J. Physiol. (1958) 141, 446–463

# THE EFFECT OF DIFFUSIONAL BARRIERS UPON THE PHARMACOLOGY OF CELLS WITHIN THE CENTRAL NERVOUS SYSTEM

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(*Received* 30 *December* 1957)

For many years it has been realized that the presence of the 'blood–brain barrier' has hindered the access of systematically administered drugs to possible central sites of action. The finding that Renshaw cells within the spinal cord are sensitive to acetylcholine and related substances, has provided a unique situation for the testing of drugs having known actions at peripheral cholinergic junctional regions. However, it seems likely that these cells are protected from some circulating drugs by at least two diffusional barriers. For example, the action of the blood–brain barrier is indicated by the finding that prostigmine is a very effective anticholinesterase when injected locally, but is almost ineffective when given intravenously (Eccles, Eccles & Fatt, 1956; D. R. Curtis, unpublished observation). The existence of a second barrier (cf. Eccles, Fatt & Koketsu, 1954; Curtis & Eccles, 1958), more intimately related to the synaptic terminals upon Renshaw cells, is suggested not only by the duration of the repetitive discharge produced by a single volley in the motor axons, but also by the relative ineffectiveness of the action of some drugs upon the first two or three responses.

The development of methods by which drugs can be applied to the immediate locality of Renshaw cells (Curtis & Eccles, 1958) overcomes the problem of the blood–brain barrier and allows an investigation to be made not only of the barriers associated with Renshaw cells, but also of the pharmacological properties of these cells.

## METHODS

The methods have been described in the previous paper (Curtis & Eccles, 1958). In several early experiments, because of the relative ineffectiveness of tubocurarine chloride (DTC) applied electrophoretically, the investigation was repeated after the electrodes had been tested on a neuromuscular preparation. A saturated solution of DTC, being approximately 0·04 M, is an extremely poor conductor and in most cases the electrode resistance exceeded 300 MΩ. With the available polarizing currents the maximum current obtainable was $25 \times 10^{-8}$ A and the possibility existed that DTC was not being passed out of the electrode in sufficient amount. However, with much

lower currents sufficient DTC could be electrophoretically injected upon the end-plate region of an isolated rat-diaphragm preparation to block the effect of acetylcholine applied electrophoretically from an adjacent barrel.

## RESULTS

### *Anticholinesterases*

When administered intravenously, prostigmine has very little action upon the cholinesterase associated with Renshaw cells (Eccles *et al.* 1956). However, when applied electrophoretically, prostigmine behaves as a typical anticholinesterase, having an action on Renshaw cells similar to that produced by eserine when administered intravenously. In Fig. 1 *A* are shown three records of the response of a single Renshaw cell to a volley evoked in the ventral root

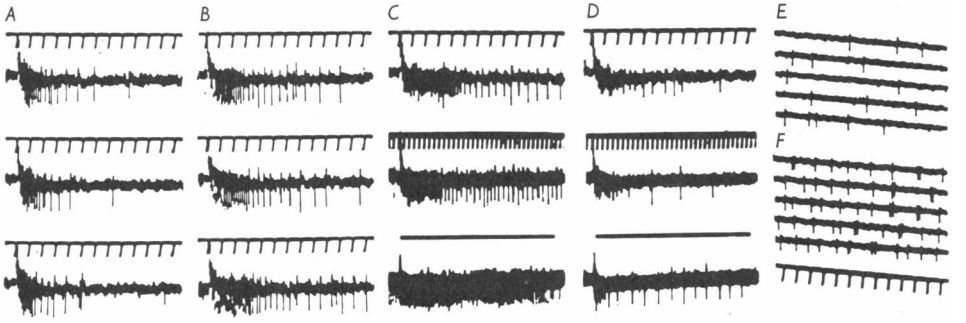


Fig. 1. *A–D*, records showing discharge evoked by synaptic stimulation of a single Renshaw cell. *A*, three control responses. A current of $2 \times 10^{-8}$ A was then passed through the prostigmine-containing barrel for 90 sec: the three responses of *B* were recorded 55, 60 and 75 sec after the commencement of this current, those of *C* at 2 sec intervals following its cessation and those of *D* 17 min later; time marker, 10 msec for *A*, *B* and upper two records of *C* and *D*, 1 sec for the remaining records of *C* and *D*. *E*, discharge produced by acetylcholine: *F* as for *E* but a current was passing prostigmine from another barrel of the electrode, having commenced 1 min previously; time marker, 10 msec.

of that segment. Following this, a current of $2 \times 10^{-8}$ A was passed through the barrel containing prostigmine bromide and the upper, middle and lower records of Fig. 1 *B* and of Fig. 1 *C* were taken 55, 60, 75 and 90, 92, 94 sec later, the backing voltage being restored to the prostigmine barrel after the records of Fig. 1 *C* were taken. The enormous prolongation of the responses is obvious, particularly in the lowest record of Fig. 1 *C*. This effect of prostigmine had a latency of 45–60 sec but persisted for a much longer time. The records of Fig. 1 *D* were taken from the same Renshaw cell 17 min after those of Fig. 1 *C*.

Not only did prostigmine increase the rate of discharge of those cells firing spontaneously, but it also increased the rate of response of the cell to applied acetylcholine. The records of Fig. 1 *E* and *F* were both evoked by a current of $7 \times 10^{-8}$ A flowing through a barrel containing acetylcholine, but for a

minute before *F* a current of $2 \times 10^{-8}$ A had been flowing through the prostigmine barrel as well, with the consequence that the acetylcholine has become much more effective. Further, but not illustrated here, prostigmine prolonged for many minutes the discharge evoked by injected acetylcholine. In the absence of prostigmine the responses usually ceased within 1 sec of the cessation of the application of acetylcholine (cf. Curtis & Eccles, 1958).

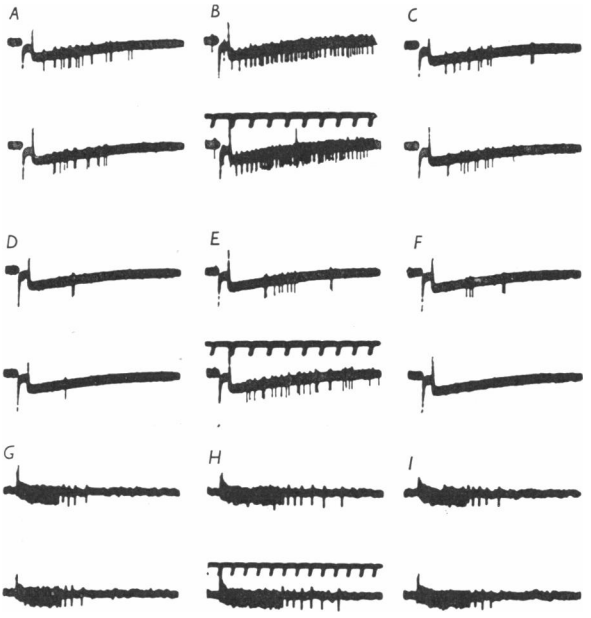


Fig. 2. Discharges evoked from a single Renshaw cell. *A–C*, current pulse of $12 \times 10^{-9}$ A flowing for 50 msec through an acetylcholine electrode. *D–F*, as for *A–C* but for a current $4 \times 10^{-9}$ A. *G–I*, synaptic stimulation by a volley in the segmental ventral root. Two successive records are illustrated for each type of stimulus. After the control records *A*, *D* and *G*, edrophonium was ejected from another barrel of the electrode by a current of $10^{-7}$ A; *B*, *E* and *H* were recorded during this; *C*, *F* and *I*, 20 sec after the edrophonium current ceased; time marker, 10 msec.

Similar results were obtained with edrophonium (Tensilon: 3-hydroxyphenyldimethylethyl-ammonium chloride, Roche Products Ltd.), which is structurally related to prostigmine and has a similar anticholinesterase activity (Randall, 1950; Nastuk & Alexander, 1954; Katz & Thesleff, 1957). When administered intravenously this drug has no effect on Renshaw cells. In Fig. 2*A* and *D* acetylcholine has been ejected from the electrode by the use of electrical pulses, the pulses being just threshold in *D*. The control response to synaptic stimulation by a volley in the ventral root is shown in Fig. 2*G*. A current of $10^{-7}$ A was passed through the electrode containing edrophonium, and 20 sec later the responses shown in Fig. 2*B*, *E* and *H* were recorded, using

the same stimuli respectively as for *A*, *D* and *G*. After these records the edrophonium current was terminated and 1 min later the responses *C*, *F* and *I* were recorded again with the appropriate stimuli.

Thus edrophonium and prostigmine behave as reversible anticholinesterases, accentuating the action of acetylcholine, whether released synaptically or injected electrophoretically. Neither edrophonium nor prostigmine prolonged the response of a cell to nicotine or carbaminocholine.

### *Blocking drugs*

Tubocurarine (DTC) is a powerful inhibitor of acetylcholine action at the neuromuscular junction (del Castillo & Katz, 1957*a*), but is virtually without effect upon Renshaw cells when administered intravenously or intra-arterially (Eccles *et al.* 1956). This has been ascribed to the blocking action of the blood–brain barrier (Eccles *et al.* 1956), but the methods used would not permit an accurate localization of the site of the barrier.

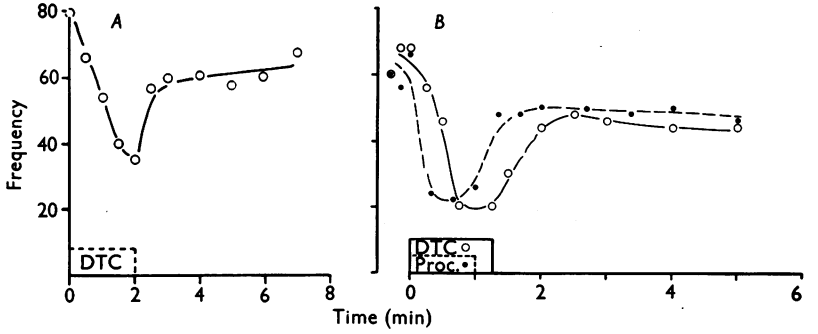


Fig. 3. *A*, maximum frequency of discharge of a Renshaw cell evoked at various intervals by equal currents passed through an acetylcholine-containing barrel for periods of 4 sec. From zero to 2 min DTC was passed from another barrel of the electrode using a current of $6 \times 10^{-9}$ A. *B*, similar curves showing the effect of procaine (•) passed for 1 min (Series of Fig. 5*A–F*) compared with that of tubocurarine (○) which was passed for 1·25 min; the current in both cases was $1{\cdot}5 \times 10^{-8}$ A. Abscissa scales differ.

When applied locally DTC was usually a relatively ineffective depressant on the responses both to synaptic stimulation and to electrophoretically injected acetylcholine. This effect was often so small that all the electrodes containing DTC were tested upon a rat-diaphragm preparation (see Methods). It was found that in spite of the high resistance of the barrels, a considerable quantity of DTC was always being passed ionophoretically.

On several occasions, however, DTC had a considerable effect upon the discharge of a Renshaw cell, evoked by acetylcholine applied from a nearby electrode. In the series illustrated in Fig. 3*A* tests were made at the indicated times by passing a current for 4 sec through the acetylcholine barrel of the

electrode. The same current was used on each occasion, control observations having indicated that under these circumstances, and provided that tests were made at intervals exceeding 15 sec, the maximum frequency of discharge of the cell attained in individual tests varied by less than 10%. A current of $6 \times 10^{-9}$ A was used to pass DTC from the electrode for 2 min. The acetylcholine response was diminished. On cessation of the DTC injection, there was an initial rapid rate of recovery, but recovery was still incomplete 5 min later.

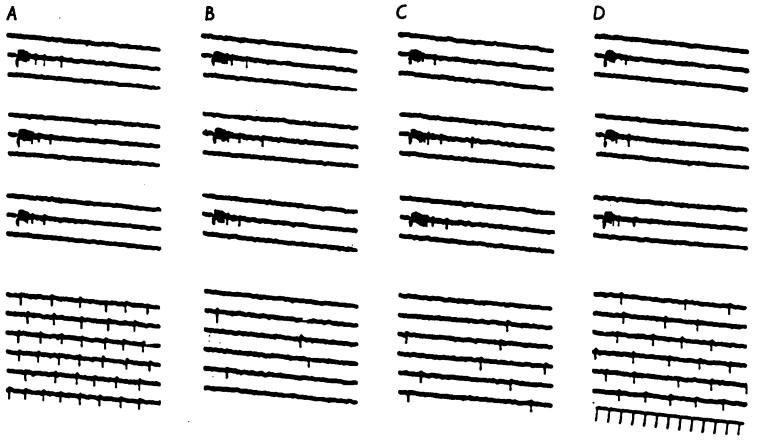


Fig. 4. In this figure, and in Fig. 5 *A–F*, each column from above downwards consists of responses to three separate ventral root volleys and the maximum discharge evoked by equal currents passed through a barrel containing acetylcholine. These responses were recorded on moving film, there being 5 msec between sweeps. *A*, control responses. DTC was then passed using a current of $3 \times 10^{-8}$ A for 65 sec and the records *B*, *C* and *D* were taken 40, 60 and 180 sec, respectively, after this current commenced; time marker, 10 msec.

Unfortunately, the effect of DTC upon the synaptically evoked responses of this cell was not recorded. However, several other cells were tested in full, one of which is illustrated in Fig. 4. In any one column are shown responses to three separate ventral root volleys and below are the responses evoked by acetylcholine in a manner similar to that used for the tests of Fig. 3*A*. In Fig. 4, after the control records *A*, a current of $3 \times 10^{-8}$ A flowed through the DTC barrel for 65 sec and the responses *B* and *C* were taken 40 and 60 sec after the onset of this current, while *D* was taken 2 min after its cessation. Although in *B* and *C* the response to injected acetylcholine was nearly abolished, there was very little alteration in the responses to synaptic excitation. Perhaps the last one or two spikes were not as regular in occurrence under the influence of DTC. A similar series is illustrated in Fig. 3*B* (○), where less DTC was used ($1{\cdot}5 \times 10^{-8}$ A for 75 sec). Again, the effect of directly applied acetylcholine was diminished. This same cell was tested with procaine, which was found by del Castillo & Katz (1957*a*) to be a powerful inhibitor of acetylcholine action

at the motor end-plate of the frog. The experiment illustrated in Fig. 5*A–F* is similar to that of Fig. 4, the upper three records showing the effect of synaptic stimulation of the cell and the lower record the result of a constant rate of electrophoretic injection of acetylcholine. Column *A* gives the control responses. A current of $1{\cdot}5 \times 10^{-8}$ A flowed through the procaine barrel for 60 sec and the records of *B* and *C* were taken 20 and 60 sec after this current

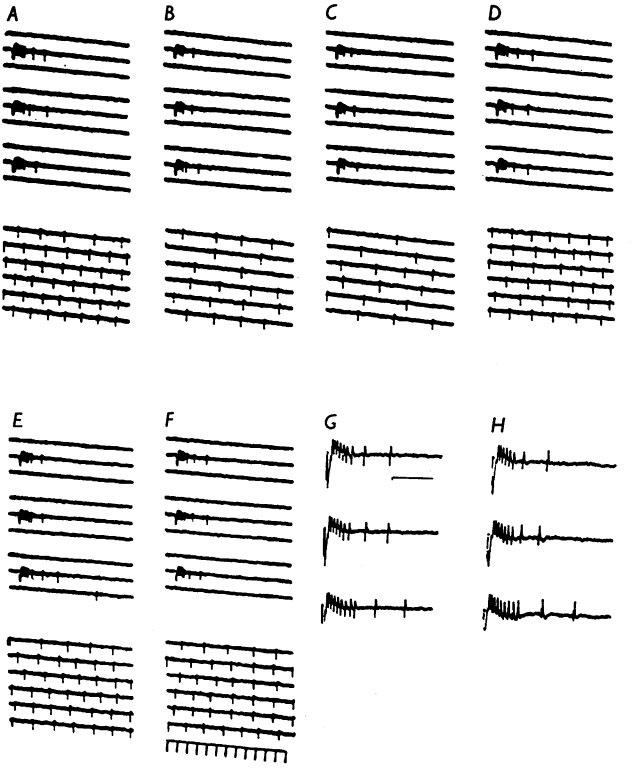


Fig. 5. *A–F* as for Fig. 4. *A*, controls. *B–F*, 20, 60, 80, 120 and 240 sec, respectively, after a current of $1{\cdot}5 \times 10^{-8}$ A commenced to flow through a barrel containing procaine for 60 sec. *G*, *H*, responses of a single Renshaw cell but evoked by maximal stimulation of the appropriate ventral root, *G* before and *H* 1 min after the administration of DTC 1 mg/kg intravenously. Time marker 10 msec.

commenced, and those of *D*, *E* and *F*, 20, 60 and 180 sec, respectively, after the current ceased. Procaine diminished the frequency of responses of the cell to acetylcholine and in *B* and *C* there was a definite reduction in the number of discharges evoked by synaptic stimulation. This series is plotted in Fig. 3*B* (●), where a comparison is made between the effects of both procaine and DTC upon the frequency of response evoked by constant rates of injection of acetylcholine. The procaine was slightly more rapid in action, but once the

drugs ceased to flow from the electrode the recovery phases were very similar. However, with both procaine and DTC, although some degree of reversibility occurred within 2–3 min, this was not complete. The effect of intravenously applied DTC upon a nearby cell in the same animal is shown in Fig. 5 *G* and *H*. The number of responses evoked by synaptic stimulation was virtually unchanged 1 min after the injection of 1 mg/kg. Fig. 6 *A*, *B* and *C* show records from a cell upon which DTC had no effect. This was from the same animal as the cell illustrated in Fig. 3 *A*. After the two control records of *A*, evoked by maximal stimulation of the segmental ventral root, a current of $6 \times 10^{-9}$ A flowed through the DTC barrel of the adjacent electrode for 5 min. The records

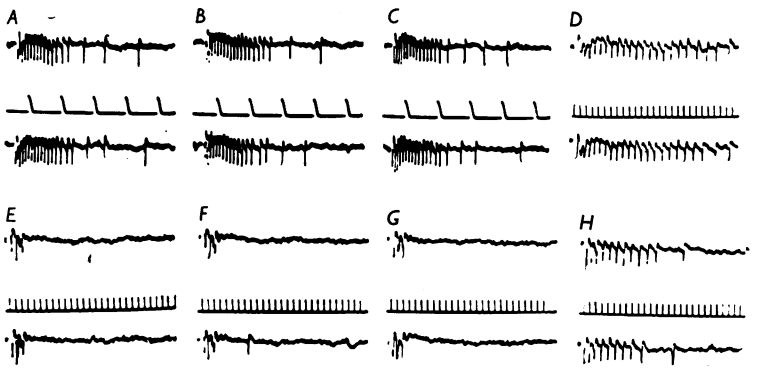


Fig. 6. Synaptic stimulation of a single Renshaw cell. Note different time marker, *A–C* 10 msec, *D–H* 1 msec. *A*, controls. DTC was then passed from an electrode with a current of $6 \times 10^{-9}$ A for 5 min. Records *B* and *C* were taken at the cessation of this current and 2 min later, respectively. *D*, controls. Dihydro-β-erythroidine was passed from another barrel for 90 sec with a current of $2 \times 10^{-7}$ A; *E*, *F*, *G*, *H*, 70, 120, 180 sec and 28 min, respectively, after this current commenced.

*B* were taken at this time and those of *C* 2 min later. Several cells tested with both procaine and DTC were discharging spontaneously at rates of 8–50/sec. Both drugs depressed these responses by approximately the same factor by which the discharges due to applied acetylcholine were affected.

Dihydro-β-erythroidine (DHE) effectively blocks the effect of intra-arterially injected acetylcholine and nicotine upon Renshaw cells (Eccles *et al.* 1956; Curtis, Eccles & Eccles, 1957). However, when synaptic stimulation is used, the first two or three responses of the cell are relatively resistant to the drug, even when large doses are employed. This has suggested that the synaptic barrier gives a degree of protection to acetylcholine released inside it and that the initial high concentration of acetylcholine is sufficient to overcome the degree of post-synaptic block that can be established. When applied locally, DHE has a similar effect upon synaptically evoked responses. The repetitive responses of a Renshaw cell (same cell as Fig. 6 *A*, *B*, *C*) are shown in Fig. 6 *D–H*.

A current of $2 \times 10^{-7}$ A flowed through the DHE barrel for 90 sec and the records *E*, *F*, *G* and *H* were taken at 70, 120, 180 sec and 28 min, respectively, after the onset of this current. There was a complete loss of the later responses of the cell, but in spite of the large amount of DHE used the first two responses persisted. The effect was reversible but recovery was incomplete at 28 min, which is further evidence that the dose was large. When smaller doses of DHE are used, the effect reverses more readily and fewer of the responses are blocked.

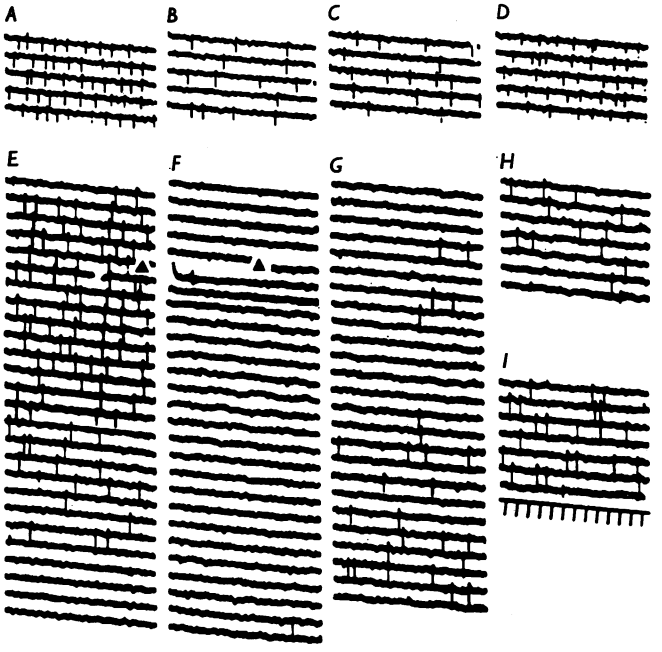


Fig. 7. *A–D*; a current flowed continuously through a nicotine electrode. After the control *A*, dihydro-β-erythroidine was passed from another barrel using a current of $8 \times 10^{-9}$ A for 15 sec; *B*, *C* and *D* were recorded 10, 25 and 75 sec after this began. *E–I* (*E*, *F* and *G* being continuous); throughout this series a constant current passed acetylcholine into the neighbourhood of the same cell, the current having commenced several seconds before the beginning of *E*. At ▲ in *E*, $8 \times 10^{-9}$ A was used to pass dihydro-β-erythroidine from the electrode until ▲ in *F*. *H* and *I* are respectively 18 and 30 sec after the cessation of the dihydro-β-erythroidine current.

Directly applied DHE blocks the effect of acetylcholine and nicotine injected from another barrel of the micro-electrode. Fig. 7 *A–D* shows this effect on a background discharge due to nicotine, and again it is reversible. Figs. 7 *E*, *F* and *G* are in continuity. A background discharge produced by acetylcholine was abolished within 2 sec by a current of $8 \times 10^{-9}$ A through the DHE barrel (first triangle). Within 4 sec of the cessation of this current (triangle in Fig. 7 *F*),

the cell again responded to acetylcholine (Fig. 7*G*). This recovery progressed slowly and was still incomplete in Fig. 7*I* after 30 sec. However, both the nicotine and acetylcholine series in Fig. 7 do not take account of the accommodation occurring when Renshaw cells are subjected to long applications of depolarizing drugs (Curtis & Eccles, 1958) and are of necessity incomplete. Nevertheless, they illustrate adequately the effect that DHE has on the responses of these cells to depolarizing agents.

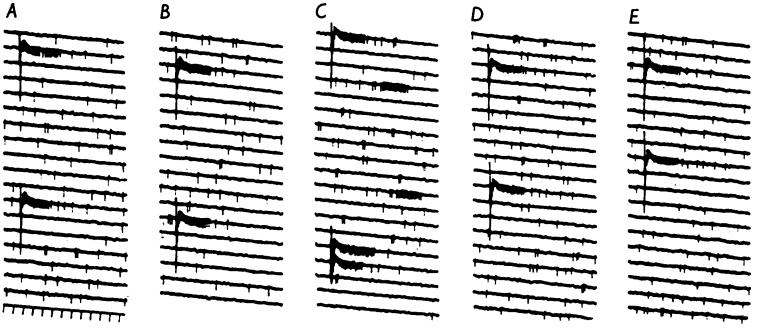


Fig. 8. Background discharges of a Renshaw cell, together with synaptically evoked responses. *A*, controls; *B–E* during and after the application of $C_{10}$; time marker 10 msec. See text.

*Decamethonium*

del Castillo & Katz (1957*b*, *c*) have shown in the frog nerve–muscle preparation that decamethonium ($C_{10}$) not only has a depolarizing action upon the post-synaptic membrane, but acts also as a competitive inhibitor of cholinesterase. This has been confirmed using the rat-diaphragm preparation (D. R. Curtis, unpublished observation), and in view of the double action of this substance its effect on Renshaw cells was determined, because it has been found to be inactive when administered intra-arterially. Fig. 8*A* shows the background discharge of a single Renshaw cell together with two series of repetitive discharges evoked by synaptic stimulation. Following this a current of $6 \times 10^{-8}$ A flowed through the $C_{10}$ barrel for 120 sec, and the subsequent records, taken in a similar fashion, were recorded at 30, 90, 150 and 210 sec after the onset of this current. There was a slight increase in the rate of background discharge, but very little alteration in the number of responses to synaptic nerve stimulation, such as would have been recorded if prostigmine had been used (Fig. 1). With the same cell, however, a more marked change was obvious when acetylcholine was used to increase the rate of discharge. In Fig. 9*A* the background discharge and five responses to stimulation of the ventral root are shown. In Fig. 9*B* acetylcholine was used to stimulate the cell and the current through this barrel ceased at the triangle. The response was relatively small and had virtually finished within 1–1·5 sec of the cessation

of the current. $C_{10}$ was then passed from the electrode with a current of $3 \times 10^{-8}$ A and after 1 min the records *C* and *D* were taken, using the same conditions as those of *A* and *B*, respectively. There was a slight diminution in the background discharge and the responses to synaptic stimulation were slightly increased in the frequency of discharge and in its duration. However, the discharge rate produced by acetylcholine was increased by a factor of 1·5 and the effect was prolonged for many seconds after the acetylcholine had been turned off, at the triangle in Fig. 9*D*. These findings suggest that $C_{10}$ has little action upon the acetylcholine released at the synapses, but behaves effectively

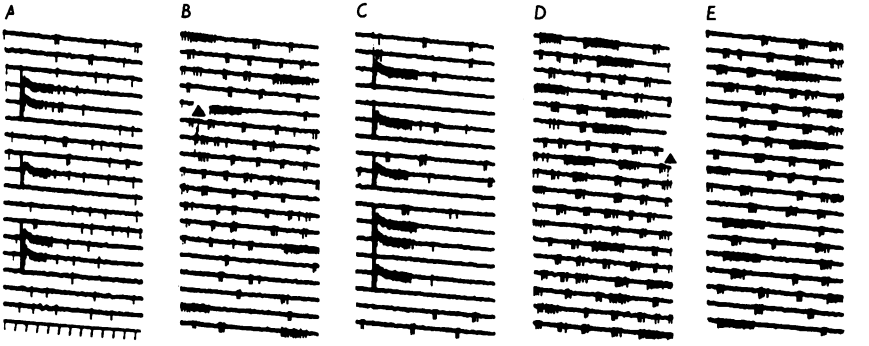


Fig. 9. *A*, background activity and synaptically evoked responses of a Renshaw cell. *B*, discharge produced by a current passing through the acetylcholine barrel which commenced 2 sec before *B* and ceased at ▲. *C*, as for *A*. *D–E* continuous, as for *B*. During *C–D*, $C_{10}$ was passed from another barrel of the electrode, this current starting 60 sec before *C*; time marker, 10 msec.

as an anticholinesterase towards acetylcholine released from an electrode. This has been confirmed in many cells, another of which is shown in Fig. 10. In Fig. 10*A*, *B*, *C* and *D* are shown the responses to acetylcholine which in each case was passed by a current pulse of $30 \times 10^{-9}$ A for 50 msec. Two seconds elapsed between sweeps, and each of the series of *A*, *B*, *C* and *D* are composed of three successive responses. After the three control responses *A*, a current of $7 \times 10^{-9}$ A flowed through the $C_{10}$ barrel while the responses in *B* were recorded, and was then turned off for the recording of *C* and *D*. The effect of acetylcholine was potentiated and prolonged by the action of $C_{10}$, which with these doses was readily reversible. However, with the same cell, very little effect even with large doses of $C_{10}$ was observed upon the responses to synaptic stimulation. The three records of Fig. 10*E* are control responses and those of *F* were recorded after a time of 5 min during which a current of $5 \times 10^{-8}$ A flowed through the $C_{10}$ barrel. There was a little prolongation and intensification of action of acetylcholine released synaptically.

On several occasions the effect of $C_{10}$ on the discharge evoked by nicotine was determined. No prolongation of nicotine action occurred but there was

on all occasions, with different cells in two animals, a definite reduction in the frequency of the response to nicotine and a small reduction in the duration of this excitation. This effect is illustrated in Fig. 11, where a comparison is made using the same cell between the effect of $C_{10}$ on the frequency of the responses due to acetylcholine (●) and nicotine (○). This will be discussed later.

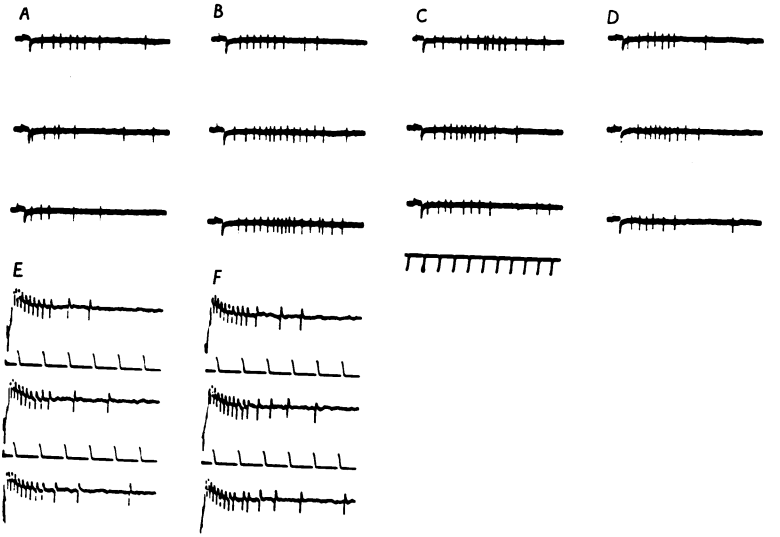


Fig. 10. *A–D*; each sweep shows responses evoked by a constant current pulse passing through an electrode containing acetylcholine; the records were taken in continuity, there being 2 sec between sweeps. After the three control records *A*, a current passed $C_{10}$ from another barrel during the three records of *B*, this current ceasing just before the first record of *C*; time marker, 100 msec. *E*, *F*, synaptic stimulation of the same cell, *E* before and *F* after a current of $5 \times 10^{-8}$ *A* was passed through the $C_{10}$ barrel for 5 min; time marker, 10 msec.

## DISCUSSION

The results reported in this paper are concerned both with the action of certain drugs on Renshaw cells and with the barriers surrounding these cells. It will be convenient to discuss these problems with the aid of a diagram (Fig. 12) which shows a single Renshaw cell (*RC*) in relation to both a multibarrelled electrode (*ME*) and a capillary (*C*) surrounded by the blood–brain barrier (*BBB*). The synaptic barrier (*SB*) surrounds the terminals of the axon collaterals (*AXC*).

Although it is clear that a 'blood–brain barrier' exists (Friedemann, 1942; Krogh, 1946; Tschirgi, 1952; Manery, 1954; Bakay, 1956; Dobbing, 1956; Davson, 1957), there is considerable confusion regarding its nature and site. It is possible that the barrier is related to capillary walls (Rodriguez, 1955) although histologically there is nothing to distinguish these capillaries from those elsewhere in the body (Patek, 1944; Woollam & Millen, 1954). Consequently, the barrier may be outside the actual vessel wall, being either the

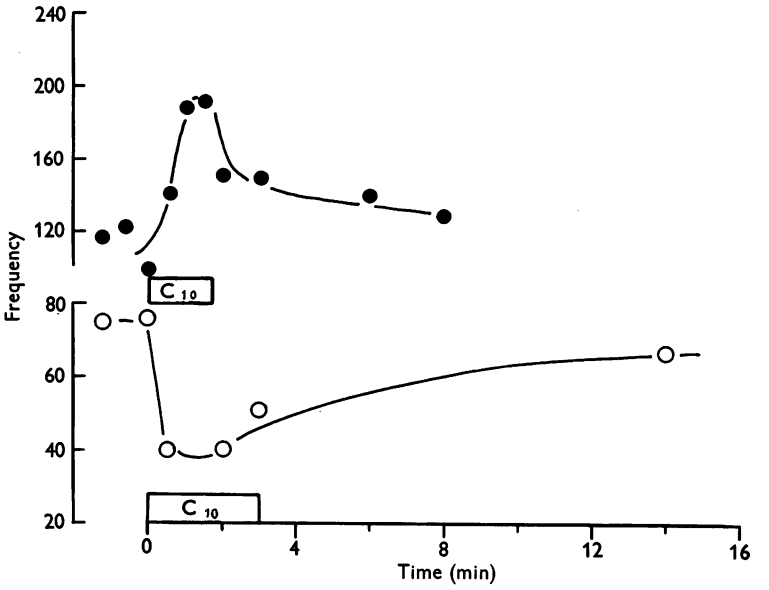


Fig. 11. The maximum frequency evoked by acetylcholine, ●, and nicotine, ○, when tested by using constant currents of either drug for 3–4 sec; note different frequency scales. As indicated, equal currents flowed through a $C_{10}$ barrel from zero time. The two series were recorded 25 min apart from the same Renshaw cell.

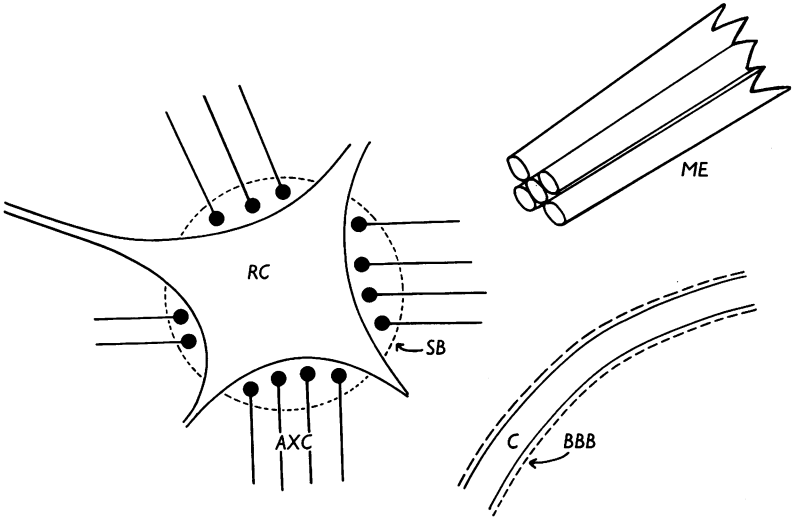


Fig. 12. Diagrammatic representation of the situation under discussion. See text.

'pia-glial' membrane (Tschirgi, 1952; Mayer & Bain, 1956) or even the ground substance between nerve cells (Hess, 1953, 1955; Woollam & Millen, 1954). It is clear, however, that the ground substance is relatively unimportant, because substances unable to pass the blood–brain barrier are quite effective when applied locally within the nervous system. There also appears to be no marked impedance to the free passage of even large molecules such as those of tetanus and botulinum toxins within the spinal cord (Brooks, Curtis & Eccles, 1957; V. B. Brooks and D. R. Curtis, unpublished observations.) It is highly probable that the blood–brain barrier is associated with the capillaries and their covering sheaths. For present purposes the actual histological location is relatively unimportant.

Although a variety of factors, including molecular weight, electrical charge and lipoid solubility (cf. Friedemann, 1937) may determine whether or not a barrier exists to any one substance, the factor of lipoid solubility is probably of prime importance when considering the substances used in this paper (Schweitzer, Stedman & Wright, 1939; Eccles *et al.* 1956). As has been shown experimentally for the surface membrane of the squid giant axon (Bullock, Nachmansohn & Rothenberg, 1946; Rothenberg, Sprinson & Nachmansohn, 1948), the blood–brain barrier might be expected to be relatively impermeable to quaternary ammonium compounds such as acetylcholine, prostigmine and tubocurarine. These substances occur only in ionic form and are therefore relatively insoluble in lipids. The salts of tertiary bases, however, are partly un-ionized and consequently are soluble in lipids. Thus no major barrier would exist for substances such as eserine, dihydro-$\beta$-erythroidine and nicotine. This separation into tertiary and quaternary compounds has gained recent support from the finding of Koelle & Steiner (1956) concerning the relative abilities of related tertiary and quaternary anticholinesterases to inactivate brain cholinesterase after intravenous and intraventricular application (cf. Mayer & Bain, 1956).

The effectiveness of the blood–brain barrier in preventing the action of a drug upon centrally located neurones can only be assessed when the drug has an action when applied locally near such cells. However, this is further complicated by the presumed presence of barriers more closely related to the synaptic areas of cells. There is good evidence for the presence of such a barrier for Renshaw cells (Eccles *et al.* 1956). A single volley in the axon collaterals causes a repetitive high-frequency discharge of a single Renshaw cell which may persist for at least 50 msec. Hence, it has been suggested that the free diffusion of acetylcholine away from the site of liberation is hindered. Further, not only is it impossible to excite a Renshaw cell at high frequencies by externally applied acetylcholine (Curtis & Eccles, 1958), but also the first two discharges in response to synaptic activation are resistant to the action of drugs that block the responses (Eccles *et al.* 1954; see below). Consequently, it is

postulated that acetylcholine is released within a barrier which not only prevents its free diffusion from the site of release but also partially protects the acetylcholine and the post-synaptic receptor sites from the action of externally applied drugs. The behaviour of Renshaw cells towards pharmacologically active agents must be viewed in the light of these two barriers and the actions of the drugs used in this study will now be discussed with due regard to the two sites of liberation of acetylcholine: within the synaptic barrier from the nerve terminals, and outside the barrier from a micro-electrode. It has been reported in the previous paper (Curtis & Eccles, 1958) that acetylcholine is not as effective a depolarizer of Renshaw cells as is nicotine, even when applied locally. This would be expected, since the synaptic barrier prevents the free diffusion of acetylcholine from within. The occasional ineffectiveness of intra-arterially injected acetylcholine (Eccles *et al.* 1956; Curtis *et al.* 1957) suggests that the blood–brain barrier also prevents the passage of acetylcholine from the blood stream to the nervous system and it is probable that the same two factors are involved in blocking the actions of succinylcholine, carbaminocholine, acetyl-$\beta$-methylcholine and arecholine.

Both prostigmine and edrophonium have virtually no action upon Renshaw cells when administered intravenously, but are effective anticholinesterases when applied near a Renshaw cell. The responses to synaptic stimulation and to acetylcholine released externally to the synaptic barrier are reversibly intensified and prolonged, suggesting that cholinesterase both within the barrier and external to it is inactivated. Thus, this barrier offers little or no resistance to the passage of these drugs, whereas the blood–brain barrier is relatively impermeable. The increase in the rate of background discharge produced by these drugs is probably due to the inactivation of cholinesterase, thereby protecting acetylcholine released spontaneously from the terminals. However, the present series of experiments does not exclude the possibility that these drugs also have a depolarizing action (cf. Katz & Thesleff, 1957).

There is little difference between the action of DHE when administered either locally or diffusely by intravenous injection. The blood–brain barrier is therefore freely permeable to this substance. However, this barrier is very effective in preventing tubocurarine from reaching the nervous tissue after intravenous administration. Unfortunately, no assessment of the barrier offered to procaine administered systemically has been made, but even when applied locally both procaine and DTC are much less effective in blocking the action of acetylcholine than is DHE. Both have very little effect when tested against the relatively large concentrations of acetylcholine released by the nerve terminals, although they can diminish the frequency with which externally applied acetylcholine can discharge a single Renshaw cell. It also is of interest that the first two or three responses to orthodromic stimulation are not suppressed even by large concentrations of DHE.

All three drugs, DTC, DHE and procaine have a curare-like action at the motor end-plate (Harvey, 1939; Unna, Kniazuk & Greslin, 1944; del Castillo & Katz, 1957*a*) and it would be expected that each would produce an inactive receptor–drug complex preventing access of acetylcholine to the post-synaptic receptors. An explanation is required for the fact that all three drugs can abolish the responses of a cell due to acetylcholine applied outside the synaptic barrier, and yet differ in their actions upon responses due to acetylcholine released from the nerve terminals. If this differential effect was due solely to a local competition between blocking agent and acetylcholine for the receptor site, it might be expected that the effectiveness of a blocking agent would depend upon the relative stability of the respective receptor–drug complexes (cf. del Castillo & Katz, 1957*a*). However, this is unlikely for two reasons. As pointed out by del Castillo & Katz (1957*a*), the dissociation of these blocking agent–receptor complexes appears to be relatively slow when compared with action of acetylcholine and it is unlikely that enough drug could be displaced in the time involved. Secondly, the rates of decay of the inhibitions of acetylcholine action by DTC, procaine and DHE when assessed at the end-plate regions (del Castillo & Katz, 1957*a*; D. R. Curtis, unpublished observation) suggest that the DTC complex is more stable than either of the other two and that the DHE complex is relatively unstable. However, the results reported here show definitely that the blocking action of DHE upon Renshaw cells, when tested by high concentrations of acetylcholine released within the synaptic barrier, is much greater than that observed with DTC and procaine. Thus the relative actions of these three drugs may depend upon the permeability of the synaptic barrier to each (cf. del Castillo & Katz, 1957*a*, p. 351). It can be postulated that the barrier is relatively permeable to DHE, and less so to procaine and DTC. Enough of these agents penetrate to block the responses due to randomly released acetylcholine from the terminals and to the relatively small amount of acetylcholine which penetrates the synaptic barrier when injected from an electrode. However, when large quantities of acetylcholine are released from the nerve terminals, insufficient post-synaptic receptor sites are occupied by DTC or procaine to cause a significant depression, whereas more are occupied by DHE. Within the barrier the relative stability of the drug–receptor complex may be important, because with the large doses of DHE used in this study it can reasonably be expected that all post-synaptic receptors might be occupied, and this block is overcome by the high acetylcholine concentration. This point might be further elucidated at the neuromuscular junction.

Decamethonium is of considerable interest because of its behaviour both as a depolarizing agent and as an anticholinesterase (Paton & Zaimis, 1949; del Castillo & Katz, 1957*c*). When injected intra-arterially, doses of 100 $\mu$g do not fire Renshaw cells (Eccles *et al.* 1956), but this test cannot be used as evidence that the blood–brain barrier is impermeable to this drug. When applied from a micro-electrode $C_{10}$ has very little effect upon the discharge of a Renshaw cell evoked synaptically. Consequently, it can be assumed that very little passes the synaptic barrier to inhibit the action of cholinesterase located near the post-synaptic receptor sites. On the other hand, however, the effect of acetylcholine applied externally to this barrier is both accentuated and prolonged, indicating that $C_{10}$ effectively inhibits the action of cholinesterase located in this region. This finding is not explicable by the differential inhibition of specific and non-specific cholinesterase by $C_{10}$, because $C_{10}$ has a greater inhibitory action upon true cholinesterase (Paton & Zaimis, 1949) which is presumably in higher concentration within the synaptic barrier than external to it. No evidence has been obtained that $C_{10}$ can depolarize Renshaw cells sufficiently to cause discharges. The slight increase observed in the rate

of background discharge is suggestive, but could be due to the anticholinesterase action of the drug in protecting acetylcholine released spontaneously from the nerve terminals. The finding that the rate of discharge produced by nicotine is diminished in the presence of $C_{10}$ suggests that $C_{10}$ occupies post-synaptic receptor sites and so blocks the action of nicotine (cf. del Castillo & Katz, 1957*c*). However, it is also possible that $C_{10}$, by indirectly facilitating the action of the quantally released acetylcholine, could produce the same result by decreasing the sensitivity of the post-synaptic receptors. Unfortunately, no assessment has been made of the effect of the blood–brain barrier upon the movement of $C_{10}$ from the blood stream to nervous tissue. The experiments cited (Eccles *et al.* 1956) were not designed to detect the effect of $C_{10}$ upon cholinesterase and the lack of a direct action of the drug upon Renshaw cells has been sufficiently explained by the synaptic barrier. However, it is possible that the blood–brain barrier is impermeable to $C_{10}$, because Irwin & Wells (1957) have been unable to demonstrate an action of succinylcholine, $C_{10}$ and DTC upon the central mechanisms controlling respiration—unless, indeed, these neurones are surrounded by a synaptic barrier.

It can be concluded from the results reported in this and the previous paper (Curtis & Eccles, 1958) that the anomalous behaviour of Renshaw cells when tested with intravenously and intra-arterially injected drugs (Eccles *et al.* 1954; Eccles *et al.* 1956) is not due to essential differences between the cholinergic synapses formed by the motor axon or its branches centrally and peripherally, but to the presence of diffusional barriers existing between the central site of release and action of acetylcholine and the sites of application of the testing drugs. It has thus been shown that acetylcholine is the chemical transmitter released at the synapse between the axon collaterals and Renshaw cells, just as it is also released at the neuromuscular junction. When the actions of drugs upon neurones are being considered, care must be taken concerning the relative permeabilities of diffusional barriers that might exist between the site of application and the presumed sites of action. The blood–brain barrier could hinder the free access of intra-arterially and intravenously injected drugs to centrally located nerve cells. In addition, however, it is probable that the synaptic regions of many neurones are surrounded by a barrier, because repetitive discharges at high frequency in response to a single presynaptic stimulus are observed for many types of neurones (cf. McIntyre, Mark & Steiner, 1956). The simplest explanation of this involves the limitation of the free diffusion of the chemical transmitter away from its site of liberation.

The characters of the two barriers investigated in this paper are entirely different. Whereas lipoid solubility may be a factor in determining the passage of a substance across the bloo–brain dbarrier, substances such as prostigmine are freely diffusible across the synaptic barrier. It appears that drugs of high molecular weight have difficulty in reaching the synaptic areas upon Renshaw

cells, since the barrier exists to maintain a high concentration of acetylcholine at the post-synaptic receptors. The possibility that the barrier excludes true cholinesterase from the locality of the synapses is rendered untenable by the finding that some anticholinesterases (prostigmine) prolong the discharge evoked by synaptic action, whereas $C_{10}$ has little effect upon this type of response. Consequently, prostigmine must inactivate cholinesterase located within the synaptic barrier.

SUMMARY

1. The pharmacological behaviour of single Renshaw cells has been tested by passing drugs electrophoretically from an adjacent multibarrelled electrode, the responses of the cell being recorded from one of the barrels.

2. Some drugs such as prostigmine, edrophonium (Tensilon), procaine, tubocurarine and decamethonium, which are relatively ineffective when administered intravenously or intra-arterially, become much more effective when applied near such cells.

3. All evidence confirms the cholinergic nature of transmission between motor axon collaterals and Renshaw cells.

4. Both the blood–brain barrier and another diffusional barrier, more closely related to the synaptic terminals upon Renshaw cells, can prevent drugs from reaching the site of release of acetylcholine and the underlying post-synaptic receptors.

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