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PHARMACOLOGICAL INVESTIGATIONS ON A CENTRAL SYNAPSE OPERATED BY ACETYLCHOLINE

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Recently it has been shown that impulses in the motor-axon collaterals synaptically excite a special group of interneurones (called Renshaw cells) lying in the ventro-medial region of the ventral horn of the spinal cord (Eccles, Fatt & Koketsu, 1954). It would be expected that synaptic transmission from these collaterals of motor axons would be mediated by the same transmitter substance that operates at the peripheral neuro-muscular junctions of these motor axons, i.e. by acetylcholine, and pharmacological investigation confirmed this expectation. However, in some respects the pharmacological findings display anomalies: for example dihydro-β-erythroidine was a powerful blocking agent, whereas D-tubocurarine was ineffective; in contrast to such anticholinesterases as eserine and TEPP, prostigmine was found to be virtually ineffective; finally there was a large variation in the excitatory action produced by intra-arterial injections of acetylcholine. The present paper gives an account of further pharmacological investigations which have been performed on this synaptic mechanism in an attempt to account for these anomalies, and also of the further exploration of the pharmacological behaviour of Renshaw cells.

The methods of investigation have been previously described (Eccles *et al.* 1954), the experimental animal being the cat. The intra-arterial injection technique has been essentially that described by Holmstedt & Skoglund (1953). Despite the ligation of all the vessels from the lower region of the aorta (below the renal arteries) except the lumbar arteries, the injection (usually 1 ml.) must be distributed to an extensive area of tissue including the lumbar musculature.

RESULTS

The excitatory action of acetylcholine and nicotine

When relatively small quantities of acetylcholine chloride (10–100 μg) were given by intra-arterial injection, it has already been reported (Eccles *et al.* 1954) that

after a latent period of about 2 sec most Renshaw cells respond by a repetitive discharge that persists for several seconds. However, there was a wide variation in their responsiveness to acetylcholine. For example, with two cells the threshold was as low as 8 μg and with nine other cells it was below 30 μg, while several cells failed to respond even to 100 μg. Furthermore, it was frequently observed that the responsiveness of Renshaw cells declined with successive injections of acetylcholine. For example, with two cells kept under observation for a long period, a threshold value of 20 μg changed gradually to one of

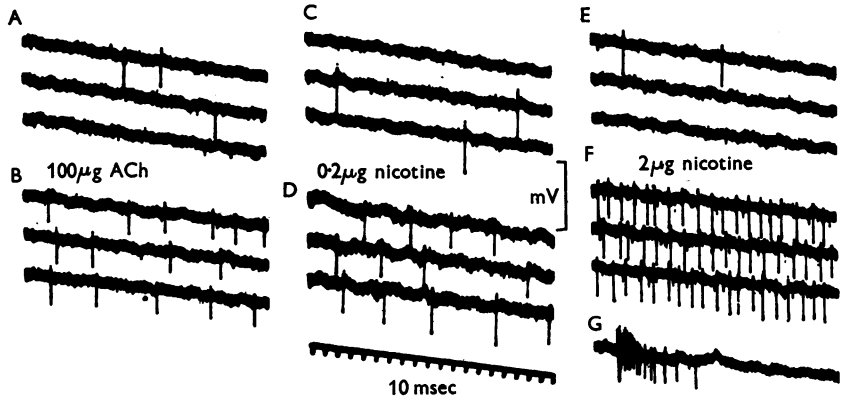

Fig. 1. Responses of a Renshaw cell recorded with a microelectrode in close proximity, downward deflexion signalling negativity. All traces are oblique because the transverse sweeps are recorded on a continuously moving film. Each sweep occupies about 200 msec, and only a few milliseconds separate the successive sweeps. Intra-arterial injection of 100 μg of acetylcholine chloride increased the slow spontaneous rate of discharge of the Renshaw cell (about 5/sec in A) to about 25/sec in the three successive traces of B which occurred at the height of the discharge. Similarly, the intra-arterial injection of 0·2 μg of nicotine increased the response of this same cell from the spontaneous rate of C to a maximum of about 20/sec in D, while 2 μg of nicotine caused the much larger effect, E to F. G shows the response of this Renshaw cell to an antidromic volley in L7 ventral root, the sweep speed being too slow for discrimination of the initial high-frequency discharge.

200 μg. This declining responsiveness to successive injections of acetylcholine has previously been reported for sympathetic ganglion cells (Feldberg & Vartiainen, 1934) and for the motor end-plate (Brown, Dale & Feldberg, 1936). Some Renshaw cells, however, even failed initially to respond to 100 μg of acetylcholine. A possible explanation of this changing responsiveness would be that vascular changes diminished the effectiveness with which the injected solution was applied to the Renshaw cells. This explanation can be tested by investigating the responsiveness of Renshaw cells to another excitatory agent, nicotine, at intervals during a serial investigation with acetylcholine injections. According to the above explanation it would be expected that there would be parallel changes in responsiveness.

All Renshaw cells that have been tested have been very sensitive to nicotine given by intra-arterial injection. The smallest effective quantity has varied from 0·2 to 2 μg of the pure alkaloid in the seven cells in which by graded dosage there has been an evaluation of the threshold. As illustrated in Fig. 1, Renshaw cells have always been much more sensitive to nicotine than to acetylcholine, 0·2 μg nicotine producing in this cell (cf. Fig. 1C with D) about the same intensity of response as 100 μg of acetylcholine chloride (cf. Fig. 1A with B), while 2 μg of nicotine evoked a frequency of discharge as high as

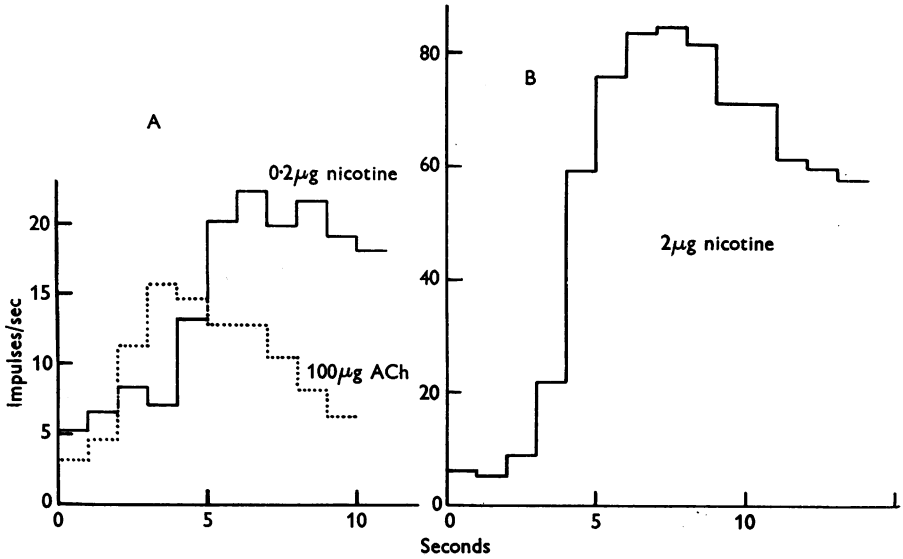

Fig. 2. The total number of discharges in successive seconds, i.e. the frequency per second, is plotted as ordinates against the times after the intra-arterial injection as abscissae. In Fig. 2A the dotted line illustrates the response to 100 μg of acetylcholine (mean of response to five injections, Fig. 1A, B being part of one), while the continuous line shows the response to 0·2 μg of nicotine (mean of responses to three injections, Fig. 1C, D being part of one). Fig. 2B, as in Fig. 2A, but mean of responses to two injections of 2 μg of nicotine, Fig. 1E, F being part of one.

100 per sec (cf. Fig. 1E with F). In addition, the response evoked by nicotine has always had a slower rising phase and a much slower decline (the 0·2 μg response of Fig. 2A), being much more prolonged than an acetylcholine-evoked response of the same frequency (the 100 μg response of Fig. 2A). Only the first 15 sec of the responses evoked by 2 μg of nicotine was photographed (cf. Fig. 2B). Total durations of several minutes have been observed for such large responses (cf. Fig. 7).

When injected intra-arterially, nicotine has usually been 50 to 200 times as effective as acetylcholine in evoking discharges of Renshaw cells. An important factor determining this high ratio is revealed by the series of Figs. 3 and 4.

Initially an injection of 200 μg of acetylcholine was required to evoke a small response (Fig. 3B) similar to that evoked by an injection of 1 μg of nicotine (Fig. 3C), i.e. nicotine was about 200 times as effective as acetylcholine. After the intravenous injection of 0·2 mg/kg of eserine sulphate, there was the typical increase in duration of the Renshaw cell discharge evoked by an antidromic volley in the motor axons (cf. Fig. 3D with A; Eccles *et al.* 1954). The anticholinesterase had presumably depressed the destruction of the trans-

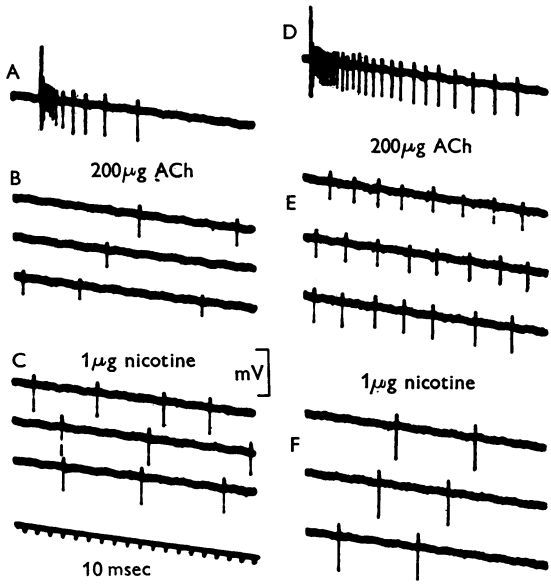

Fig. 3. Responses of another Renshaw cell recorded as in Fig. 1, but there was no spontaneous discharge. A is response to a single antidromic volley in L7 ventral root, while B and C show three successive sweeps at the height of the responses evoked by intra-arterial injection of 200μg of acetylcholine and 1μg of nicotine respectively. Several minutes after the intravenous injection of 0·2 mg/kg eserine, the responses A, B and C were changed to D, E and F. Inadvertently with these latter records there was a delay of almost 200 msec between successive sweeps.

mitter substance (presumably acetylcholine) by cholinesterase. Correspondingly, the injection of 200 μg of acetylcholine now evoked a more intense and prolonged response (Fig. 3E), while there was even a slight diminution in the response evoked by 1 μg of nicotine (Fig. 3F). The further intravenous injection of 1·0 mg/kg of eserine caused the Renshaw cell to discharge without additional application of acetylcholine for many minutes at a frequency of about 15/sec. This effect is regularly observed after the injection of large doses of anticholinesterases, being presumably due to the action of acetylcholine spontaneously liberated from the synaptic terminals. When this spontaneous discharge had ceased, the responses of Fig. 4A–D were recorded. The very

prolonged discharge (about 3 sec in duration) evoked by a single antidromic volley (Fig. 4A) showed that the cholinesterase activity had been depressed to a low value. Correspondingly, there was a large increase in the effectiveness of injected acetylcholine, 50 μg giving a large response (Fig. 4B), while 20 μg gave much the same response (Fig. 4C) as 1 μg of nicotine (Fig. 4D). It will be observed that the effectiveness of nicotine had not been significantly changed by the large dose of anticholinesterase.

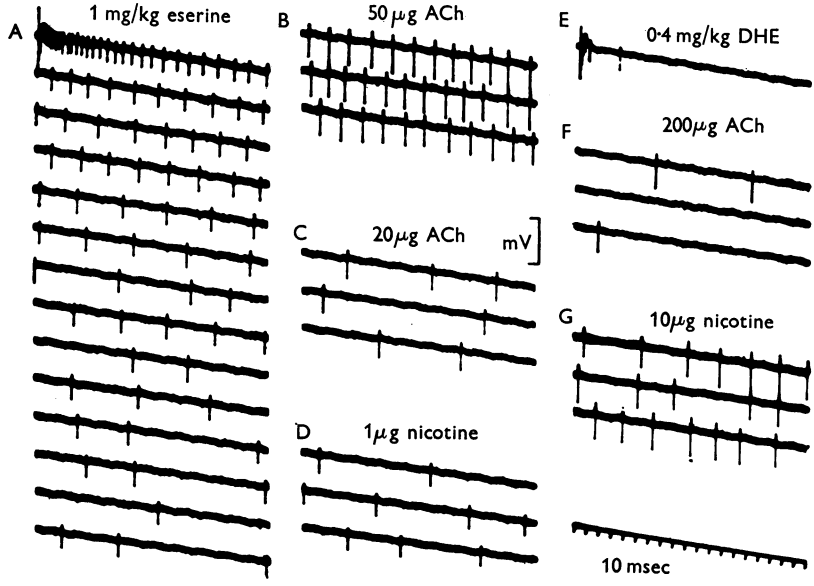

Fig. 4. Recording of same Renshaw cell as in Fig. 3, but several minutes after the further intravenous injection of 1·0 mg/kg eserine sulphate. A shows response evoked by a single antidromic volley, while B–D show respectively three successive records at the heights of the responses evoked by the intra-arterial injections of 50 μg acetylcholine, 20 μg acetylcholine and 1 μg nicotine. Records E–G were obtained during the maximum of the depression produced by the intravenous injection of 0·4 mg/kg dihydro-β-erythroidine hydrobromide, E being evoked by the antidromic volley and F and G by the intra-arterial injections of 200 μg acetylcholine and 10 μg nicotine respectively.

Thus the injection of 1·2 mg/kg of eserine increased the sensitivity of Renshaw cells to acetylcholine by about 10 times, the ratio to nicotine sensitivity having been increased from 1 in 200 to 1 in 20. This selective effect of eserine can be attributed to its action as an anticholinesterase. The absence of any significant change in the response of the Renshaw cell to nicotine indicates that in the doses used eserine had no direct effect on the excitability of Renshaw cells. The anticholinesterase not only increased, but also prolonged, the Renshaw cell discharge evoked by a given injection of acetylcholine. These effects on another Renshaw cell are illustrated in Fig. 5, where

the time courses of responses evoked by 30 μg of acetylcholine are plotted before and after two doses of eserine. However, the responses evoked by acetylcholine were still much briefer than those evoked by nicotine (cf. Figs. 2, 7). Possibly there was still enough active cholinesterase to account for this difference, but heavier dosage with anticholinesterase caused a rapid background discharge of the Renshaw cell and so made the later stages of a response indeterminable. One other feature of interest is that, even when no other

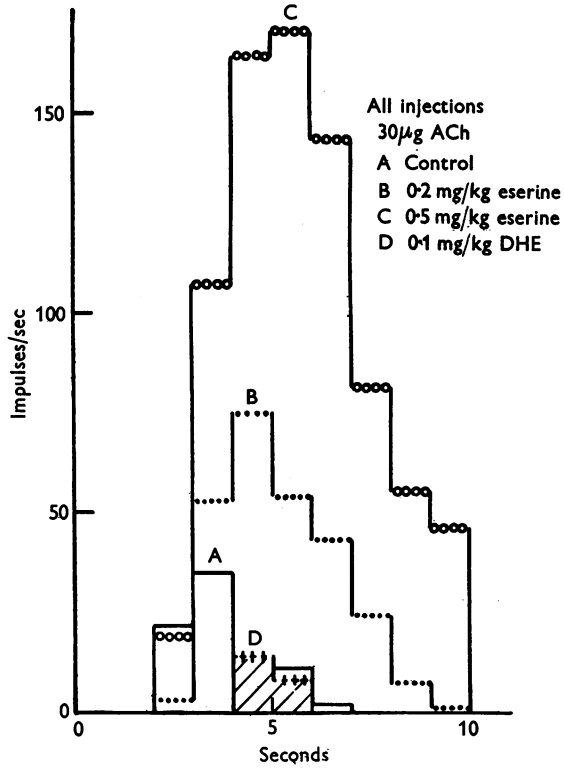

Fig. 5. Time course of the responses evoked in a different Renshaw cell by the intra-arterial injection of 30μg acetylcholine, plotted as in Fig. 2. Throughout there was no spontaneous discharge. The response before eserine is shown by A, B and C showing responses after the intravenous injection of 0·2 and a further 0·5 mg/kg eserine respectively. Finally, D shows the response after the intravenous injection of 0·1 mg/kg dihydro-β-erythroidine.

drugs had been injected, there were large differential changes in the sensitivity of the Renshaw cells to acetylcholine and to nicotine. Invariably the sensitivity to nicotine has increased during a series of injections, often by a factor of 10, while at the same time there has usually been a decline in the sensitivity to acetylcholine. This divergence is at present inexplicable.

In contrast to the selective change in sensitivity produced by an anticholinesterase, the intravenous injection of dihydro-β-erythroidine hydrobromide caused approximately the same change in the excitability of the Renshaw cells to nicotine and to acetylcholine. For example, after the intravenous injection of 0·4 mg/kg of dihydro-β-erythroidine, 10 μg of nicotine evoked the response of Fig. 4G, which is little more than that evoked by 1 μg prior to the injection (Fig. 4D), and 5 μg of nicotine was quite ineffective. At the same time the sensitivity to acetylcholine was also greatly reduced, 200 μg evoking a smaller response than did 20 μg prior to the injection (cf. Fig. 4F with C). The response evoked by an antidromic volley was also reduced characteristically (cf. Eccles *et al.* 1954) so that virtually only the short initial burst remained (cf. 4E with A). It appears that the intravenous injection of 0·4 mg/kg of dihydro-β-erythroidine had diminished the sensitivity of the Renshaw cells to both nicotine and acetylcholine to about the same degree (about 10 times). This effect parallels the action of D-tubocurarine (and presumably dihydro-β-erythroidine) at the synapses of sympathetic ganglia.

The effect on motoneurones of the various changes in Renshaw cell discharges can be seen in an experiment in which the microelectrode was recording simultaneously from a Renshaw cell and the axon of a rhythmically discharging motoneurone (Fig. 6A). When an intra-arterial injection of 20 μg nicotine greatly increased the frequency of the Renshaw cell discharge (Fig. 6B), there was first a slowing of the motoneurone discharge and later suppression of all discharge, as is shown in the plotted course of the Renshaw cell and motoneurone discharges (Fig. 7). As the frequency of the Renshaw cell discharge gradually declined, there was some return of the motoneurone discharges (Fig. 6C and the intermediate part of Fig. 7), but recovery to the initial frequency did not occur until an intravenous injection of 0·1 mg/kg of dihydro-β-erythroidine (DHE) virtually suppressed the Renshaw cell discharge (Fig. 6D and the latter part of Fig. 7). These changes are readily explicable by the inhibitory action which Renshaw cells have been shown to exert on motoneurones (Eccles *et al.* 1954). If this information were not available, the changes in motoneuronal responses could be taken to indicate that nicotine had a direct depressant action on motoneurones and that this effect was blocked by dihydro-β-erythroidine. Possibly the depressant action which nicotine was observed to have on spinal reflexes (Schweitzer & Wright, 1938; Bülbring & Burn, 1941) is explicable by its excitatory action on the Renshaw cells with the consequent inhibition of motoneurones.

Several other substances (succinylcholine, decamethonium, mecholyl and arecholine) having an action resembling acetylcholine on other tissues have been tested by intra-arterial injection. The first two are nicotinic and the other two muscarinic in action. All have been uniformly without effect on Renshaw cells. In view of the wide variation in the effectiveness of acetylcholine when

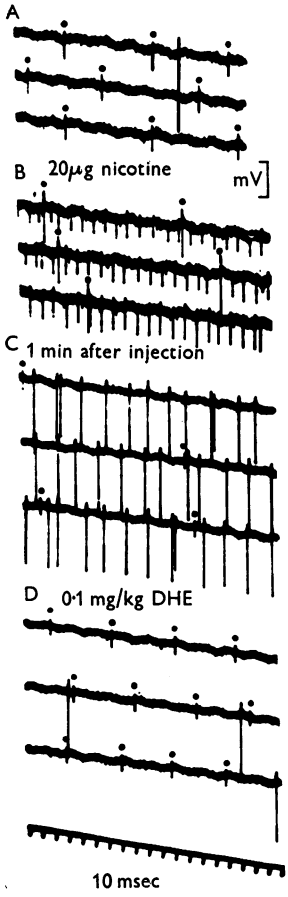

Fig. 6.

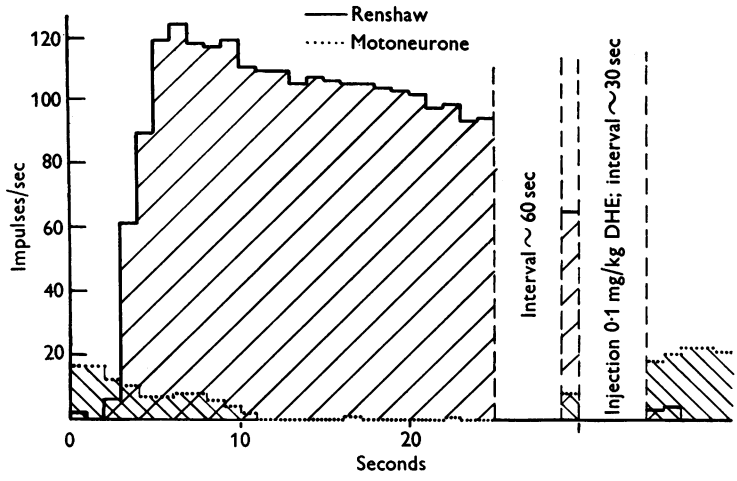

Fig. 7.

Fig. 6. Combined records of Renshaw cell and motoneurone recorded as in Fig. 1, both being identified by the characteristic responses evoked by an antidromic volley in L7 ventral root. Throughout, the motoneuronal spikes are indicated by dots placed just over their diphasic component. A shows three successive records of spontaneous discharges, which were at a regular rhythm of about 15/sec for the motoneurone, but only rarely for the Renshaw cell—the single large spike. B shows the height of the reponse to an intra-arterial injection of 20μg of nicotine. The Renshaw cell now gives the small spike at over 100/sec (note one double response), while the motoneurone gives the larger spike with a pronounced diphasic form. Presumably a small movement was responsible for the alteration in the relative sizes of the two types of spike. C shows response over 1 min after the injection, the frequency of the Renshaw cell discharge having fallen to about 60/sec (note three double discharges). Finally, D shows effect of an intravenous injection of 0·4 mg/kg dihydro-β-erythroidine in slowing the Renshaw cell discharge with consequent recovery of the motoneuronal discharge.

Fig. 7. Time course of responses of Renshaw cell (continuous lines) and motoneurone (dotted lines), plotted as in Fig. 2, for the series illustrated in Fig. 6A–D. Characteristic diagonal hatchings are used to distinguish the two responses.

given intra-arterially, further tests are desirable. However, with two Renshaw cells succinylcholine chloride was ineffective in a dose 10 times as large as an effective dose of acetylcholine chloride, and when the cholinesterase was largely inactivated by eserine, succinylcholine was found to be ineffective in a dose that was 50 times the effective dose of acetylcholine. Doses of 100 to 200 μg of the other three substances were similarly ineffective.

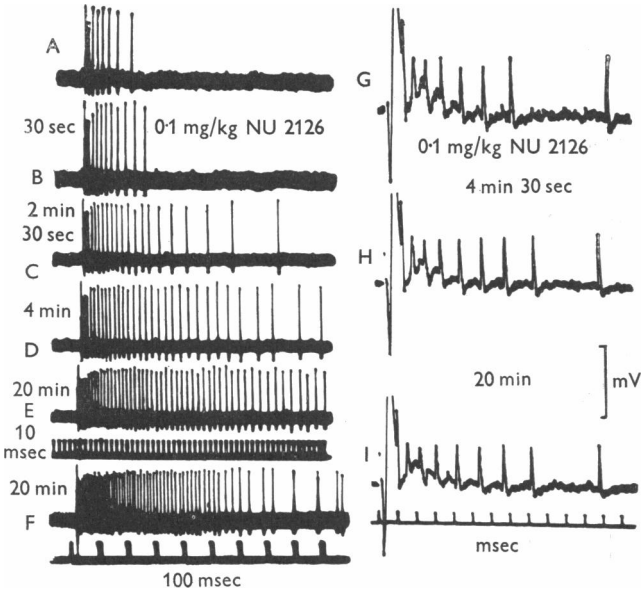

Fig. 8. Renshaw cell discharge evoked by maximum antidromic volley in L7 ventral root, A and G being before and B–F and H and I being after intravenous injection of 0·1mg/kg of NU 2126. The interval after the injection in minutes is marked at the side of each record. Upward deflexion signals negativity of the microelectrode, i.e. the reverse of Fig. 1.

Action of anticholinesterase drugs

In the previous paper (Eccles *et al.* 1954) there was an illustration of the effect of eserine in greatly prolonging the duration of the discharge which an antidromic volley evoked from a Renshaw cell. Fig. 8 illustrates the similar effectiveness of another anticholinesterase drug, dimethyl carbamate of 3-hydroxy-2-dimethylaminomethyl pyridine dihydrochloride (NU 2126), which has been found to be a very potent anticholinesterase at the amphibian neuro-muscular junction (Eccles & Macfarlane, 1949). The characteristic slow onset of the effect is seen in Fig. 8B to E. The prolongation and increased frequency of the later phases of the discharge in Fig. 8A to F contrast with the unchanged initial high frequency phase of the discharge (Fig. 8G, H, I).

The anticholinesterase, tetraethyl pyrophosphate (TEPP) resembled eserine and NU 2126 in its action on the Renshaw cell response evoked by an anti-

dromic volley (Fig. 9), but it appeared to be only about half as effective as these two very potent substances. Di*iso*propylfluorophosphate, DFP, also had a similar action, but had to be given in a dose as large as 1 mg/kg.

In contrast to these anticholinesterases, prostigmine was much less effective, even in a dose as large as 2 mg/kg. For example, in Fig. 10 an injection of 1 mg/kg of prostigmine bromide caused only a small prolongation of the responses evoked by a single antidromic volley and by a brief train of antidromic volleys (compare Fig. 10C with A and D with B). In contrast, a few minutes later the injection of one-tenth the amount of eserine had a greater effect (compare Fig. 10E with C and F with D). The comparison is best made with one of the later traces of Fig. 10B, D and F. For example, in the fourth trace there were respectively 10, 13 and 23 responses. However, in two of the eight Renshaw cells so investigated prostigmine was observed to have an anticholinesterase action comparable with that of eserine.

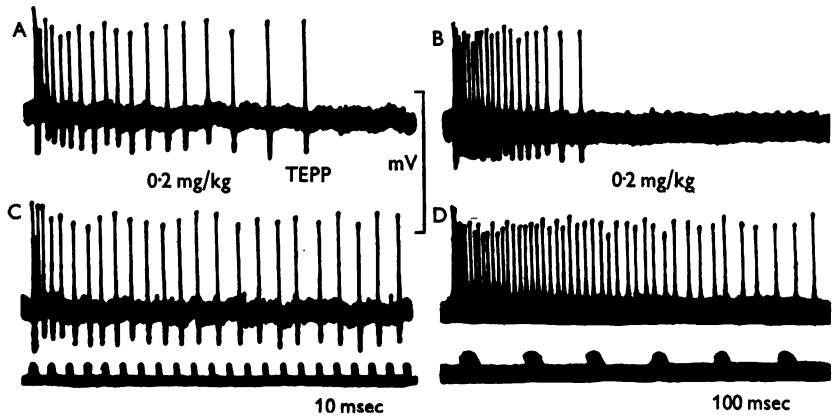

Fig. 9. As in Fig. 8, but to illustrate effect of the anticholinesterase, TEPP. A and B before and C and D after injection of 0·2 mg/kg of TEPP.

One possible explanation of the relative ineffectiveness that is usually observed for prostigmine given by intravenous injection is that a barrier to its diffusion surrounds the blood vessels of the central nervous system. There is evidence that this diffusional barrier exists for many substances (Krogh, 1946; Tschirgi, 1952; Manery, 1954). This possibility has been tested in a few preliminary experiments by directly injecting prostigmine into the neighbourhood of the Renshaw cell that is under observation. The recording microelectrode has also functioned as the injecting cannula. With the present technique any one Renshaw cell can be subjected to the action of only one drug, so at best it is only possible to make a very rough comparison of the effectiveness of different drugs. However, prostigmine would appear to be at least as effective as eserine when applied to the environment of all the Renshaw

cells that have been tested. Possibly the relative ineffectiveness that is usually observed for intravenous injections of prostigmine is attributable to a barrier that obstructs diffusion out of the blood vessels in the spinal cord. However, further experimental investigation is desirable.

The effect of intravenous injection of octamethyl pyrophosphoramide (OMPA) was tested on two Renshaw cells. In one cell, 40 min after injecting 2 mg/kg OMPA, there was a barely noticeable increase in the response to an antidromic volley in motor axons. At this time a further 4 mg/kg was injected, and 30 min later the discharge was observed to be prolonged, but only to a relatively minor extent. This very small effect of OMPA, in contrast to eserine or TEPP, is consistent with the finding of DuBois, Doull & Coon (1950) that OMPA fails to inhibit cholinesterase in the central nervous system, though effective in other tissues.

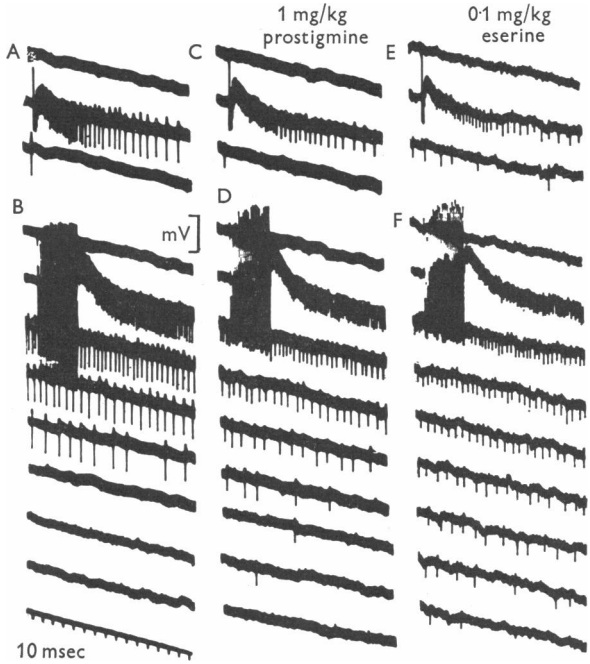

Fig. 10. Responses of a Renshaw cell recorded as in Fig. 1, and evoked by a single antidromic volley in L7 ventral root (A, C, E) or by a brief train of such antidromic volleys (27 at 560/sec in B, D, F). The sweep speed is too slow for identification of the individual spikes immediately after the single antidromic volley. An initial trace of the base-line is shown before all responses. A, B are before, and C, D 10 min after, the intravenous injection of 1·0 mg/kg prostigmine bromide, when the effect was at a maximum. The small anticholinesterase action is indicated by slight increase in duration of both responses. A larger anticholinesterase effect is seen in E and F (compare with C and D) 10 min after the further intravenous injection of 0·1 mg/kg eserine sulphate. The progressive diminution of the spike potential is presumably due to a small movement of the microelectrode.

Action of other drugs

It has already been reported (Eccles *et al.* 1954) that, when given intravenously in doses as low as 0·1 mg/kg, dihydro-β-erythroidine hydrobromide had a powerful depressant action on the repetitive discharge which a motor axon volley evoked from a Renshaw cell, whereas atropine sulphate was only slightly effective and D-tubocurarine chloride ineffective even when these drugs were given in much larger doses. Intravenous injection of gallamine triethiodide (Flaxedil) (1·2 mg/kg) and dimethyl-D-tubocurarine iodide (1 mg/kg) have now also been found to be ineffective. The only other blocking agent

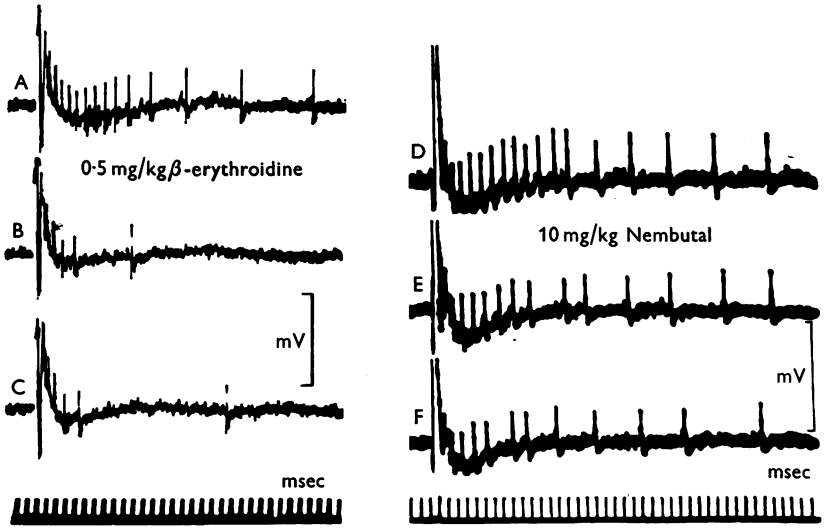

Fig. 11. A–C. Responses of a Renshaw cell recorded as in Fig. 7, A being before and B, C after the intravenous injection of 0·5 mg/kg β-erythroidine hydrochloride. D to F. As in Fig. 11A–C, but for another Renshaw cell, D being before and E, F after the intravenous injection of 10 mg/kg pentobarbitone sodium.

which has been effective in depressing transmission on to Renshaw cells was β-erythroidine hydrochloride. Fig. 11A to C illustrates the depressant action produced by this substance, which is observed to act similarly to the dihydro-compound, though it is only about one-fifth as effective. A similar difference in effectiveness of the two compounds has also been observed for the depressant action on neuromuscular transmission (Bovet & Bovet-Nitti, 1948).

Hexamethonium iodide and α-lobeline hydrochloride were given intra-arterially in doses of 100 μg, but did not have any significant effect on Renshaw cells.

Strychnine chloride in an intravenous dose of 0·1 mg/kg has been observed to have either no effect or a slight potentiating action on the Renshaw cell

discharge to a ventral root volley. It seems probable that in those cases where a potentiation of the discharge occurred the action of strychnine was not directly on the transmission process on to the Renshaw cells, but rather the effect arose on account of a large increase in the spontaneous discharge of interneurones, some of which impinged upon the Renshaw cell, thus raising its level of excitability. The evidence that Renshaw cells can be excited by pathways involving interneurones and that synaptic contact on them is not made exclusively by motor axon collaterals has been given in a previous paper (Eccles *et al.* 1954).

When given intravenously in doses of 10 mg or more, pentobarbitone sodium exerted a depressant effect on the responses of Renshaw cells evoked by an antidromic volley (Fig. 11D to F). As with other depressants, the effect is restricted to the later stages of the response. Presumably this relatively slight effect is merely an example of the depressant action of this anaesthetic on all types of central synaptic transmission.

DISCUSSION

Though in general conforming with a cholinergic transmission, the synaptic transmitter action from motor-axon collaterals to Renshaw cells has some anomalous features. It has been possible, experimentally, to show that there is likely to be some kind of diffusional barrier which prevents prostigmine from acting as an anticholinesterase when it is injected into the circulation. Possibly the ineffectiveness of D-tubocurarine may be similarly explained. Furthermore, the varying effectiveness of the diffusional barrier around blood vessels may explain the large range of variation observed for the ratio of acetylcholine to nicotine sensitivity with different Renshaw cells and even with the same cell at different times. The ineffectiveness of intra-arterial injections of succinylcholine could be similarly explained.

Schweitzer, Stedman & Wright (1939) pointed out that quaternary ammonium compounds such as prostigmine, D-tubocurarine and acetylcholine occur only in ionic form, and hence are insoluble in lipids. On the other hand, the salts of tertiary bases will be partly unionized and hence soluble in lipids. Eserine is one such tertiary base, while other compounds with similar solubilities would be dihydro-β-erythroidine, β-erythroidine, NU 2126 and nicotine. It has since been shown experimentally that the quaternary ammonium compounds, prostigmine and acetylcholine, cannot penetrate the surface membrane of the squid giant axon, whereas the tertiary bases, eserine and trimethylamine, penetrate readily (Bullock, Nachmansohn & Rothenberg, 1946; Rothenberg, Sprinson & Nachmansohn, 1948). The simplest explanation of our experimental results would thus be that blood vessels in the spinal cord are surrounded by a sheath which has the properties of a lipid membrane, i.e.

allowing amines to diffuse through but acting as a barrier to the diffusion of quaternary ammonium compounds.

There is now much evidence that a very effective diffusional barrier surrounds blood vessels of the central nervous system, which are thus distinguished from other blood vessels (Krogh, 1946; Tschirgi, 1952; Manery, 1954). Probably this barrier is to be identified as the pia-glial membrane (Patek, 1944; Woollam & Millen, 1954). Thus our explanation would ascribe to this diffusional barrier the special properties of being permeable to tertiary and lower order amines and relatively impermeable to quaternary ammonium compounds. The direct application of D-tubocurarine to the environment of a Renshaw cell would provide a crucial test of this explanation.

There is evidence also for a diffusional barrier that is much more intimately related to the synaptic terminals on Renshaw cells. The long duration of the repetitive discharge evoked by a single volley in the motor-axon collaterals shows that the acetylcholine liberated by a single impulse must persist in the environment of a Renshaw cell for at least 50 msec under normal conditions and for several seconds when the cholinesterase is inactivated (cf. Figs. 8–10). Since the intervals between successive discharges lengthen from the first very brief interval (usually 0·6–0·7 msec), it may be assumed that the excitatory action of the liberated acetylcholine begins to decline within 1 msec of its onset. If the acetylcholine were diffusing freely away from its site of liberation with the mobility obtaining in an aqueous medium, its concentration at the synaptic region would fall to a negligible value in a few milliseconds even when cholinesterase was inactivated (cf. Fatt, 1954; Ogston, 1955). Under such conditions the observed decline of the transmitter action was hundreds of times slower (cf. Figs. 4A, 8, 9, 10); hence it seems necessary to postulate that some diffusional barrier exists around the synaptic regions.

It has been invariably observed that the first few discharges have been resistant to the action of pharmacological agents, both those that block and those that potentiate (cf. Fig. 8G–I). This contrast between the initial and the later discharges indicates that the acetylcholine is initially effective by virtue of its high concentration close to the focus of liberation. As it is gradually dispersed by diffusion through the barrier, the rhythmic discharge slows, being then attributable to the more extensive action of a very much lower concentration. At this stage it is acting in a zone which is less protected by diffusional barriers and which consequently is more readily accessible to pharmacological agents, particularly the amines. Similar postulates of an initial focal action followed by a more diffuse action have already been proposed for cholinergic actions at the neuromuscular junction (Eccles, Katz & Kuffler, 1942; Eccles & Macfarlane, 1949; Fatt & Katz, 1951; Fatt, 1954) and at the ganglionic synapse (Eccles, 1944). This prolonged transmitter action contrasts with the action of other transmitters in the central nervous system, which are effective for a very few

milliseconds at the most, for example the excitatory and inhibitory transmitters on motoneurones (Coombs, Eccles & Fatt, 1955*a*, *b*) and the excitatory transmitter for group I*a* intermediate neurones (Eccles, Fatt & Landgren, 1955). At such synapses the removal of the transmitter is as fast as would be expected for diffusion of a substance having a coefficient of diffusion of the same order as known transmitter substances, e.g. acetylcholine.

SUMMARY

1. The techniques of intra-arterial injection and direct micro-injection have been employed in the further investigation of the pharmacological properties of the interneurones (called Renshaw cells) that are excited cholinergically by impulses in the motor-axon collaterals.

2. When applied by intra-arterial injection, nicotine evoked a prolonged discharge of Renshaw cells even in a dose as small as 0·2 μg, the threshold dose never being above 2 μg. Acetylcholine was less effective, often by a factor of hundreds. Furthermore, in contrast to nicotine, its effectiveness declined with successive injections.

3. Anticholinesterases increased the effectiveness of injected acetylcholine, causing even a tenfold lowering of the threshold dose, whereas there was no significant change in the effectiveness of nicotine. Dihydro-β-erythroidine greatly depressed the sensitivity of Renshaw cells to both nicotine and acetylcholine.

4. Various acetylcholine analogues were found to be ineffective when given by intra-arterial injection.

5. There has been a further investigation of the relative ineffectiveness of prostigmine as an anticholinesterase when it is given by intravenous injection. In two of the eight experiments prostigmine was as effective as eserine, but in the others it had at most only a small action. However, when directly injected into the environment of a Renshaw cell, prostigmine acted as a powerful anticholinesterase. It is suggested that surrounding blood vessels in the central nervous system there may be a diffusional barrier to prostigmine and also to D-tubocurarine. Several other anticholinesterases were found to be effective when given intravenously.

6. β-erythroidine was found to resemble dihydro-β-erythroidine in depressing synaptic transmission to Renshaw cells, but appeared to be less effective. Various other depressants of cholinergic transmissions were found to be ineffective.

7. Pentobarbitone sodium had a small depressant action, which presumably is an example of its general anaesthetic action on central synapses.

8. In addition to the postulated barrier around blood vessels, it is suggested that it is necessary also to postulate a barrier that greatly impedes the diffusion

of acetylcholine from the synaptic regions. This barrier may also account for some of the anomalous pharmacological properties of this cholinergic synapse.

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