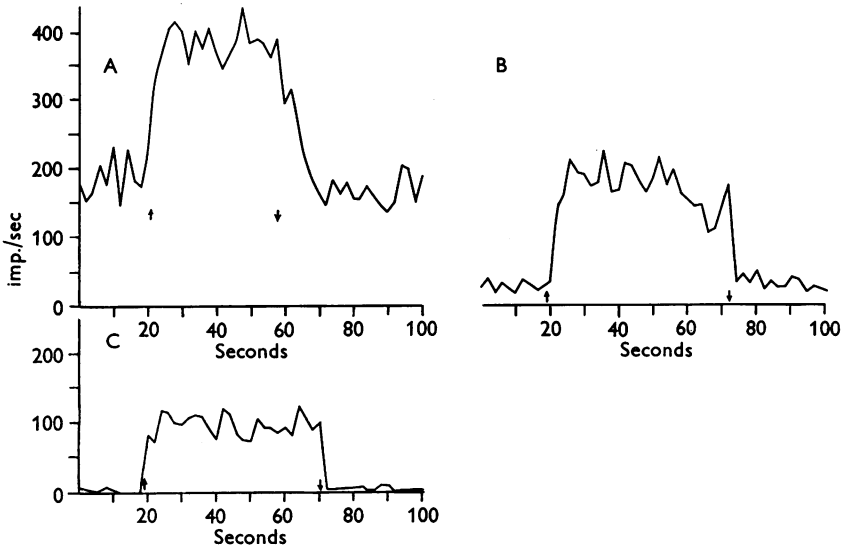

Fig. 1. Changes in carotid nerve impulses during sympathetic stimulation. Preparation *in situ*. A, B and C, stimulation of preganglionic sympathetic trunk at 10/sec. A, recording from the whole nerve; B, pressoreceptors eliminated by dissection; C, recording from carotid nerve centrally severed 6 days previously.

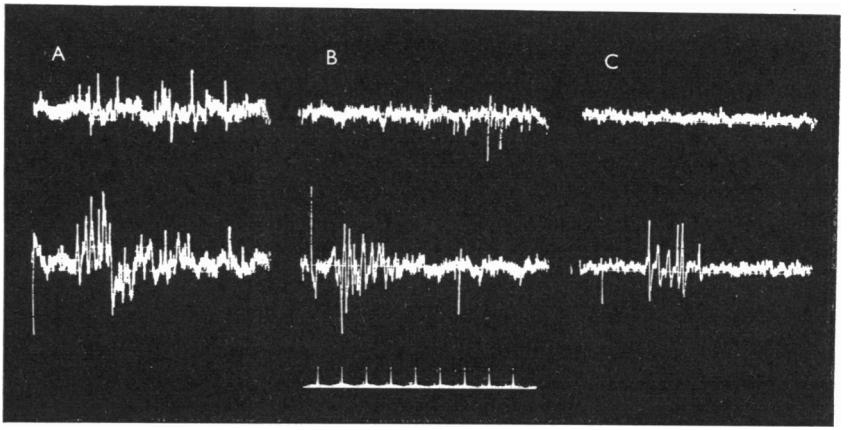

Fig. 2. Effect of single pre-ganglionic shocks on carotid nerve impulses. Preparation *in situ*. In all columns the upper traces represent base-line discharge; lower traces show the evoked sympathetic potentials. A, recording from normal carotid nerve; B, carotid nerve centrally severed 48 hr previously; C, recording 6 days after central section of carotid nerve. Time marker, 10 msec.

Stimulation of post-ganglionic fibres. Since preganglionic sympathetic stimulation increased the counts from the carotid nerve, sometimes showing a short period of facilitation and a gradual return to resting discharge frequency, it was suspected that a small sympathetic effect on chemoreceptor discharge might be present. In order to study this effect a series of experiments was performed in the animal and another series *in vitro*. In both cases the superior cervical ganglion was removed and the ganglio-glomerular nerve was placed on stimulating electrodes. Removal of the ganglion was carried out in order to avoid ganglionic facilitation during repetitive stimulation. Recording was done in the animal from small carotid-nerve filaments containing no pressoreceptor fibres; *in vitro* recording was done from the whole nerve but pressoreceptor discharges were eliminated by keeping the artery unstretched and by removing the adventitia (cf. Alvarez-Buylla, 1954; Eyzaguirre & Lewin, 1961*b*).

Stimulation of the ganglio-glomerular nerve *in vivo* usually did not produce any change in the frequency of chemoreceptor discharge. In a few instances, however, a small but definite increase was obtained. In these experiments the relatively slow potential recorded from the carotid nerve and evoked by ganglio-glomerular stimulation was not recorded by the counter. The instrument was gated in such a way as to avoid relatively slow waves. Such an experiment is illustrated in Fig. 3A. Between the arrows the ganglio-glomerular nerve was stimulated at 10/sec and a slow increase in chemoreceptor frequency was obtained.

The small increase in chemoreceptor activity produced by sympathetic stimulation observed in the cat could be explained either by a direct sympathetic action on these receptors or by glomerular vasoconstriction (Daly *et al.* 1954). These possibilities were elucidated by studying the effect of sympathetic stimulation on carotid-nerve activity in the excised preparation. Such an experiment is illustrated in Fig. 3B. The relatively low background discharge may be readily explained, since the preparation was bathed with saline equilibrated with 94 % O_2 and 6 % CO_2. Between the arrows the ganglio-glomerular nerve was stimulated at 10/sec. No changes in chemoreceptor activity could be detected. In all cases studied *in vitro* chemoreceptor frequency remained unchanged during sympathetic stimulation. It is concluded that the small increase in chemoreceptor discharge obtained in the animal is due to glomerular vasoconstriction, since direct sympathetic effects on chemoreceptor discharges have not been found.

The complex potential evoked in the carotid nerve by preganglionic sympathetic stimulation

The experiments already described showed the presence of a polyphasic potential complex in the carotid nerve during sympathetic stimulation

(Fig. 2). However, owing to the abundant background discharge, such potentials were clearly recorded in the animal only after elimination of a number of afferent fibres by dissection or by degeneration. An analysis of the sympathetic potentials obtained from the whole carotid nerve was possible in excised preparations when the artery was unstretched, the adventitia removed and the preparation bathed in saline equilibrated with a high concentration of oxygen. With these procedures the sensory discharge background was drastically reduced and appropriate placing of the recording leads brought the sympathetic potentials into full view.

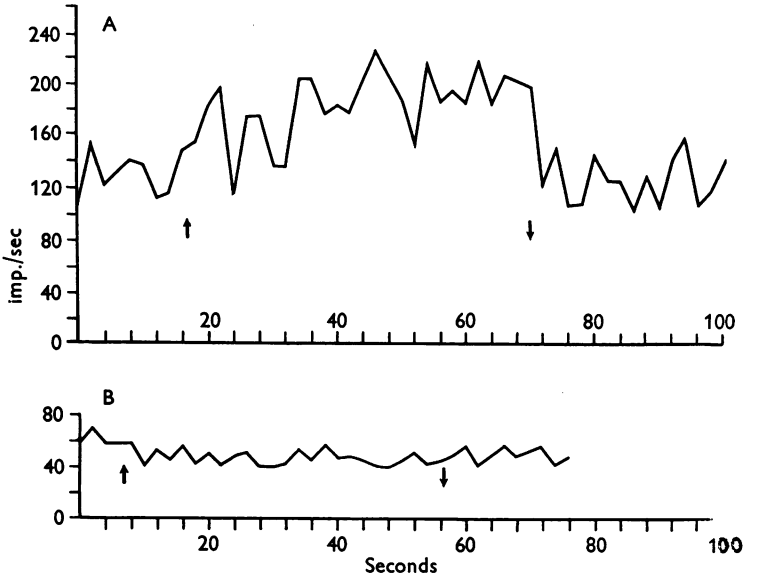

Fig. 3. Increase of chemoreceptor discharge during ganglio-glomerular stimulation in the animal and lack of effect *in vitro*. A, recording from carotid nerve *in situ*. B, recording from carotid nerve *in vitro*. In A and B ganglio-glomerular nerve stimulated at 10/sec.

The carotid-nerve potential of sympathetic origin was studied during stimulation of the preganglionic fibres with single and double shocks at varying intervals. Potentials from the carotid nerve and from the sympathetic ganglion were simultaneously recorded by independent leads which were connected to the double-beam oscilloscope through separate amplifiers. The ganglionic potentials were registered by placing the leads on the ganglion, one at each pole. Figure 4 illustrates an experiment in which single shocks of increasing intensity were delivered at 0·6/sec. In the illustration the upper trace shows the potentials recorded from the carotid nerve, while the lower trace shows the potentials recorded from the sympathetic ganglion. Submaximal preganglionic volleys produced a

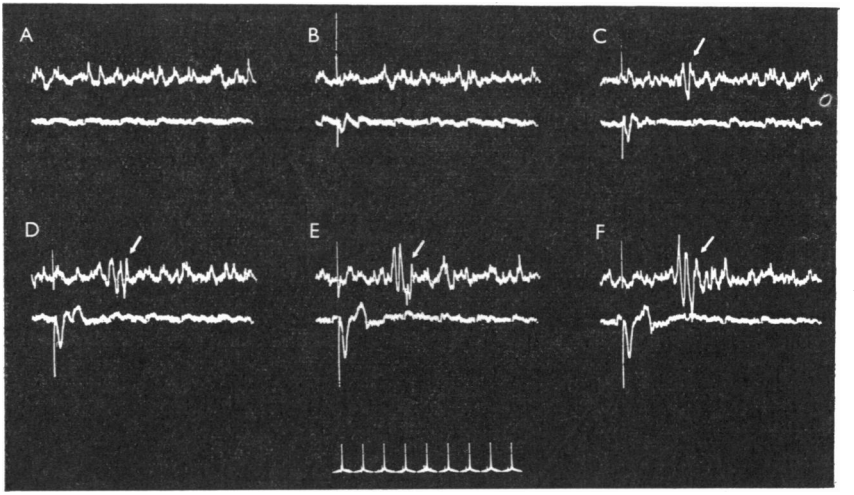

Fig. 4. Effects of preganglionic shocks of increasing intensity on carotid-nerve impulses. The upper traces show recording from carotid nerve while the lower ones show potentials obtained from the superior cervical ganglion. Excised preparation. A, base lines; B, C, D, E, and F, potentials obtained by increasing stimulus strength. Arrows indicate the carotid nerve potential of sympathetic origin. Time marker, 10 msec.

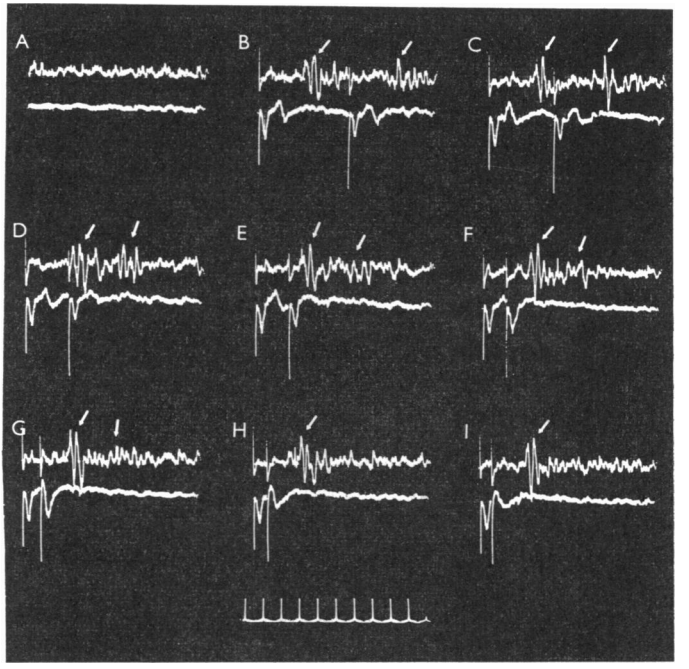

Fig. 5. Same as Fig. 4 but this time double shocks at varying intervals were applied to the preganglionic sympathetic fibres. Note the dispersion and disappearance of the second carotid nerve potential at close stimulating intervals. Time marker, 10 msec.

small polyphasic action potential in the carotid nerve, which increased in amplitude as the stimulus strength was increased. The total duration of this complex was about 10 msec (arrows). This temporal dispersion indicates that maximal preganglionic volleys activate fibres of different conduction velocities. The intervals between the different peaks of the polyphasic complex were too long (more than 2 msec) to be accounted for only by synaptic delay. When in the same experiment two shocks were given at decreasing intervals, the second carotid-nerve potential became smaller and temporal dispersion even more noticeable (Fig. 5). Figure 5B and C shows that at shock intervals of 50 and 45 msec respectively the polyphasic response of the carotid nerve (upper beam) is relatively well preserved after the second shock, but there is a deficit in the amplitude of the second ganglion potential (lower trace). When the second shock was given at intervals of 18 msec a smaller and highly polyphasic potential of longer duration was recorded from the carotid nerve following a smaller ganglion potential. When the shocks were separated by an interval of 10 msec the second carotid-nerve potential was even smaller and more dispersed (Fig. 5G). At shorter intervals (8 and 6 msec respectively) the second carotid-nerve complex became even more dispersed and was hardly noticeable (Fig. 5H and I).

Neuronal origin of the sympathetic fibres in the carotid nerve

Experiments were designed to study whether or not the sympathetic fibres in the carotid nerve had their synaptic origin in the superior cervical ganglion. In the excised preparation the tissues were lifted into oil and the sympathetic ganglion was lowered into a chamber containing a solution of a ganglionic blocking agent. The sympathetic preganglionic fibres were stimulated by maximal shocks at 0·6/sec. One pair of recording electrodes registered activity from the carotid nerve while another pair of leads was placed on the ganglio-glomerular nerve.

Metubine (dimethyl-tubocurarine iodide; Lilly) in concentrations of 5×10^{-6} to 5×10^{-4} (w/v) or hexamethonium (hexamethylenebis(trimethylammonium chloride); Burroughs Wellcome) in concentrations of 5×10^{-6} (w/v) were employed. The results obtained with either substance were similar: the sympathetic ganglion was blocked and the complex potential evoked in the carotid nerve by preganglionic sympathetic stimulation disappeared. Such an experiment is illustrated in Fig. 6. Figure 6A shows the base-line discharge. In figure 6B the upper trace illustrates the evoked carotid-nerve action potential complex lasting about 12 msec. The lower trace shows the potential registered from the ganglio-glomerular nerve. Between B and C, hexamethonium was applied to the ganglion. In D, 7 min after application of the drug, the carotid-nerve

complex is reduced in amplitude and more dispersed. The ganglio-glomerular nerve potential is also smaller. These phenomena continue their course in E and F. In G the carotid-nerve complex has disappeared almost entirely while very little is left of the ganglio-glomerular potential. After complete ganglionic block the stimulating electrodes were placed on the ganglio-glomerular nerve and single shocks evoked a large potential in the carotid nerve (not illustrated).

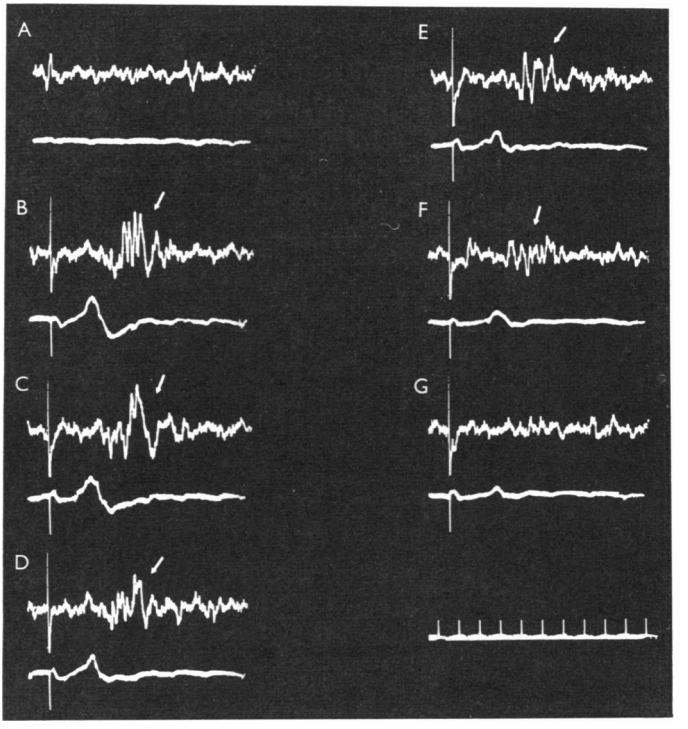

Fig. 6. Effect of hexamethonium applied to the superior cervical ganglion on the carotid-nerve potential of sympathetic origin. Upper traces show the carotid-nerve impulses while the lower traces show the potentials obtained from the ganglio-glomerular nerve near the ganglion. Excised preparation. A, base line; B, control record showing the effect of a single shock applied to preganglionic sympathetic fibres; C, 3 min after local application of hexamethonium (5×10^{-6} (w/v)); D, 7 min after the drug; E, 11 min after C_6; F, 14 min after the drug; G, 34 min after C_6. Time marker, 10 msec.

Essentially similar results were obtained in the animal when recording was done from filaments of a normal nerve or one in which the afferent fibres had degenerated. Hexamethonium in doses of 5 mg/kg given intravenously completely abolished the carotid-nerve sympathetic potential in a few seconds. After several minutes the potential returned to normal.

From these experiments it is concluded that most of the sympathetic fibres giving rise to activity in the carotid nerve had their origin in the superior cervical ganglion.

Absence of synapses in the carotid-nerve sympathetic pathway at the glomerular level

The preceding section showed that the majority of the carotid-nerve sympathetic fibres must have their somata in the superior cervical ganglion. However, the possibility exists that some synapses with ganglion cells could have been missed. Consequently, experiments were designed to test

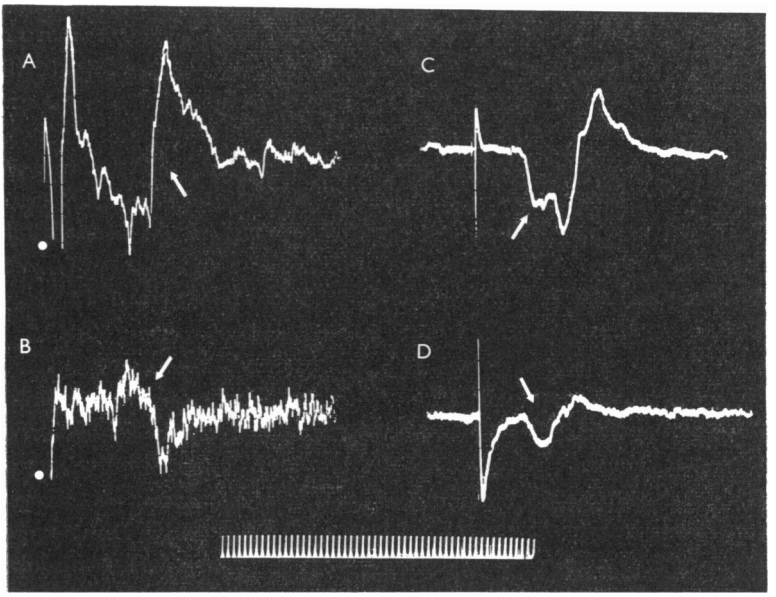

Fig. 7. Recording of C-fibre potentials from carotid and ganglio-glomerular nerves. Excised preparation. A and C show the sympathetic potential recorded from the carotid nerve during stimulation of the ganglio-glomerular nerve. B and D show the sympathetic potentials recorded from the ganglio-glomerular nerve during stimulation of the carotid nerve. Note the coincidence of peaks as shown by arrows. Time marker, 1 msec.

for the presence of synapses in the carotid-nerve sympathetic pathway in or around the glomus. Experiments were performed to search for a synaptic barrier to 'antidromic' stimulation and cholinergic blocking agents were applied to the glomus.

'Antidromic' stimulation. In a set of experiments the excised preparation was left in oil while stimulating electrodes were placed on the ganglio-glomerular nerve and potentials recorded from the carotid nerve. After a characteristic potential complex was obtained the stimulating and recording

leads were reversed, care being taken to avoid displacement of the electrodes. In both instances the cathode was nearer to the first recording electrode. Such an instance is exemplified in Fig. 7. Figure 7A illustrates the potentials obtained by single maximal shocks delivered to the ganglio-glomerular nerve while potentials were recorded from the carotid nerve; B shows the reverse situation. It is evident in this column that the first complex (dot) elicited by 'orthodromic' stimulation is not properly recorded during 'antidromic' stimulation because it rides partly on the artifact. But the second 'orthodromic' complex (arrows) is clearly present in the 'antidromic' situation and their peaks coincide exactly. In both cases, however, the 'antidromic' potentials are considerably smaller, owing to the less favourable recording conditions. Indeed, the ganglio-glomerular nerve is short and thick, possessing a strong sheath which contains many fibres that do not travel in the carotid nerve (Eyzaguirre & Uchizono, 1961). Figure 7C, obtained from a different preparation, shows the 'orthodromic' potential presenting two peaks which correspond to the slowest components already described (potentials marked by arrows). In this experiment, the earlier wave, shown in the left-hand column (dot) was not obtained. Figure D shows the 'antidromic' potential (arrow) whose peak corresponds to the first peak of the deflexion obtained in C. The second peak of C did not appear clearly in D although it was visible when high amplification was used.

The smaller size of the potential recorded from the ganglio-glomerular nerve as compared to that obtained from the carotid nerve could be explained because of unfavourable recording conditions. However, the possibility still remained that a small number of synapses could have been missed in the 'antidromic' situation. Consequently, a second series of experiments was performed as shown below.

Application of cholinergic blocking agents. The excised tissues were lifted into oil while the glomus was lowered into a small chamber containing a solution of a cholinergic blocking substance. Care was taken to eliminate by dissection most of the connective tissues surrounding the carotid body to improve penetration of substances. Hexamethonium (5×10^{-5} (w/v)), Decamethonium (Decamethylenebis(trimethylammonium bromide); Burroughs Wellcome) in doses of $2{\cdot}5 \times 10^{-6}$ (w/v) or atropine sulphate (10^{-5} (w/v)) were applied in different experiments. Results were on the whole negative, since none of these substances appreciably changed the carotid nerve potential evoked by ganglio-glomerular stimulation. Essentially similar results were obtained in the animal when these substances were injected intravenously while the ganglio-glomerular nerve was stimulated and potentials were recorded from the carotid nerve. These drugs would be expected to block any type of cholinergic synapses if present.

Results presented in this section show that cholinergic synapses do not occur in the carotid-nerve sympathetic pathway once the fibres leave the superior cervical ganglion and reach the glossopharyngeal nerve. Some myelinated fibres, however, leave the ganglion to innervate some microganglia located near the glomus. The short post-synaptic fibres from the microganglia innervate some glomerular blood vessels (de Castro, 1926) but they do not seem to continue in the carotid nerve (see also Eyzaguirre & Uchizono, 1961).

Conduction velocity of the sympathetic fibres in the carotid nerve

In order to have an estimate of the conduction velocity of the fibres producing the sympathetic component of the carotid-nerve complex, the ganglio-glomerular nerve was stimulated and action potentials recorded from the carotid nerve in the excised preparation. The distance between the stimulating and recording electrodes was kept at 5 mm as measured with a calibrated eyepiece. In general, stimulation of the ganglio-glomerular nerve produced one or two complexes in the carotid nerve, apparently due to activation of two distinct fibre groups. One group had a conduction velocity of about 1·8 m/sec (potential marked by a dot in Fig. 7), the other 0·3–0·4 m/sec (potentials marked by arrows in Fig. 7). A better view of the potentials elicited by fibres having slower conduction velocity is shown in Fig. 8, where shocks of increasing intensity were delivered. In fig. 8A and B, a potential of increasing amplitude was obtained with a shock–peak latency of 13·2 msec. Stronger shocks brought in a second potential with a latency of 17·8 msec, which started to appear in C and which is quite evident in D, E and F. The conduction velocity of the fibres involved was calculated as 0·4 m/sec for those forming the first complex and 0·3 m/sec for those eliciting the second complex. It must be understood, however, that the conduction velocity estimates are only approximate; the electrodes could not be displaced to take measurements at different points in the nerve, which was too short for such a manoeuvre.

The experiments illustrated in Figs. 7 and 8 show that stimulation of the ganglio-glomerular nerve evokes activity in two distinct fibre groups, which may be recorded from the carotid nerve. Assuming that conduction velocity in such fibres is directly related to diameter by a factor of 1·7 (Gasser, 1955) one may estimate that one group of fibres has a diameter of about 1 μ while the other group has a diameter of from 0·1 to 0·3 μ.

Further information on the two types of slowly-conducting fibres was obtained by dissecting the carotid nerve into small filaments and recording the response obtained by stimulation of the ganglio-glomerular nerve. In several instances unitary activity of sympathetic fibres was obtained, since

the potentials showed a sharp threshold and all-or-none characteristics. Such an instance is illustrated in Fig. 9. Figure 9A is the base-line discharge from a small carotid-nerve filament. B and C show the small potential (marked by dots) evoked by stimulation of the ganglio-glomerular nerve. The conduction velocity of this unit was 1·66 m/sec. Figure 9D, E and F are from a different experiment. D shows the base-line discharge,

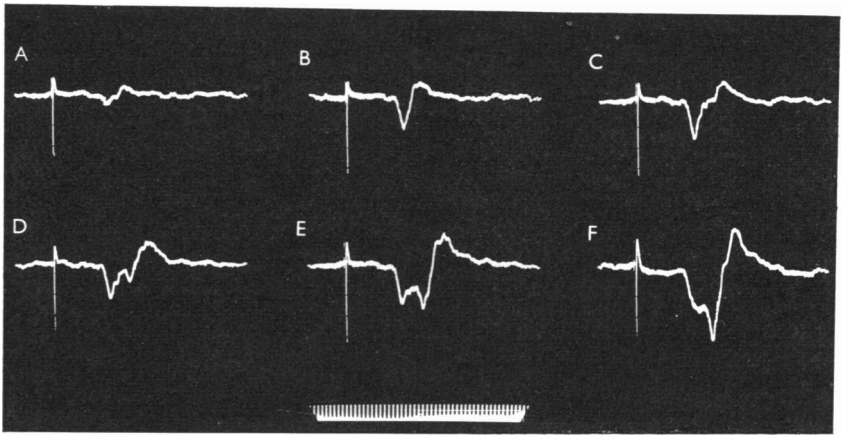

Fig. 8. Stimulation of ganglio-glomerular nerve and recording from carotid nerve. Excised preparation. From A to F, shocks of increasing intensity applied to the nerve. Time marker, 1 msec.

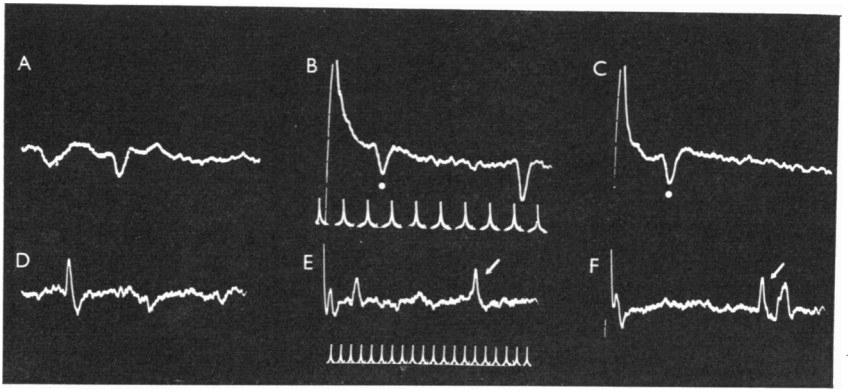

Fig. 9. Recording of single C-fibre potentials from carotid nerve. Excised preparation. Stimulation of ganglio-glomerular nerve at 1/sec. The two rows illustrate results obtained from two different experiments. A, B and C taken from same experiment. A, base line; B and C, a small C-fibre potential is recorded near the artifact (dots). D, E and F taken from different experiment. D, base-line discharge; E and F illustrate a C-fibre potential obtained from carotid nerve (arrows). Close to the artifact (E and F) a potential from myelinated fibre conducting at 5 m/sec is seen. Note similarity of C-fibre potential with normally occurring sensory discharge. Time markers, 1 msec.

while E and F illustrate the effect of single shocks delivered to the ganglio-glomerular nerve. A potential was evoked (arrows) from a unit which conducted at 0·34 m/sec, which clearly falls into the C-fibre group.

The fact that the amplitude of chemoreceptor impulses in Fig. 9 is of the same order of magnitude as that evoked by sympathetic stimulation poses the question whether or not in this case the afferent discharges were conveyed by sensory C fibres. Non-myelinated sensory elements have been described in the rabbit's carotid nerve (Douglas & Ritchie, 1956). C fibres, presumably subserving a sensory function, also occur in the homologous nerve of the cat (Eyzaguirre & Uchizono, 1961).

Lack of membrane potential changes in glomerular cells during sympathetic stimulation

De Castro (1926) has indicated that sympathetic fibres from the superior cervical ganglion are distributed in the glomerular interstitial spaces without making contact with the major glomerular cells. However, the possibility exists that stimulation of the sympathetic nerves might produce a detectable change in the membrane potential of the glomerular cells. Consequently, the glomerular cells were impaled with micro-electrodes and the sympathetic nerves were stimulated by placing electrodes on the cervical sympathetic trunk or on the ganglio-glomerular nerve. A total of 450 cells were impaled in this series. They showed low resting potentials (10–50 mV), the mode of the frequency–distribution curve being 20 mV. Sympathetic stimulation did not change the membrane potential of any of the cells studied.

In all probability the impaled cells belonged to the so-called 'chief' cells, since these are the largest and most numerous of glomerular cells (Adams, 1958). Their diameter ranges from 6 to 10μ (Ross, 1959). The other cellular elements in the carotid body (sustentacular cells, etc.) are probably too small to allow easy penetration of the micro-electrode. The low resting potentials obtained could have been due partly to injury produced by the insertion of the micro-electrode. However, resting potentials appeared suddenly during penetration and were fairly well maintained for periods long enough to test the effect of sympathetic stimulation.

From a physiological point of view these experiments have not decided the question of a synaptic contact between sympathetic fibres and glomerular cells. It is possible that sympathetic action might elicit some form of activity in these cells which does not appear as a resting-potential change.

DISCUSSION

The primary purpose of this study was to determine whether or not the carotid-body chemoreceptors are provided with an efferent control through fibres originating in the superior cervical ganglion. The observations reported here show that the role played by cervical sympathetic nerves in modulating the chemoreceptor discharge is small. Sympathetic effects when present seem to be entirely vascular. This finding falls into line with the observations of Daly *et al.* (1954), who found sometimes an 87 % increase in glomerular vascular resistance when the cephalic end of the cut cervical sympathetic trunk was stimulated. Direct sympathetic effects on the chemoreceptors seem to be absent, since, in the excised preparation stimulation of the cervical sympathetic nerves did not change the frequency of the chemoreceptor discharge or the membrane potential of the 'chief' glomerular cells.

In the search of an efferent control of the chemoreceptors one would have to look into possible efferent contacts provided by fibres of the carotid nerve. In this connexion it is worth noting de Castro's observation (1926, 1951) that degeneration of the ganglio-glomerular nerves does not change appreciably the fine non-myelinated plexus which innervates the arteries and arterioles of the glomus. It is his contention that a great part of the vascular innervation is provided by small non-myelinated fibres originating from a few ganglion cells interspersed among carotid-nerve fibres near the glomus. The preganglionic fibres of these structures have apparently a medullary origin. Whether or not activity of some of these (or other) fibres may modulate the chemoreceptor discharges is unknown.

A positive finding in this study is the presence of a nervous pathway of sympathetic origin in which fibres originating in the superior cervical ganglion course toward the glomus, where numerous fibres continue in the carotid nerve and then join the glossopharyngeal nerve. Some of the fibres, however, leave the glomus to innervate other regions in the neck (Eyzaguirre & Uchizono, 1961). Most of the carotid-nerve sympathetic fibres are non-myelinated, judging from their conduction velocities, and fall into two definite groups. One group conducts at 0·3–0·4 m/sec while the other group conducts at 1·6–1·8 m/sec. Their final destination and function is unknown.

SUMMARY

1. The effect of sympathetic stimulation on the carotid body chemoreceptors discharge was investigated both in the animal and on the carotid body *in vitro*. In the animal sympathetic stimulation may induce a small increase in chemoreceptor discharge. This effect is due to vasoconstriction,

since chemoreceptor discharges are not influenced by sympathetic activity *in vitro*.

2. Stimulation of preganglionic sympathetic fibres produces a polyphasic potential in the carotid nerve after a constant latency. This potential persists in the nerve even 6 days after severance of the glossopharyngeal nerve. The fibres giving rise to the carotid-nerve sympathetic potential have their somata in the superior cervical ganglion, since hexamethonium (injected into the animal or applied locally) blocks such a response.

3. The fibres producing the carotid-nerve sympathetic potential are mainly non-myelinated (C fibres). Two groups of fibres have been found: one group has a conduction velocity of from 1·6 to 1·8 m/sec while the second group of fibres conduct at 0·3–0·4 m/sec. Their final destination or function is unknown. This sympathetic pathway seems to pass by the glomus without the interposition of cholinergic synapses in or around the carotid body.

4. The membrane potential of the 'chief' glomerular cells (10–50 mV) remains unchanged during sympathetic stimulation.

5. It is concluded that the sympathetic control of the carotid body chemoreceptors is provided only through vascular effects. Other possible efferent pathways are discussed.

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