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THE ACTION OF ACETYLCHOLINE ON CONDUCTION IN MAMMALIAN NON-MYELINATED FIBRES AND ITS PREVENTION BY AN ANTICHOLINESTERASE

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Although acetylcholine excites nerve *endings* (Brown & Gray, 1958; Douglas & Gray, 1953; Zotterman, 1953; Gray & Diamond, 1957; Diamond, 1959; Douglas & Ritchie, 1960), there is a lot of experimental evidence which suggests that it does not act on the main part of the fibre running in the nerve trunk. For example, it does not initiate impulses when injected close-arterially into mammalian nerve trunks or when applied to amphibian single nerve fibres (Jarrett, 1956), it does not depolarize the fibre or block conduction (Lorente de Nó, 1944; Straub, 1955) and it does not alter the membrane conductance of squid giant axons (Hodgkin, 1947). However, in a recent series of experiments where acetylcholine was injected close-arterially into the cat's skin to study its excitatory effect on the endings of sensory non-myelinated fibres of mammalian cutaneous nerves (Douglas & Ritchie, 1960), a striking change was often observed in the shape of an antidromic C potential recorded from the nerve near the point where it left the skin. The type of change observed suggested that the after-potential had been altered. The affected region of the nerve was close to the skin, and it was observed that often the close-arterial injection not only reached the skin, but also perfused the nerve. It thus seemed likely that externally applied acetylcholine *could* alter the electrophysiological properties of mammalian C fibres, but with the techniques then used no definite conclusions could be reached. The present experiments use a technique more suited to this particular problem and deal with the action of acetylcholine on non-myelinated fibres in isolated mammalian nerves.

METHODS

Adult rabbits were anaesthetized with urethane (1·6 g/kg) given into a marginal ear vein as a 25% (w/v) solution. A length of about 60–70 mm of a cervical vagus nerve was excised and then desheathed under the microscope (magnification $\times 25$) by dissection with sewing needles sharpened to fine scalpels. In several experiments a similar length of the thoracic

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vagus near the level of the diaphragm was used; according to Evans & Murray (1954) this nerve contains hardly any myelinated fibres (less than 0·3 % compared with about 13 % in the cervical portion of the vagus). The desheathed nerve was then mounted on the sucrose-gap apparatus (Fig. 1) which was devised by Stämpfli (1954) and described in detail by Straub (1956, 1957); this apparatus had previously been used to study the C fibres in mammalian cervical sympathetic trunks (Ritchie & Straub, 1957). A single electrical shock delivered from an RF isolation unit (Schmitt, 1948) to minimize shock artifact, could be applied through any two of four indwelling platinum electrodes (E_1, E_2, E_3, and E_4); usually E_1 and E_2 were used with the cathode at E_1. Rectangular pulses of current of 0·05 msec duration were used to excite the myelinated fibres and pulses of 0·5 msec duration to excite the non-myelinated fibres. The potentials were recorded at the junction between the fluid

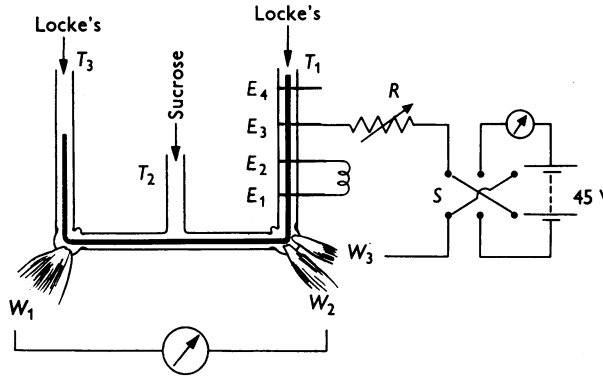

Fig. 1. The sucrose-gap apparatus showing the arrangement of electrodes for stimulating (E_1 and E_2), for recording (W_1 and W_2) and for applying external currents (W_3 and E_3). S is a reversing switch, and R a series resistor (0·1–100 MΩ). For detailed description, see text.

which flowed through the fine Perspex tube (T_1) and the isotonic sucrose solution in the middle Perspex tube (T_2); the distance from this junction to the stimulating cathode at E_1 was about 5 mm. These potentials were recorded relative to the potential which existed at the interface between the sucrose solution and the Locke's solution flowing through the Perspex tube (T_3) and which was assumed to remain constant throughout the experiment. The action potential and changes in the resting membrane potential were picked up through a pair of non-polarizable Ag–AgCl cotton wick electrodes (W_1, W_2), fed into a low-grid-current cathode-follower and then into a directly coupled amplifier. The potentials were displayed on a cathode-ray oscillograph and photographed on film. In some experiments small currents were passed through a Ag–AgCl cotton wick electrode (W_3) dipping into the pool between T_1 and T_2 and one of the indwelling platinum electrodes (E_3 or E_4). This current could be varied by means of a series resistor (R) and could hyperpolarize or depolarize the fibre at the point of recording by extra-polar spread, depending on the setting of a reversing switch (S). The rate of flow of the perfusion fluid was measured on a small flowmeter: it was usually about 0·5–1 ml./min. The temperature of the perfusion fluid near the recording sucrose–Locke's solution interface was measured by means of a fine thermocouple: it was usually about 30–35° C. The perfusion fluid in T_1 could quickly be changed by means of a tap (not shown in the diagram).

The composition of the Locke's solution used was (mM): NaCl, 154; KCl 5·5; $CaCl_2$, 2·2; dextrose, 5·0. It was buffered in a few early experiments by adding $NaHCO_3$ (final concentration, 1·9 mM) and bubbling into it a 5 % CO_2, 95 % O_2 mixture; but in most experiments it was buffered by adding a sodium phosphate buffer (1 or 2 mM) to bring the pH to about 7·0.

This latter solution was equilibrated with oxygen. The acetylcholine solutions used in the experiments were always freshly prepared from new phials of acetylcholine chloride (Merck) usually within a few minutes of testing its effect.

RESULTS

The vagal C potentials

The typical vagal compound action potential obtained with the present technique is illustrated in Fig. 2*a*. It consisted of two main elevations. The smaller was usually merged with the stimulus artifact, and was derived from the fast-conducting myelinated B fibres; the main component, which was usually between 5 and 20 mV in amplitude, was the C potential derived from the non-myelinated fibres. In this and in all subsequent records of the compound action potential the record of the combined B and C potentials obtained by using a strong shock was superimposed on a

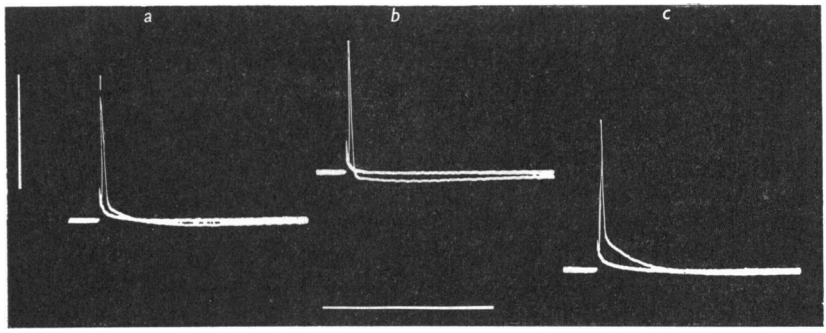

Fig. 2. The compound vagal action potential of the rabbit's cervical vagus nerve. The B potential is lost in the stimulus artifact. The main elevation is the C potential of the non-myelinated fibres. The records were taken: (*a*) in the normal nerve; (*b*) during the passage of a depolarizing current of 15 μA between W_3 and E_3 (Fig. 1); (*c*) during the passage of a hyperpolarizing current of 15 μA. The vertical bar represents 5 mV and the horizontal bar 250 msec. The temperature of the nerve was 35·2° C.

record of the B potential only, which was obtained immediately beforehand and which thus provided a base line for the C potential. The C spike was followed by a large after-positivity which in a series of 20 *fresh* nerves in phosphate-buffered Lock's solution varied from 0 to 7·5 % of the spike height (except in one nerve where it was 15·5 %), the average being $4{\cdot}0 \pm 0{\cdot}8$ % (S.E.) of the spike. No preparation ever gave the large positive after-potentials, up to 30 % of the spike height, which Gasser (1950) postulated in his reconstruction of the C potential of the saphenous nerve. The after-positivity tended to become more pronounced in preparations which had been mounted for some time. It was enhanced by

depolarizing the fibre by external currents (Fig. 2*b*) and abolished by hyperpolarizing the fibre (Fig. 2*c*). These latter observations suggest that the origin of this after-positivity is the same as that suggested by Frankenhaeuser & Hodgkin (1956) for the positive phase of the spike in the squid axon. They point out that if the resting membrane potential is below the potassium equilibrium potential (which it usually is) the rise in the membrane permeability to potassium ions persisting immediately after the spike (Hodgkin & Huxley, 1952) will give rise to a transitory hyperpolarization.

The action of acetylcholine on the vagal C fibres

The effect of acetylcholine on the resting potential. When the Locke's solution in T_1 bathing the nerve was switched to a solution containing acetylcholine in a concentration of 3×10^{-4} (w/v), i.e. a concentration of about 1·6 mM, there was a rapid fall in the membrane potential (Fig. 3) after an initial delay of about 20 sec, which represented the time taken to clear the dead space of the switching apparatus. On being returned to

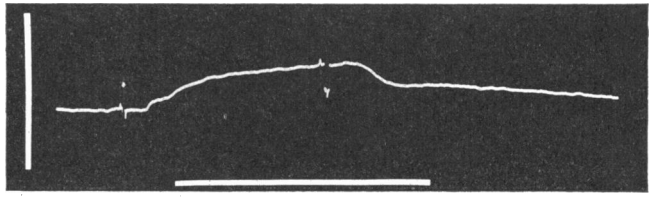

Fig. 3. The effect of acetylcholine on the resting membrane potential of the rabbit's cervical vagus nerve. At the first marker the perfusing fluid was changed from Locke's solution to one containing 3×10^{-4} acetylcholine. At the second marker perfusion with the original Locke's solution was resumed. An upward deflexion indicates depolarization. The vertical bar represents 5 mV and the horizontal bar 3 min. The temperature of the nerve was 35·1° C.

Locke's solution the membrane repolarized, although recovery was relatively slow and was often not complete. The depolarization was usually measured 3 min after switching to Locke's solution. It nearly always occurred (one exception was observed in sixteen experiments, when there was a slight hyperpolarization which may have been due to amplifier drift). In experiments on sixteen different nerves the average depolarization after 3 min was $1{\cdot}23 \pm 0{\cdot}19$ mV. However, the maximum depolarization sometimes occurred earlier than this, as in the experiment of Fig. 7.

With a smaller concentration of 10^{-4}, little or no change in the resting potential was observed. In seven of the experiments described above, where a concentration of 3×10^{-4} was used, a concentration of 6×10^{-4}

was also tested. It was found to produce, on the average, about 140% of the depolarization that the smaller, standard, concentration produced. In three experiments a concentration of 10^{-3} was tested, producing an even greater depolarization, the average being about 180% of that obtained with a concentration of 3×10^{-4}.

Because of the small degree of short-circuiting of the fibres with the sucrose-gap technique these *measured* membrane potential changes are assumed to be close to the *actual* membrane potential changes. The absolute value of the resting potential could not, unfortunately, be determined, for with the particular sucrose-gap (T_2 in Fig. 2) which was used in the present experiments the nerve preparation only partly filled the lumen. This meant that when the preparation was perfused with isotonic potassium chloride solution instead of Locke's solution, in an attempt to measure the resting potential of the fibres, a large part of the recorded depolarization must have been caused by the more rapid diffusion of potassium than of sodium into the sucrose solution. Indeed, when the nerve preparation was replaced by a piece of thread of the same or smaller diameter, depolarizations of over 20 mV were obtained by changing the perfusion fluid from an isotonic sodium chloride solution to an isotonic potassium chloride solution; similar *hyper*polarizations were obtained on changing the perfusion fluid from an isotonic sodium chloride solution to an isotonic lithium chloride solution, as would be expected from the order of the mobilities of the lithium, sodium and potassium ions. The depolarization with acetylcholine in the nerve experiments, however, reflected an effect of the drug on the nerve membrane and was not a diffusion potential, for the diffusion effect with acetylcholine (and with choline) was found to produce a hyperpolarization, as would be expected with ions less mobile than the sodium ion. The small concentrations of acetylcholine used in the present nerve experiments did not in fact produce any appreciable diffusion potential.

The effect of acetylcholine on spike height. The depolarization of the C fibres by acetylcholine was accompanied by a fall in spike height. This is illustrated in Fig. 4, which shows the results obtained in two typical experiments. The upper left-hand record *a* is the control record taken shortly before one of the nerves was perfused with a concentration of 3×10^{-4} acetylcholine. The middle record (*b*) was taken 3 min after the change over had taken place. It is clear that not only is there a depolarization of about 2·03 mV (indicated by the shift in the level of the record) but that there is also a fall of about 2·55 mV (i.e. 18%) in spike height. The lower 3 records show the effect of a 3 min exposure to a lower concentration, 10^{-4}, of acetylcholine (*e*) and the subsequent recovery on resuming perfusion by Locke's solution (*f*). In observations made repeatedly on

more than thirty different nerves, this reduction in spike height was *always* obtained on exposure of the preparation to an acetylcholine concentration of 3×10^{-4}; recovery was usually complete within 6 min of the nerve being returned to Locke's solution. In eighteen nerves where the acetylcholine was tested on the fresh preparation in phosphate-buffered Locke's solution, the average fall after 3 min exposure to acetylcholine was 2·79 mV, with a standard deviation of 1·15 mV (S.E. $\pm$ 0·27); this corresponded to an average fall of 32·2 % in the spike height, with a

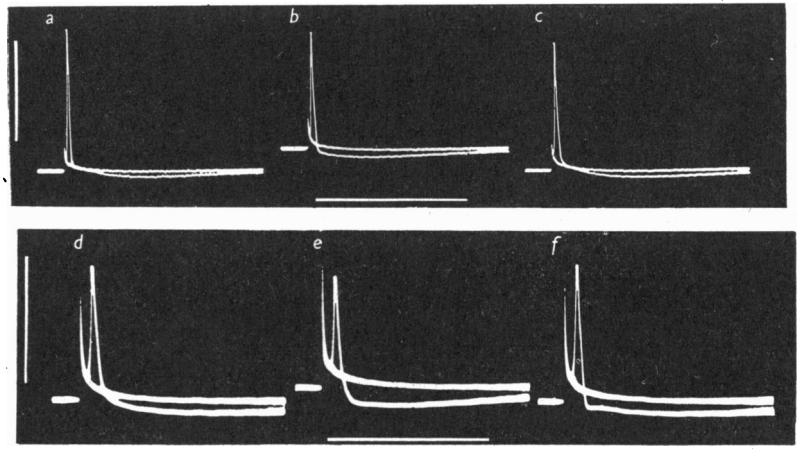

Fig. 4. The effect of acetylcholine on the C potential of the rabbit's cervical vagus nerve. The upper three records (*a*, *b* and *c*) were taken from one preparation and the lower three (*d*, *e* and *f*) from another. Record *a* was taken just before and record *b* 3 min after exposure of the nerve to 3×10^{-4} acetylcholine; record *c* was taken 15 sec after *b* with the preparation still in the acetylcholine solution, but hyperpolarized by passing 7·7 μA between W_3 and E_3 (Fig. 1). Record *d* was taken just before, and record *e* 3 min after exposure to 10^{-4} acetylcholine. Record *f* was taken 6 min after the drug had been removed. In this and all subsequent figures (except Fig. 6 and 11) the vertical displacement of the records reflects the changes in resting membrane potential. In the upper set of records the vertical bar represents 10 mV and the horizontal bar 250 msec; the corresponding bars in the lower set represent 5 mV and 100 msec, respectively. The temperature of the nerve in the upper set was 35·3° C; the temperature of that in the lower set was not measured.

standard deviation of 7·5 % (S.E. $\pm$ 1·8 %). Higher doses produced larger falls; this is illustrated in Fig. 5 which shows the results of one experiment on a particularly sensitive preparation, where the response to four different concentrations of acetylcholine was tested. In seven experiments where a concentration of 6×10^{-4} was used, the reduction in size of the action potential was on average 45 % greater than the reduction with the standard concentration of 3×10^{-4} obtained about 10 min beforehand; in five

similar experiments where a concentration of 10^{-3} was used the corresponding reduction was 90% greater than with 3×10^{-4}. In some other experiments where only a concentration of 10^{-3} was tested complete block occurred. A concentration of 10^{-4} was usually ineffective, but as has been shown in Fig. 4*e*, it could on occasion reduce the spike.

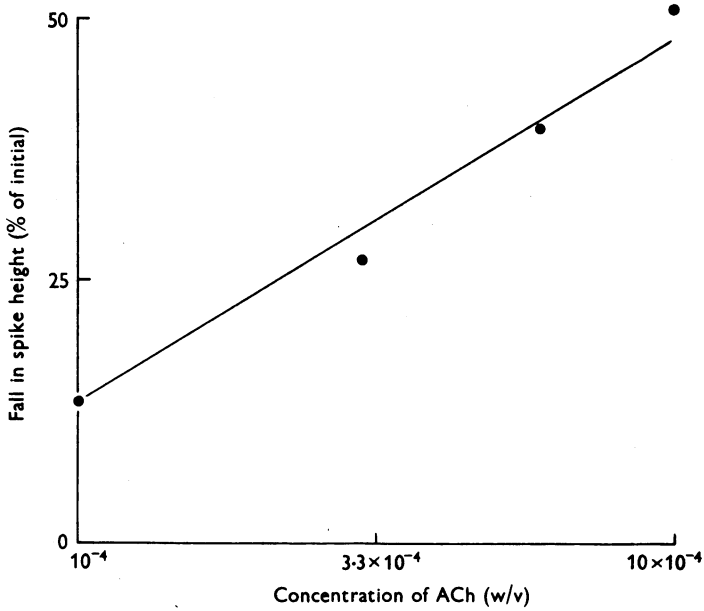

Fig. 5. The relationship between the concentration of acetylcholine in the perfusion fluid and the reduction in the height of the C potential of the rabbit's vagus nerve. Same nerve as in Fig. 4 (lower set). Note that the concentration scale is logarithmic.

The effect of acetylcholine on conduction velocity. Even in records of the C potential such as those in Fig. 4, which were taken on a slow time base, it could be seen that acetylcholine delayed the spike. This effect is seen particularly clearly with a faster time base, as in the experiment of Fig. 6. In this experiment each of the four indwelling platinum stimulating electrodes was used in turn as the stimulating cathode so that an accurate estimate of the conduction velocity could be obtained by the graphical method illustrated in the figure. The peak of the C potential in this preparation was found to be conducted at 0·99 m/sec at 35° C. Ten minutes after the preparation had been exposed to a concentration of 6×10^{-4} acetylcholine a similar determination of conduction velocity showed the peak to be conducted at only 0·57 m/sec, i.e. a slowing of over 40% had occurred.

Since bundles of nerve fibres rather than single axons were used in the

present experiments, this slowing in conduction must have contributed to the fall in spike height, for the more time that is allowed for temporal dispersion to occur, the smaller the action potential of the whole nerve becomes (Ritchie & Straub, 1956). A similar explanation accounts for the further observation, illustrated in Fig. 6, that the percentage fall in spike height was greater with long conduction distances than with short conduction distances. Thus with E_1 as the stimulating cathode the smallest conduction distance was obtained, and acetylcholine was found to produce

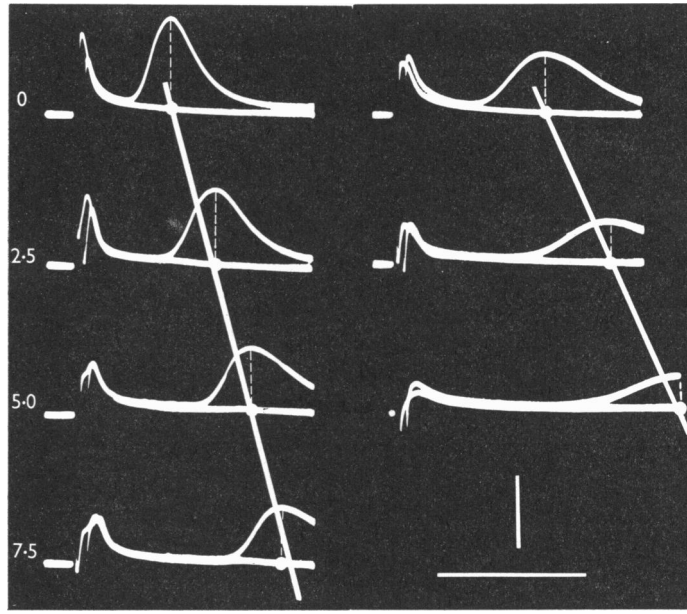

Fig. 6. The effect of acetylcholine on the conduction velocity of the non-myelinated fibres of the rabbit's vagus nerve. The four left-hand records were taken in Locke's solution. They were obtained by using E_1, E_2, E_3 and E_4 (Fig. 1) in turn as the stimulating cathode. The numbers at the side of each trace indicate the distances in millimetres of the stimulating cathode from E_1. The vertical positioning of each trace in the composite figure is proportional to this distance. The three right-hand traces are the corresponding records (using E_1, E_2 and E_3) obtained 10 min after the preparation was exposed to a concentration of 6×10^{-4} acetylcholine. The vertical bar represents 5 mV and the horizontal bar 10 msec. The temperature of the preparation was 34° C.

a 34·9 % fall in spike height (top pair of records), whereas with E_2 and E_3 as the stimulating cathodes the corresponding effects were 45·5 and 45·9 %, respectively: with E_4 as the stimulating cathode the reduction in spike height was 65·2 %.

The effect of acetylcholine on the positive after-potential. It can be seen in Fig. 4 that acetylcholine, in addition to depolarizing the fibre and

reducing the spike, has changed the form of the positive after-potential. The main change, which nearly always occurred when the after-potential could be resolved in the records, was that its peak occurred earlier; the acetylcholine also often caused the positive after-potential to end slightly earlier and to increase its amplitude at its peak. These changes were particularly pronounced in early experiments with the bicarbonate-buffered Locke's solution, but this may have been due to chance rather than to an effect of the bicarbonate ion.

The effect of hyperpolarizing currents on the action of acetylcholine

Depolarization of the fibre with external currents reproduces many of the effects of perfusion with acetylcholine. This can be seen by comparing the C potential of the depolarized fibres in Locke's solution shown in Fig. 2*b* with the C potential obtained when the same preparation was exposed to a concentration of 3×10^{-4} acetylcholine shown in Fig. 4*b*. The records are remarkably similar to one another; in both the spike height is reduced and the after-positivity enhanced. It therefore seemed worth while to find out whether or not the action of acetylcholine could be reversed by hyperpolarizing the fibres with externally applied currents. The results of one such experiment are illustrated in Fig. 4*c*, where it can be seen that the after-potential changes produced in the presence of acetylcholine have been reversed by means of a small hyperpolarizing current. However, the spike height, though increased by this procedure, was never returned to its original level. It seems therefore that the effects of acetylcholine in reducing spike height may not be entirely caused by the acetylcholine depolarization.

The time course of the action of acetylcholine

The resting and action potentials. In most experiments the nerve was stimulated infrequently, once every 3 min. The long interval between stimuli was chosen because, as Gasser (1950) has emphasized, previous activity alters the shape of the spike. Any such alteration as a result of the stimulation technique would have confused the interpretation of the action of acetylcholine. In a few experiments, however, the stimulation frequency was increased to 4 shocks/min to allow the time courses of the effects of acetylcholine on spike height and on resting potential to be obtained. The first portion of a series of records taken in such an experiment is shown in Fig. 7. The records show that the reduction in spike height and the depolarization occur *pari passu*. This is seen particularly clearly when the time courses of the depolarization and of the reduction in spike height are plotted together, as in Fig. 8. In this experiment there was actually some recovery in the membrane potential even when the

fibre was still exposed to the acetylcholine; this recovery in membrane potential was accompanied by a partial recovery in size of the spike.

In experiments such as that illustrated in Fig. 8 the size of the effect on the resting potential recorded 3 min after exposure to the drug was smaller than the maximal effect occurring about 1 min after the exposure. This early maximum was usually followed by a more slowly developing effect. Thus, in most of the experiments where records were taken only

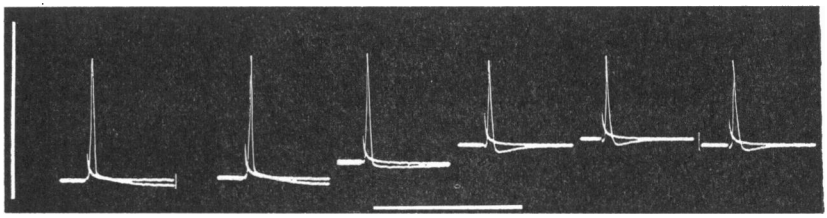

Fig. 7. Records showing the early time course of the action of acetylcholine on the non-myelinated fibres of the rabbit's vagus nerve. The records were taken every 15 sec. The left-hand record is the control and the switch to perfusion with 3×10^{-4} (w/v) acetylcholine occurred immediately after this record was taken. The vertical bar represents 10 mV and the horizontal bar 250 msec. The temperature of the nerve was 34·8° C.

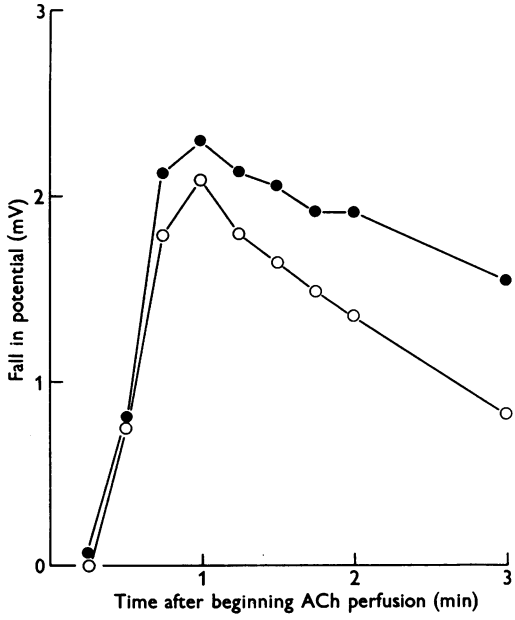

Fig. 8. The time course of the action of acetylcholine (3×10^{-4}) on the resting membrane potential (open circles) and on the amplitude of the C potential of the rabbit's vagus nerve (solid circles). The temperature of the nerve was 34·8° C.

every 3 min, the depolarization tended to increase slowly in successive records. This was parallelled by a continued increase in the effect of acetylcholine in reducing spike height. This is illustrated by the experiment of Fig. 9, which shows that in a preparation where a 3 min exposure to 3×10^{-4} acetylcholine produced a typical 32·6 % fall in spike height, longer exposures of 6 and 9 min produced falls of 50·0 and 58·9 % of spike height, respectively.

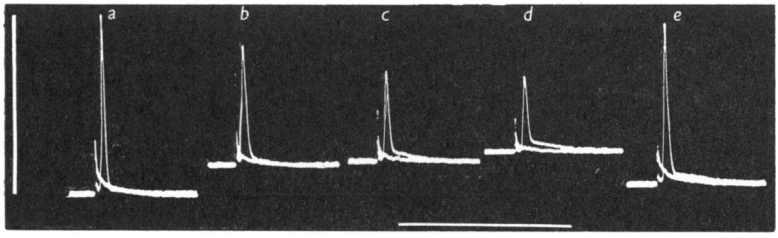

Fig. 9. The effect of prolonged exposure to acetylcholine on the non-myelinated fibres of the rabbit's cervical vagus nerve. Record *a* was taken before, and records *b*, *c* and *d* were taken 3, 6 and 9 min respectively after perfusion with 3×10^{-4} acetylcholine was begun. Record *e* was taken 12 min after the preparation was returned to Locke's solution. The vertical bar represents 5 mV and the horizontal bar 250 msec. The temperature of the nerve was 34·2° C.

The positive after-potential. Figure 7 illustrates a further typical finding in these experiments. The positive after-potential was particularly pronounced within the first minute after switching to perfusion by an acetylcholine solution, becoming larger in amplitude and more transitory than the type of after-potential which was recorded after exposure of 3 min or more (as in Fig. 4*b*).

Conduction velocity. The time course of the effect of acetylcholine on conduction velocity was determined by observing the progressive changes in the latency of C potential after exposure of the fibres to acetylcholine. As can be seen in Fig. 10 (solid circles) the latency changes occurred in two phases: there was first a slight reduction and then an increase. The effect of acetylcholine on spike height (open circles) also occurred in two phases. These correspond with the early and late phases previously illustrated in Figs. 8 and 9, which also indicate that there is an early and a late phase of depolarization.

A comparison of Figs. 8 and 9 and 10 makes it clear that the two phases of reduction in spike height, of depolarization and of latency changes correspond in time with one another. There is a rapid phase which reaches maximum about 1 min after switching to perfusion with acetylcholine (i.e. within $\frac{1}{2}$ min of the drug reaching the preparation) and a much slower phase which requires more than 5–6 min to reach its maximum.

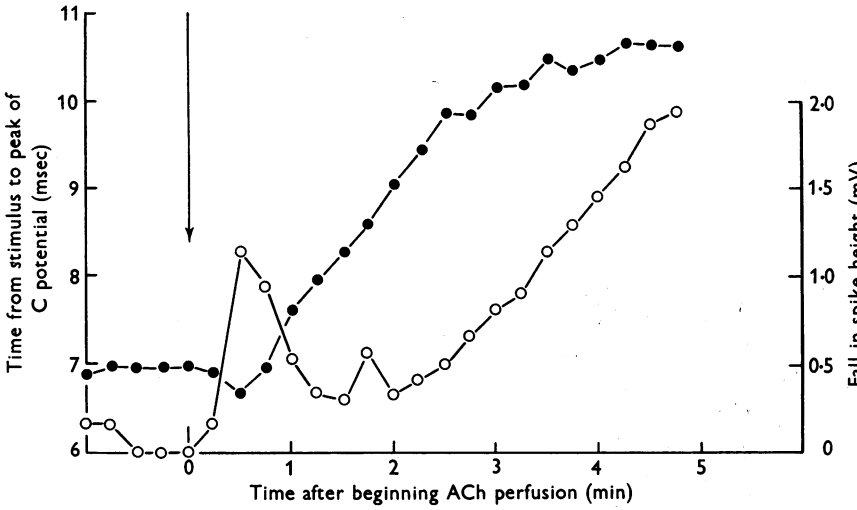

Fig. 10. The time course of the effect of acetylcholine on the conduction velocity of the non-myelinated fibres in a rabbit's cervical vagus nerve. The abscissae are the times after first exposing the preparation to a concentration of 6×10^{-4} acetylcholine, and the ordinate is the latency of the peak of the C potential (solid circles) and the reduction in spike height (open circles). The temperature of the nerve was 34° C.

The effect of acetylcholine on the myelinated B fibres

The action of acetylcholine on the myelinated vagal B fibres was not studied systematically because our main interest lay in its action on the C fibres. But it was often observed that the concentrations of acetylcholine which reduced the C potential also reduced the B elevation of the compound action potential. This reduction might have been caused by a direct action of acetylcholine on B fibres; or it might have occurred because the partially depolarized C fibres presented a smaller short-circuiting resistance to the B fibres than did the C fibres in Locke's solution, thus reducing the B potential. This latter possibility has not yet been tested directly. There is no doubt, however, that the reduction of the C potential is a direct effect of acetylcholine and is not caused by an increased short-circuiting of affected B fibres, for typical effects of acetylcholine were obtained in several experiments on the lower portion of the thoracic vagus nerve at the level of the diaphragm, which contained hardly any myelinated fibres (Evans & Murray, 1954) and which was found in the present experiments to have an insignificant B potential.

The effect of eserine on the action of acetylcholine

The action of acetylcholine in depolarizing nerve fibres seems in some ways analogous to its action in depolarizing various junctional tissues,

such as the motor end-plate and the ganglionic synapse. It was expected, therefore, that anticholinesterases would potentiate the action of this drug on nerve fibres, as it does elsewhere, by preventing the tissue break-down of acetylcholine. We were therefore surprised to find that in the presence of eserine salicylate ($1–10 \times 10^{-5}$, w/v), acetylcholine had a *smaller* effect on the spike height (Fig. 11). The effect of eserine in blocking the action of acetylcholine may be the same as that reported by Dettbarn (1959) who found that it blocked the depolarizing action of various lipid-soluble quaternary ammonium ions on the myelinated fibres of the frog.

Whereas eserine in weak concentrations (10^{-5} or less) had no action by itself on spike height, eserine in stronger concentrations ($3 \times 10^{-5}–10^{-4}$) always reduced the spike (by an average of 10·6 % in 3 min in four experiments and by greater amounts at longer times); there was, however, no detectable depolarization with these concentrations. An even stronger concentration (10^{-3}) produced a complete block in conduction. This block was partially reversed by applying a hyperpolarizing current, as Wright (1956) had previously noted. Concentrations of eserine as low as this (0·08–0·2 mM to reduce spike height, and 2 mM to produce block) have not previously been reported to be effective on myelinated fibres (Langley & Dickinson, 1890; Lorente de Nó, 1944; Straub, 1955; Wright, 1956) or on squid nerve fibres (Bullock, Nachmansohn & Rothenberg, 1946).

DISCUSSION

The main conclusion to be drawn from these experiments is that acetylcholine does have a definite action on certain peripheral nerve axons; it depolarizes the axons of vagal non-myelinated fibres, it reduces their spike height, it slows conduction in them and it causes the positive after-potential in these fibres to become more rapid in onset and often to become larger in amplitude. The changes in the after-potential would, of course, account for the original observation of the effect of acetylcholine on nerve fibres made by Douglas & Ritchie (1960). In their experiments, electrical shocks were applied to the cat's saphenous nerve high in the thigh and the evoked antidromic action potentials were recorded *diphasically* by a pair of electrodes on a small nerve branch just before it entered the skin near the knee. They noted that the conventional negative followed by positive deflexions of the diphasic records were succeeded by a long-lasting apparent negativity. This could have been due to the appearance of a negative after-potential at the recording electrode nearer the stimulating pair. However, another possibility is that it was due to the enhancement of a positive after-potential at the other, more distant, recording electrode; either an increase in its peak height or a more rapid onset of the after-potential would be sufficient. Since

acetylcholine has now been shown to have *both* these effects on the positive after-potential and since the more distant recording electrode was the more likely to be reached by the close arterial injection (being nearer the skin) it seems that the second possibility is the one which most likely accounts for the phenomenon observed by Douglas & Ritchie (1960).

The question which immediately arises is why no depolarization, reduction in spike height, or blockage of nerve conduction was noted previously. Clearly, it has not been due to oversight, for many studies have been made to see whether or not such effects occur (e.g. Lorente de Nó, 1944; Straub, 1955) particularly because of the important role which acetylcholine is sometimes assumed to play in nervous conduction (see Nachmansohn, 1959). One possible explanation for the success of the present experiments in demonstrating an action of acetylcholine is that surface phenomena (such as membrane potential changes) are better seen in non-myelinated fibres because they have a particularly large surface:

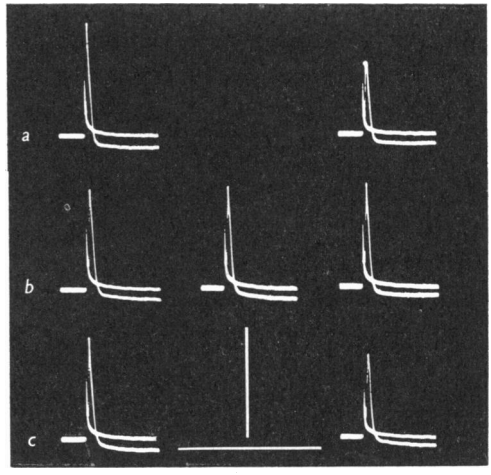

Fig. 11. Records of the C potential of the rabbit's cervical vagus nerve taken to test the effect of eserine on the action of acetylcholine on the C fibres. The records on the left-hand side were all taken with the nerve in Locke's solution; those on the right-hand side were all taken after 6 min exposure to a concentration of 3×10^{-4} acetylcholine. Each set of records has been brought to the same base line. The top pair of records, *a*, shows the effect of acetylcholine on the untreated nerve. Between taking the records on the left-hand side and the middle records in *b*, eserine (10^{-5}) was added to the perfusion fluid and was still present when the effect of acetylcholine was subsequently tested (right-hand records). The pair of records in *c* was taken 33 min after the last of the records in *b* during which time the fibre was bathed in Locke's solution (with no eserine in it). Note the partial recovery in the action of acetylcholine. The vertical bar represents 5 mV and the horizontal bar 250 msec. The temperature of the nerve was 34·2° C.

volume ratio. This ratio is much larger in the small non-myelinated vagal fibres than in the larger fibres used previously, such as the myelinated fibres of amphibia or the squid fibres. It seems from these experiments, and from others now being made on the effects of neurotropic drugs on nerve fibres (P. Greengard & J. M. Ritchie, unpublished), that these small fibres may be intermediate, as far as their pharmacological reactions are concerned, between the more commonly studied peripheral axons which tend to be relatively insensitive to applied drugs and the nerve-cell bodies with their dendrites.

The present experiments were carried out on nerve bundles rather than on single fibres. It is therefore difficult to determine whether the effect of acetylcholine on the membrane of an individual fibre is really graded, getting greater as the concentration of the drug is increased, or whether the observed decrease in the C potential is caused by an increasing *number* of fibres becoming *completely* blocked. Furthermore, because temporal dispersion occurs under the experimental conditions, a reduction in the C potential could have been caused without any fall in spike height of the individual fibres either if the spike became shorter in duration or if the conduction velocity decreased. The depolarization by itself must have led to a decrease in the spike height of the individual fibres, for in addition to lowering the potential from which the spike started it must also have reduced the amount of overshoot by partially inactivating the sodium carrier mechanism (Hodgkin & Huxley, 1952). How much the observed decrease in the C potential of the whole nerve is due to this factor and how much it is due to the increased dispersion allowed by the decreased conduction velocity is not known at the moment.

Our experiments do not yet allow us to decide how acetylcholine acts, whether, for example it acts by causing a non-specific increase in membrane permeability to all ions as it does at the motor end-plate (Fatt & Katz, 1951) or whether, for example, it increases the membrane permeability to one specific ion such as the sodium ion. However, our experiments do indicate something about the type of receptor involved. Whatever this receptor is, it is blocked by the anticholinesterase eserine. This suggests that the receptor is similar to, though not necessarily the same as, cholinesterase itself: and that the reaction between acetylcholine and the receptor cannot occur in the presence of an anticholinesterase. Whatever the explanation, the experiments clearly demonstrate a paradoxical effect of an anticholinesterase, namely that it inhibits rather than enhances the effect of applied acetylcholine. This is not, however, the first time that such an effect has been noted, for Feldberg & Vartiainen (1935) observed that although eserine, when injected into the cat's superior cervical ganglion in small amounts (concentration in the perfusion fluid, 10^{-6}) enhanced

the excitant action of a subsequent injection of acetylcholine, a stronger concentration (10^{-4}) rendered the ganglion cell unresponsive to acetylcholine. This effect, which they attributed to a nicotine-like action of eserine, may well have been caused by an action of eserine similar to that described in the present paper. Furthermore Quilliam & Strong (1949) observed in isolated perfused hearts which had been exposed to DFP and which consequently were very sensitive to applied acetylcholine, that subsequent exposure to the anticholinesterases eserine or prostigmine decreased this sensitivity.

One possible explanation which would account for our results is that the effects of acetylcholine on the resting and action potentials depend on the action of some of the products of hydrolysis of acetylcholine. We have tried to test this possibility by examining the effects of perfusing the fibre with choline chloride, with sodium acetate and with solutions where the pH had been lowered. Both the choline and the more acid Locke's solutions reduced spike height, but the effects produced were rather small, the fall being only 10–20 % even when the preparation was perfused with a relatively large concentration of choline chloride (15 mM) or when the pH was changed by as much as 0·5 unit; sodium acetate (15 mM) had little or no effect on spike height. These changes in choline, acetate and hydrogen ion concentration are much larger than could be produced in the bulk of the solution or on the axoplasm generally; but the possibility that changes of this size occur locally in particular regions of the membrane cannot be excluded. These experiments therefore do not as yet shed much light on the mode of action of acetylcholine on the nerve axon.

It is tempting to speculate that the sensitivity of peripheral nerve endings to acetylcholine (Brown & Gray, 1948; Douglas & Gray, 1953; Douglas & Ritchie, 1960) reflects the sensitivity of non-myelinated axons shown in the present studies, for whether or not the fibres are myelinated their endings are non-myelinated. The nerve endings are more sensitive than the nerve axons by a factor of at least 10; the threshold response of the endings is obtained with a close-arterial *injection* of 10^{-5} acetylcholine, whereas the threshold response of the fibre is obtained with *perfusion* with a concentration of about 10^{-4} of acetylcholine; but this difference might possibly be explained on anatomical grounds. However, there is one important difference between the two preparations. Acetylcholine initiates conducted impulses at sensory nerve endings, but there is no evidence that it does so in axons from experiments where it was injected into normal nerve trunks (Diamond, 1959), or from the present perfusion experiments even when the amounts were big enough to lead eventually to complete block in conduction. Furthermore, the excitatory

action of acetylcholine at the endings is certainly not reduced by anticholinesterases (Brown & Gray, 1948). This is just the opposite to what occurs with the axon, where the action of acetylcholine has now been shown to be much reduced by the anticholinesterase eserine. It seems therefore that acetylcholine acts in quite different ways at the two sites, reacting with quite different types of receptor.

SUMMARY

1. The sucrose-gap technique has been used to record the compound action potentials of desheathed bundles of vagal fibres and to study the action of acetylcholine on the numerous non-myelinated fibres in this nerve.

2. The action potential of the C fibres is followed by a long-lasting after positivity whose peak amplitude is about 4·0 % of the spike height.

3. When the preparation is perfused with acetylcholine (3×10^{-4} w/v) the resting potential falls by more than 1 mV, the spike amplitude is reduced by about 30 %, the conduction velocity is slowed and the after-positivity is enhanced.

4. Larger effects are obtained with larger concentrations; threshold effects are observed with a lower concentration, 10^{-4}.

5. High concentrations of acetylcholine (10^{-3}) sometimes completely block conduction.

6. These changes are rapidly reversed when the acetylcholine is removed.

7. Eserine in high concentration has a similar effect on the spike to acetylcholine. In a concentration of from 3×10^{-5} to 10^{-4} it reduces spike height: a concentration of 10^{-3} completely blocks conduction.

8. The effects of acetylcholine are abolished or much reduced by the anticholinesterase eserine.

9. The action of eserine is reversible.

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