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THE CHOLINERGIC NATURE OF THE NERVES TO THE ELECTRIC ORGAN OF THE *TORPEDO* (*TORPEDO MARMORATA*)

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SINCE the first evidence [Dale, Feldberg & Vogt, 1936] for the theory that the transmission of motor-nerve impulses to striated muscles at the motor end-plates is mediated by the release of acetylcholine, many facts have been brought forward supporting this conception. The experiments described in this paper were undertaken in order to discover whether the nerves to the electric organ of the *Torpedo* act in a similar way. This organ, in the *Torpedo* and in some other fishes, may be regarded as a collection of modified motor end-plates lacking the contractile structure of voluntary muscle fibres.

The electric organ of the *Torpedo* consists of a great number of prisms arranged side by side, each prism being built up of about 500 superposed plates, which are covered on their ventral surfaces by a terminal nerve net. At the moment of the discharge the ventral sides of all plates become negative to the dorsal nerve-free sides. Various hypotheses have been formed to explain this special electrogenic phenomenon. Recently,

[1] It was originally intended to publish our experiments in the *Archives Internationales de Physiologie* together with those of Dr Nachmansohn in which he compared the cholinesterase content of the electric organ of different species of fishes and in other tissues of the *Torpedo*. A short communication by all three of us was published in the *Proceedings* of this *Journal* (1940, **97**, 3). In the meantime Dr Nachmansohn has found it more convenient to publish the main part of his results elsewhere (*Science*, 1940, **91**, 405; *J. Neuro-Physiol.* 1941, **4**, 348). I am therefore publishing the results of the experiments of Prof. Fessard and myself in this *Journal*, publication in the *Archives Internationales* having become impossible. The text is in the main a translation from an agreed French manuscript, with some small alterations and additions which are the outcome of subsequent discussions.

the possibility that the discharge results from release of an electrically active substance has been considered on the basis of researches from two independent sources.[1]

Auger & Fessard [1938, 1939*a*], working on isolated prisms of the organ, concluded from the latency and form of the discharge following their electrical stimulation that excitation was always indirect. They were able to confirm Garten's [1910] original observation of the impossibility of obtaining a discharge from the whole organ by mechanical or even electrical stimulation after degeneration of its nerves. The fact that the denervated organ was inexcitable even by the stimulus of an electric shock rendered it difficult to imagine that the nerve impulse in the normally innervated organ should transmit its excitation by means of its electrical variation only. From analogy with the action of nervous impulses at motor end-plates of striated muscle they thought the most likely cause for the discharge to be the effect of a polarizing or a depolarizing substance, such as acetylcholine, released by the arrival of the nerve impulse at the terminal nerve net. Results obtained with eserine, curare and atropine on isolated prisms were in agreement with this hypothesis.

The finding of an extremely high concentration of cholinesterase in the electric organ of the *Torpedo* [Marnay, 1937; Nachmansohn & Lederer, 1939] represented the other and more direct evidence in this direction. Marnay & Nachmansohn [1937] had found that the concentration of cholinesterase in striated muscle was greater in those parts of the fibre containing the end-plates, suggesting a high concentration of the enzyme at these regions. As the end-plates are analogous to the elements of which the electric organ of the *Torpedo* is composed, Nachmansohn considered the possibility of an identical mechanism of transmission of the nervous impulses in both structures, and, at his suggestion, Marnay examined the cholinesterase content of the electric organ.

Our experiments were planned to determine whether the electric organ contains acetylcholine; if so, whether the substance is liberated

[1] We are grateful to Sir Henry Dale for drawing our attention to T. R. Elliott's brilliant anticipation of this mode of action. Elliott, who was the first to conceive the idea of chemical transmission in the autonomic nervous system, discussed this possibility for the electric organ in 1914 in his Sydney Ringer Memorial Lecture and even made an attempt to extract the active substance. He writes: 'I have tried in vain to discover an active substance in the muscle plates of striped muscles. And Professor Herring was also disappointed when he examined for this purpose the electrical organs of the skate which are exaggerated motor end-plates.... But it is hard to forgo the belief that such discoveries lie in the lap of the future.'

during stimulation of its nerves; and whether an arterial injection of acetylcholine into the organ has an electrogenic effect. In addition, observations were made on the effect of eserine on the discharge.

Methods

Extraction of acetylcholine. Small pieces of the fresh organ were minced in a mortar with sand in acidified saline solution to which was added eserine 1 in 10,000. The mixture was brought to boiling-point, cooled, made up to a given volume and assayed on the eserinized rectus abdominis muscle of the frog. In some instances the tissue, after being cut into small pieces, was thrown into boiling water containing no eserine, but acidified by HCl to *p*H of about 4. The mixture was filtered and the residue again minced in a small volume of acidified water with sand, boiled and added after filtration to the main extract, the whole mixture being then made up to a given volume. This extract remained active for several days when kept in the cold.

Perfusion of the electric organ. For the study of the effects of arterial injections of acetylcholine and its liberation during nerve stimulation part of the electric organ was perfused. The organ is supplied by four arteries which enter it with the nerves and are accompanied by the veins. Perfusion was carried out from the artery accompanying the second nerve. Injections of Indian ink showed that this artery supplied an area innervated by the first and second nerves, the artery supplying the former being very small. The *Torpedos* were killed by pithing the central nervous system; the electric organ was then cut out, leaving the artery to be cannulated intact and connected with the fish until the last moment. The artery was ligated and cut centrally, thus completely isolating the organ, which was then washed in sea water. A fine glass or metal cannula was tied into the artery and perfusion started at once. In those experiments in which the liberation of acetylcholine was studied the organ was placed in a suitably-shaped paraffin basin which had at its lowest point an outlet for collecting the venous effluent. On the inner surface of the basin a number of small pieces of cork were pinned to keep the organ out of close contact with the basin and prevent retention of fluid. The rate of perfusion was 3–6 c.c./min. The fluid left the organ mainly through the great veins, which were cut in the course of preparation. The fluid was collected in 3–15 min. samples.

The composition of the perfusion fluid varied. In the experiments designed for demonstrating the release of acetylcholine we used first, as recommended by Führer, a salt solution rich in urea (25 g. urea, 20 g.

NaCl, 1 g. KCl, 1·4 g. $CaCl_2$, 0·83 g. $MgCl_2$ and 0·17 g. $NaHCO_3$ made up to a final volume of 1100 c.c.). It was found, however, that the urea interfered with the assay of acetylcholine on the leech muscle, and for that reason the fluid was later changed to one containing, in 1100 c.c., 35 g. NaCl, 0·7 g. KCl, 0·1 g. $CaCl_2$ and 0·17 g. $NaHCO_3$. In the perfusions made for observing the effects of arterial injections of acetylcholine, the urea was retained but $MgCl_2$ was often omitted, and the concentrations of KCl and $CaCl_2$ made up to the same values as in the urea-free fluid. Apart from the fact that, in the absence of urea, pronounced oedema developed during prolonged perfusion, there was no difference in the results obtained with the different salt solutions. Eserine, when required, was added to the perfusion fluid in a concentration of 1 in 300,000 to 1 in 150,000.

The venous perfusate was tested for acetylcholine on the eserinized leech muscle. Since the salt concentration of the perfusion fluid was too strong for this purpose, it was diluted with four volumes of distilled water before testing.

Arterial injections of acetylcholine. Some extra precautions had to be taken to enable a rapid, close-range arterial injection to be made without mechanical disturbance of the organ. The perfusion cannula, which was also used for the injections, consisted of a syringe needle provided with a tap, the shaft being shortened so that the total dead space was less than 0·05 c.c. and the tip being made blunt and provided with a groove to take the ligature. The needle was fixed and held rigidly in position by a special clamp. The rubber tube leading from the reservoir was attached to the separated nozzle from an all-glass syringe, the tip of which fitted easily into the butt of the needle cannula, and could be quickly removed when necessary. When it was desired to make an arterial injection, the tap of the needle cannula was closed, the glass nozzle from the perfusion system was disconnected, and the nozzle of a syringe, filled with fluid to be injected, was fitted tightly to the butt of the cannula. The tap was then opened, the fluid from the syringe rapidly injected and the tap closed again. A change of nozzles then enabled the perfusion to be promptly restarted, but this was found to be unnecessary between repeated injections of the same solution.

Stimulation of the nerves. Since the area perfused from the second artery extended to that supplied from the first nerve, the first two nerves were stimulated together. In large animals the diameter of the nerve trunks was so great as to render it advisable to divide the nerves into finer bundles, thus facilitating a more homogeneous distribution of the

stimulating current. Single or repeated shocks (condensor discharges) were applied to the nerves. For repeated stimulation a rotary commutator was used; the rate of stimulation varied between 2 and 20 per sec., usually about 15. The stimuli were always supramaximal. Use was often made of local mechanical stimulation, which is, in fact, a stimulation of the terminal nerve fibres, in order to test the survival of the perfused organ or to measure roughly the changes in sensitivity during the perfusion. For that purpose light pressure was exerted on the skin with an ebonite rod.

Observation and recording of discharges. The electrodes used for leading off were always zinc plates of a few square centimetres, one side being coated with an insulating varnish the other side being placed under the ventral surface of the organ in good contact with the skin. They were connected to the amplifier by flexible leads so as to avoid transmission of the mechanical vibrations set up at the moment of an injection. The changes in potential were led to a direct-current amplifier, but sometimes, to avoid continuous readjustment necessitated by slow changes in polarization and by various other disturbances, we used condenser coupling with a time constant of about 1 sec. The variations recorded following arterial injections of acetylcholine were recorded with this arrangement and have therefore undergone some distortion. The diphasic form of the wave in Fig. 5, for instance, is not a real effect of the injected acetylcholine, but is due to the peculiarities of the recording arrangement.

The amplification was adapted in each case to the size of the deflexion under examination. A sensitivity of 10 μV. was often necessary for the discharges caused by mechanical stimulation and for the discharges occurring spontaneously under certain conditions. The proper range for detecting the electrical variations caused by small doses of acetylcholine was 0·1–10 mV. Of the total discharge on stimulation of the nerves, a small fraction only was allowed to enter the amplifier. The arrangement allowed quick changes from one sensitivity to another.

Coupled with the amplifier was a Dubois electromagnetic oscillograph for photographic registration and a cathode-ray tube for observing the deflexions during the experiment.

The usual precautions were taken to avoid electrical and mechanical disturbances. The injection experiments were made in a chamber with metal walls. The person making the injections wore rubber gloves, and made certain each time that his movements at the moment of injection did not produce any appreciable variation.

Results

Acetylcholine content

Eserinized saline extracts of the electric organ equivalent to a small amount of fresh tissue had a strong stimulating action when tested on the frog's rectus muscle. No such effect was obtained with saline extracts prepared without eserine or with eserinized saline extracts made alkaline by NaOH and kept in this condition for several minutes. For instance, in Fig. 1 are shown the effects of solutions containing the equivalent of 1 mg. fresh tissue per c.c. of an eserinized saline extract (*B* and *F*), the equivalent of 2·5 mg. tissue per c.c. of the same extract after treatment

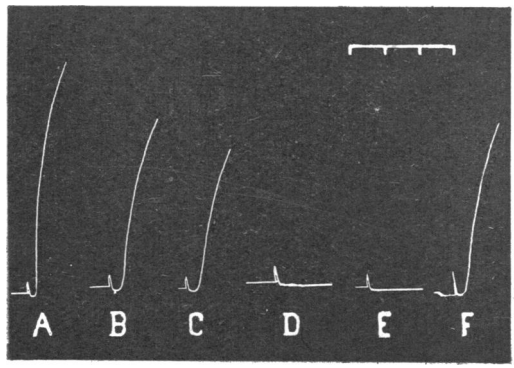

Fig. 1. Responses of the eserinized frog's rectus muscle to acetylcholine chloride (*A* and *C*) and to extracts of the electrical organ. *B*, *D*, *F* = eserinized saline extract; *D* treated with alkali. *E* = saline extract. Time in minutes. (Details see text.)

with NaOH, but neutralized before testing (*D*) and the equivalent of 2·5 mg. tissue per c.c. of a saline extract, made without eserine from another piece of the same organ (*E*). Only the eserinized saline extract, not treated with alkali, caused a contraction which was weaker than that produced by a solution of acetylcholine chloride containing 0·1 μg./c.c. and stronger than one containing 0·067 μg./c.c. (*A* and *C*). In the absence of eserine the active principle was not destroyed when the tissue was extracted with boiling acidified water (*p*H about 4) or with trichloracetic acid. These facts rendered it highly probable that the active principle was acetylcholine. The conclusion was substantiated by a more complete pharmacological analysis of an extract made with boiling acidified water. The extract was neutralized before testing. Like acetylcholine, small amounts of the extract caused contraction of the eserinized muscle of the body wall of the leech and of the frog's rectus abdominis, inhibited the

frog's heart and lowered the arterial blood pressure of the cat when injected intravenously, and, when injected in larger amounts into the central stump of the coelic artery after evisceration of the cat and giving atropine, caused a rise of arterial pressure due to output of adrenaline from the suprarenals. Atropine abolished the depressor action, the cardio-inhibitor effect, and the contraction of the frog's rectus muscle. There was good quantitative agreement between the depressor, cardio-inhibitor and leech-muscle stimulating actions of the extract, when assayed in terms of the actions of acetylcholine on the same tissues. The extract was inactivated when left for a few minutes with dog's plasma which is rich in cholinesterase; this inactivation did not occur when eserine was added to the plasma. These results appear to justify the conclusion that the active principle was acetylcholine, and for routine estimations it was thought sufficient to assay the eserinized saline extracts on the frog's rectus muscle.

The electric organs were thus found to contain 40–100 μg. of acetylcholine per gram of fresh tissue. In one fish in which the organs weighed 175 g. each, the acetylcholine chloride equivalent was nearly 90 μg./g., so that the total acetylcholine content of both organs together amounted to about 30 mg. Since the electric organ is an extremely wet tissue, containing 92% of water, the acetylcholine content per unit of dry weight is probably the highest yet observed in any organ.

Liberation of acetylcholine during nerve stimulation

When the venous effluent from the electric organ perfused with eserinized salt solution was tested in a fivefold dilution on the leech muscle, the samples collected during the beginning of the resting perfusion had some stimulating effect which diminished progressively from sample to sample, until eventually the fluid became inactive. Such an effect was also observed with the first samples of perfusate from the one experiment in which no eserine had been added to the perfusion fluid, suggesting that the effect was possibly not due to acetylcholine. Stimulation of the nerves was not started until the samples had either become inactive, or exerted only very slight effects on the leech muscle. When the nerves were then stimulated for a few minutes, the perfusate collected during the stimulation period caused a strong contraction of the leech muscle, as illustrated by the experiment of Fig. 2. All samples were diluted fivefold before testing. The effects of two consecutive samples collected before the stimulation of the nerves are shown at *A* and *B*. There is a slight contraction at *A*, but none at *B*. The strong

contraction at *C* was produced by the sample collected during a 3 min. period of stimulation at a frequency of 12 per sec. The 3 min. sample collected immediately after the period of stimulation was again nearly inactive (*D*). The next 3 min. sample was inactive (*E*). This was the usual result. In one experiment only did the sample collected in the first few minutes after the stimulation cause strong contraction, although weaker than that produced by the sample collected during stimulation. When the nerves were restimulated after the venous effluent had again become

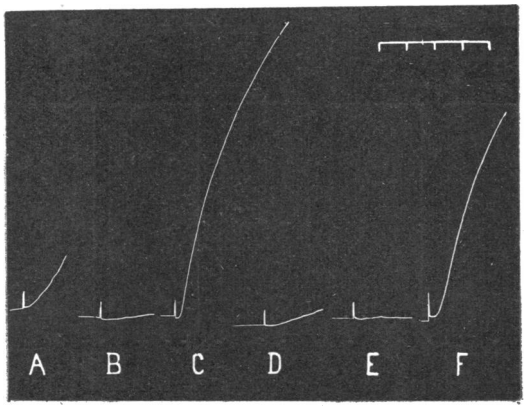

Fig. 2. Responses of the eserinized leech muscle to acetylcholine 1 in 150 millions (*F*) and to perfusate from electrical organ perfused with eserinized salt solution. *A* and *B* samples collected before, *C* during, *D* and *E* after stimulation of the nerves. All samples tested in fivefold dilution. Time in minutes.

inactive, the stimulation sample regained its stimulating effect, but this was usually less pronounced than that observed with the sample collected during the first stimulation period.

It appears justifiable to attribute the activity of the stimulation sample to the presence of acetylcholine. The high acetylcholine yield obtained on extraction of the organ is in favour of this conclusion. We have further shown that the active principle released by stimulation was destroyed by alkali and that it did not appear in the absence of eserine from the perfusion fluid. Fig. 3 gives a record of the one experiment done without eserine. Again the first sample had some stimulating effect on the leech muscle (*B*), which diminished greatly with the second sample (*C*). The sample collected during the period of stimulation showed only a slight increase in activity (*D*) which was negligible in comparison with the strong activity of the stimulation sample in the experiment illustrated

in Fig. 2. *E* shows the effect of the sample collected immediately after the stimulation.

When the active samples were assayed against acetylcholine it was found that in addition to their direct stimulating action they decreased the sensitivity of the leech muscle to subsequent doses of acetylcholine. The depression lasted for a period of several minutes. The effect has not been further examined, but it rendered an accurate quantitative comparison difficult. It was possible, however, to obtain an approximate estimate of the concentration of acetylcholine in the undiluted samples. In different experiments it varied between 1 in 18 million and 1 in 100 million. In Fig. 2, for instance, the effect of a fivefold diluted sample

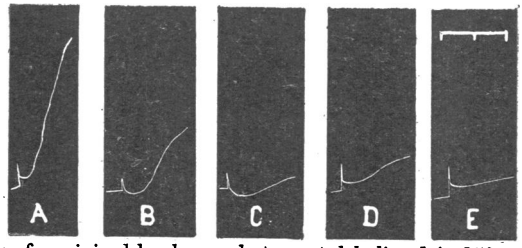

Fig. 3. Responses of eserinized leech muscle to acetylcholine 1 in 150 millions (*A*) and to perfusate from electrical organ perfused with non-eserinized salt solution. *B* and *C* samples collected before, *D* during and *E* immediately after stimulation of the nerves. All samples tested in fivefold dilution. Time in minutes.

tested at *C* was stronger than that caused by a solution of acetylcholine 1 in 150 million tested at *F*. A closer approximation gave an acetylcholine concentration of the undiluted sample of over 1 in 20 million. Since the venous outflow exceeded 3 c.c./min., the amount of acetylcholine released per minute was at least 0·15 μg. Only in the superior cervical ganglion of the cat, an organ in which also the concentration of synaptic junction is very high in relation to the tissue volume, have concentrations of acetylcholine been recovered in the perfusion effluent, during stimulation, of so high an order as those which we observed in some of the few experiments which we were able to make on the electric organ of the *Torpedo*.

Effects of eserine on the nervous discharge from the perfused organ

The effects of eserine were similar to those obtained by Auger & Fessard [1939*a*] on the isolated prisms of the electric organ.

After the addition of eserine to the perfusion fluid, the discharges obtained from stimulation of the nerve trunks, or from light pressure on

the skin with an ebonite rod, were modified in a characteristic manner. There was a great prolongation in the descending phase of the electrical deflexion of the single discharge. On the other hand, with continuous stimulation of the nerves to the perfused part, at a frequency of 5–15 per sec., fatigue set in much more rapidly than in corresponding experiments without eserine. In the absence of eserine such repeated stimulations could be continued for 10–15 min. without great diminution in the electrical responses; in the presence of eserine they diminished within 2–3 min. and disappeared after a further 2–3 min. When stimulation was then discontinued for 20–30 sec., the first stimuli on renewal of stimulation produced good responses, but stimulation quickly became ineffective again, and with renewed periods of rest and restimulation, the successive periods of recovered excitability became shorter.

In some experiments the perfusion was started without eserine and, while the arrangement for observing the discharges remained in place, the perfusion fluid was changed to one containing eserine. In these instances we obtained the definite impression that at the commencement of the eserine perfusion, the sensitivity of the organ to stimulation by slight pressure on the skin with an ebonite rod increased, but no accurate quantitative examination of this phenomenon was made. During this period the removal of the pressure from the skin was often followed by a period in which spontaneous discharges occurred, either isolated or in groups. At later stages of a prolonged perfusion with eserine the organ became definitely much less sensitive to mechanical stimulation.

Arterial injections of acetylcholine into the perfused organ

Perfusion without eserine. The arterial injections of relatively strong doses of acetylcholine into the perfused organ, connected with an amplifier, caused a potential change in the same direction as that produced by the normal discharge, but lasting much longer. The sensitivity to acetylcholine varied in different experiments; in some experiments it was necessary to inject 100 μg., in others effects were obtained with 20 and even with 10 μg. Repeated injections of the same dose gave comparable responses. Control injections of saline produced no changes in potential or slight deviations in the opposite direction. Fig. 4 illustrates the effects of 200, 100, 20 and 5 μg. of acetylcholine (at I, II, IV and V). The first two doses of acetylcholine were injected in a volume of 0·5, the third in a volume of 0·2 and the fourth in a volume of 0·1 c.c. It will be seen that the degree and duration of the electrical variation are dependent upon the dose. In comparing the responses the fact has to be taken into

account that the sensitivity of the amplifier was increased four times after the second injection. The maximal variation after 200 μg. amounted to about 0·7 mV. and after 20 μg. to less than 0·1 mV. 5 μg. were without effect (V).

It is difficult to compare these variations in potential with those obtained during the normal discharge, since we do not know the number of plates involved in the response. The discharge obtained from a small number of plates, by light pressure on the skin with an ebonite rod, produces variations of a few hundredths of a millivolt and requires a great increase in amplification.

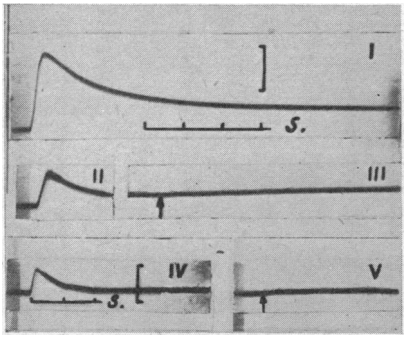

Fig. 4.

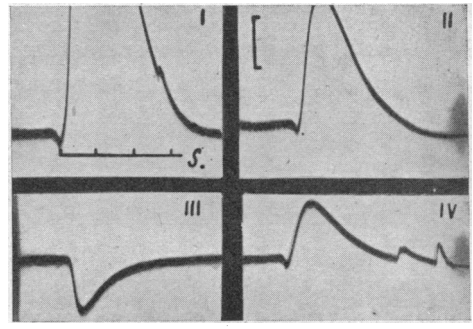

Fig. 5.

Fig. 4. Electrogenic effects of 200, 100, 20 and 5 μg. of acetylcholine (I, II, IV and V) injected arterially into electrical organ perfused with non-eserinized salt solution. At III injection of 0·5 c.c. perfusion fluid. Between II and III sensitivity increased fourfold. 0·5 mV. indicated at I, 0·1 mV. at IV. Time in seconds.

Fig. 5. Electrogenic effects of 10, 5 and 2·5 μg. of acetylcholine (I, II and IV) injected arterially into electrical organ perfused with eserinized salt solution. At III injection of 0·5 c.c. perfusion fluid. 0·5 mV. indicated at II. Time in seconds.

The striking difference is the shortness of the normal discharge, as compared with the long-lasting deviation following an injection of acetylcholine. The normal discharge takes 2–6 msec., whereas the deviation caused by 20 μg. of acetylcholine lasts more than a second, and that caused by 200 μg. has not come to an end after several seconds. With doses over 50 μg. there is always some residual depolarization. With a decrease in dosage the ascending phase of the discharge becomes more abrupt and there is a great shortening of the descending phase.

Perfusion with eserine. The effects of acetylcholine were examined during the first stages of perfusion with eserine. Under these conditions the sensitivity of the organ to acetylcholine was found to be much greater

than during perfusion without eserine. The experiment of Fig. 5 shows that under eserine it is possible to obtain an effect even with 2·5 μg. of acetylcholine, injected in 0·2 c.c. (IV), the maximal change in potential being 0·5 mV. The main wave is sometimes followed by small secondary undulations, such as those seen in the record. In the experiment of Fig. 5 a control injection of perfusion fluid caused a deviation in the opposite direction (III), probably due to the mechanical disturbance, which it is difficult to avoid completely with a rapid injection. The effect becomes smaller when a control injection is made more slowly. It is seen also when acetylcholine is injected, causing a slight deflexion downwards which precedes the response to acetylcholine. It therefore involves a slight deformation of the response, but it has, on the other hand, the advantage of marking the moment of injection and demonstrating the existence of a latency of a few tenths of a second between injection and the start of the acetylcholine response. The change produced by 5 μg. of acetylcholine (II) was stronger than that produced without eserine by 200 μg., as shown in the experiment of Fig. 4. With 10 μg. (I) the potential change is greater than 3 mV., in fact the height and duration of the deviations in Fig. 5 are shortened a little, since for this experiment the amplifier was coupled with a condenser with a time constant of about 1 sec.

In two experiments we have used a very sensitive amplification in order to be able to see if there are brief and small discharges superimposed on the slow variation of polarization, which was kept down by inserting a small capacity condenser. Sometimes we observed groups of brief discharges at the moment of the injection of acetylcholine, but the effect was not regularly obtained and occurred sometimes with control injections. It is therefore probably to be attributed to a mechanical effect of the injection. It appears likely that the shock on the adjoining tissue when the arteries are suddenly filled at the moment of injection is sufficient to provoke some rhythmic discharges from a few plates.

Discussion

Our experiments have confirmed our initial hypothesis of the cholinergic nature of the nerves to the electric organ of the *Torpedo*. The strong concentration of cholinesterase, as shown earlier by Marnay and by Nachmansohn & Lederer, and of acetylcholine in these organs which have a 92% water content, the appearance of acetylcholine in the venous effluent of the perfused organ during stimulation of its nerves

and the electrogenic effect of arterial injections of small doses of acetylcholine, each a suggestive fact in itself, would not separately be sufficient to establish the hypothesis. Taken in conjunction, however, they provide strong evidence in its support and make it possible to accept the idea that the electrical phenomena in these organs are mediated by a cholinergic nervous mechanism.

From these results, viewed in conjunction with the effects of nerve degeneration, the actions of drugs and the latency of the response, we may picture the natural discharge as an effect, on the polarization of the functional interfaces, of minute amounts of acetylcholine released simultaneously at the ventral surfaces of all the plates and then destroyed within the brief refractory period. There is no necessity for postulating a special, independently excitable structure, comparable to the contractile structure of the striated muscle fibre. The liberation of acetylcholine appears to be the final event, devoid of any further stimulating action, and responsible in itself for the sudden change in polarization which constitutes the discharge of the organ.

The effects of eserine on the response to nervous stimulation may be explained on this assumption. If we compare its effects with those obtained on the response of striated muscle to motor nerve impulses [Brown, Dale & Feldberg, 1936; Briscoe, 1936; Bacq & Brown, 1937], there is a striking parallel and a characteristic difference. In both tissues the responses to stimuli repeated at sufficiently short intervals are depressed, those to adequately spaced single stimuli are enhanced, but the enhancement is of a different kind. In the muscle the single twitch is changed into a brief, waning tetanus which can be explained on the assumption that the released acetylcholine, and the consequent change in polarization of the end-plates, persists through several successive refractory periods of the muscle fibre. Only the end-plate being present in the electric organ, no discontinuous discharge can be expected, delay in the destruction of acetylcholine on the active interfaces merely lengthening the wave of potential, particularly in its descending phase. This, in fact, was the observed change brought about by eserine. It is interesting to note that the form of the natural discharge from the electric organ of the ray [Auger & Fessard, 1939*b*] resembles that from the electric organ of the *Torpedo* after eserine. The cholinesterase concentration in the electric organ of the ray being only a small fraction of that found in that of the *Torpedo* [Nachmansohn, 1940], the resemblance can be explained as due to delayed destruction of acetylcholine in both instances. The rapid onset of fatigue to repeated stimulation after eserine resembles so

closely the paralysing or curare-like action which eserine exerts on the responses to rapidly repeated nerve impulses in striated muscles, as well as in the sympathetic ganglion [Brown & Feldberg, 1936] as to suggest identical mechanisms in all three instances. For the muscle, as well as for the ganglion, there is good evidence for attributing at least part of the effect to the paralysis produced by excess of acetylcholine, when allowed to act for a sufficiently long time on the motor end-plates or the ganglion cells. We have not as yet made the experiment required to obtain direct evidence for a similar action of acetylcholine on the plates of the electric organ, but the results with eserine are at least suggestive.

If we picture the normal discharge from the electric organ as an action of the released acetylcholine on the state of polarization of the functional interfaces, there is no necessity to expect that an arterial injection of acetylcholine will produce a discontinuous response, as in striated muscle. In striated muscle an injection of acetylcholine causes a repetitive activity in the form of an asynchronous tetanus [Brown, 1937]. The analogy of the continuous change in the state of polarization occurring at the plates of the electric organ, when acetylcholine is injected into its arterial system, strongly suggests that the repetitive series of excitatory waves and corresponding tetanus observed in the voluntary muscle, when acetylcholine is similarly applied, represent the discontinuous response of the fibres to a continuous change in the state of polarization of the end-plates. The same analogy would apply to the contrasted responses of the electric organ and of the voluntary muscle to a single nerve volley under eserine. In the electric organ repetitive discharges are obtained only by repetitive stimulation of the nerve.

The striking difference between the response of the electric organ to a single nerve volley and that produced by arterial injection of acetylcholine is the long duration of the latter, which lasts for several seconds, compared with the few milliseconds of the former. On the other hand, with injected acetylcholine the voltage is much smaller than that of the response of the whole organ to a nerve volley, although it may be several hundred times greater than the partial discharge from a small number of plates, as brought into activity, for example, by exerting light pressure on the organ. To interpret these differences we have to take into account the fact that an arterial injection of acetylcholine is unable to imitate the liberation of acetylcholine occurring at the moment of the nearly simultaneous arrival of an impulse at all the nerve endings. If this liberation represents, as we assume, the final effect of the nerve impulse, the form and duration of the potential deviation will depend

on the rapidity and simultaneity with which the acetylcholine appears and disappears at the active interfaces. Both processes proceed more slowly under the artificial condition of injection than under the normal condition of nervous excitation. The most abrupt injection is long compared with the shortness of a nerve volley. In addition, the acetylcholine, forced through the vessels, will reach the spaces between the plates before reaching the active regions, and, once accumulated in these spaces, or even having impregnated the whole organ, it will take some time to disappear. This process will be further delayed in the presence of eserine. In addition to these factors which are operating on each plate, further lengthening of the response must be caused by temporal dispersion, which exists indeed to some extent even with nervous stimulation, but is naturally much more pronounced with injection, owing to the transmission of the fluid through vessels of different lengths. This can easily be shown by making an arterial injection of Indian ink into the perfused organ, after removal of the skin; the time necessary for the ink to spread through the exposed part is of the order of a second. Such a lack of synchronism would by itself explain the long duration of the deflexion and its relatively small amplitude. The reduction of amplitude in comparison to that of the natural discharge does not result solely from the fact that there is a smaller number of plates discharging at each given moment, and having additive effects, but also, and to a greater extent, from the fact that the mass of the plates inactive at any one moment forms a passive conducting medium, into which the discharges of the active plates may be diverted. Hence only a fraction of the real potential is led off by the electrodes.

It seems useless at the present moment to push further the interpretation of the form of the electrical deflexion produced by injected acetylcholine, since we do not know the number of plates which become activated and we have no data enabling us to state precisely how much of the lengthening of the deviation is due to the factors operating at single plates, and how much to those due to temporal dispersion. The latter certainly play an important role as revealed by the fact that both phases of the deflexion are progressively shortened with diminution of the dose of acetylcholine, with which the number of activated plates decreases. An increase in the duration of the electrical variation on each plate, however, must also play an important role. Apart from the arguments already put forward in favour of this assumption, there is the fact that the slow wave of potential change usually does not show any oscillations. This complete smoothness of the wave is not easily reconciled

with the idea that they are built up by overlapping of single normal discharges of the plates, each having a duration only one thousandth of that of the wave produced by injecting acetylcholine.

New experiments are necessary to interpret quantitatively the character of the response to injected acetylcholine, but there is no inherent difficulty in understanding why the electrical response to it is not repetitive, and why it differs in duration and amplitude from the normal discharge.

The comparison of the electric organ with motor end-plates of striated muscles was to a great extent responsible for the trend our experiments have taken. The evidence, however, once obtained, also throws light on, and gives additional support to, the theory of chemical transmission of motor impulses at the end-plates of striated muscles.

Summary

The cholinergic nature of the nerves to the electric organ of the *Torpedo*, indicated by earlier observations, appears to be well established by the following observations:

(1) The electric organ yields on extraction 40–100 μg. of acetylcholine per gram of fresh tissue, corresponding to more than 0·1 % of the dry weight.

(2) During stimulation of the nerves to the organ, perfused with an eserinized saline solution, an alkali unstable substance which stimulates the eserinized leech muscle appears in the venous effluent. This does not appear in the absence of eserine from the perfusion fluid. It is concluded that the substance so liberated is acetylcholine.

(3) Eserine lengthens the descending phase of the single nervous discharge from the perfused organ and causes rapid fatigue of the response to repetitive nerve stimulation. Similar effects have been obtained by Auger and Fessard on isolated prisms.

(4) An arterial injection of acetylcholine into the perfused organ has an electrogenic effect. When the perfusion fluid contains eserine such an effect can be obtained with as little as 2·5 μg. of acetylcholine.

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