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THE ACTION OF PROCAINE AND ATROPINE ON SPINAL NEURONES

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Procaine has a curare-like action at the end-plate region of muscle, reducing the effectiveness of synaptically released and iontophoretically applied acetylcholine (Harvey, 1939; del Castillo & Katz, 1957). Furukawa (1957) has also demonstrated a direct effect upon the spike-generating mechanism of muscle fibres, an observation which is in accordance with the more recent findings of the action of procaine on nerve fibres (Shanes, Freygang, Grundfest & Amatniek, 1959; Taylor, 1959).

It has been reported that, when applied iontophoretically to Renshaw cells in the mammalian spinal cord, procaine depressed their activation by acetylcholine (Curtis & Eccles, 1958*b*). This observation led readily to the assumption that procaine was preventing the access of acetylcholine to the subsynaptic receptors of these cells. However, procaine may also have a more direct action upon the membrane of Renshaw cells; consequently an investigation was carried out in order to determine its effect upon both cholinoceptive Renshaw cells and non-cholinoceptive interneurones and motoneurones. In addition, a study was made of the action of atropine, another compound which blocks the action of acetylcholine and which possesses, moreover, certain structural similarities to cocaine.

METHODS

All methods have been reported in previous publications (Curtis & Eccles, 1958*a*; Curtis, Phillis & Watkins, 1959, 1960*a*). Motoneurones, interneurones and Renshaw cells of the lower lumbar segments of the spinal cord of the cat were identified by their responses to volleys entering the spinal cord via the dorsal and ventral roots. The cord was transected at the lower thoracic level, the animal being lightly anaesthetized with pentobarbital sodium.

Procaine and atropine were passed iontophoretically as cations from saturated aqueous solutions of procaine hydrochloride and atropine sulphate respectively in double, five-barrel or co-axial electrodes. The double and five-barrel electrodes permitted the extracellular recording of spike potentials of neurones, whereas the co-axial electrode assembly allowed the simultaneous recording of intracellular responses of motoneurones whilst applying these substances extracellularly (cf. Curtis *et al.* 1959). Other substances used in this investigation were acetylcholine, which was passed iontophoretically as a cation from an aqueous solution of acetylcholine bromide, and glutamic acid, which was passed as an anion from a solution

in NaOH at pH 8 (cf. Curtis *et al.* 1960*a*). These substances were prevented from diffusing out of the electrodes by the application of suitable backing potentials. When co-axial electrodes were used, intracellular spike potentials recorded by the inner electrode were considerably attenuated owing to the high input-capacitance of the recording system. Cells impaled by these electrodes usually had resting potentials in the range −45 to −55 mV, in comparison with those of −60 to −70 mV found when single-barrel electrodes were used (cf. Coombs, Eccles & Fatt, 1955). These low potentials were presumably due to damage caused by the electrode. However, such potentials could be maintained for periods of 30 min–1 hr and the responses to both procaine and atropine resembled those given by cells with high resting potentials.

RESULTS

Procaine

It has been demonstrated that Renshaw cells, when fired by ventral root volleys, are activated synaptically by acetylcholine (Curtis & Eccles, 1958*b*). The depressant action of procaine upon this firing has been confirmed. Renshaw cells are also excited in a repetitive manner by the anions of certain of the acidic amino acids, in particular glutamic, aspartic and cysteic acids (Curtis *et al.* 1960*a*). It has been postulated that this excitation is mediated through receptors other than those specialized for acetylcholine, because dihydro-β-erythroidine (DHE), although effectively blocking the cholinergic excitation of Renshaw cells, is without action upon the firing by these excitant amino acids. Unlike DHE, procaine blocks the excitation of Renshaw cells produced by the excitant amino acids. The Renshaw cell which was excited by a ventral root volley in Fig. 1A also responded to acetylcholine (Fig. 1B) and glutamate ion (Fig. 1C). These responses were recorded by means of the central barrel of a five-barrel electrode. One minute after a current of 100 nA began to pass procaine from another barrel of the electrode, the same types of stimulation were applied. The response to the ventral root stimulus (D) was partially blocked, and the responses to acetylcholine (E) and glutamate ion (F) were completely suppressed. Two minutes after the cessation of the procaine application, almost complete recovery had occurred, G, H and I corresponding to A and D, B and E, C and F respectively.

These results might suggest that, as glutamate ion excited these cells without attachment to acetylcholine receptors, procaine may not only block such cholinoceptive sites but also the 'amino-acid receptors'. This postulate is unlikely, owing to the structural differences between the two series of excitant substances, and it is therefore possible that procaine has a more direct action on those processes producing the spike potential. This latter postulate could be tested by applying procaine to interneurones and motoneurones of the spinal cord, for these cells have no cholinoceptive synapses (Curtis, D. R., Phillips, J. W. and Watkins, J. C.—unpublished observations). It was found that the spikes evoked by both orthodromic

stimulation and the excitant amino acids were blocked by procaine. The responses of Fig. 2A–D were recorded from an interneurone lying in the dorsal horn of the seventh lumbar segment, in response to a single maximal stimulus applied to the ipsilateral peroneal nerve. These extracellular records were obtained by using the NaCl-containing barrel of a double-barrel elec-

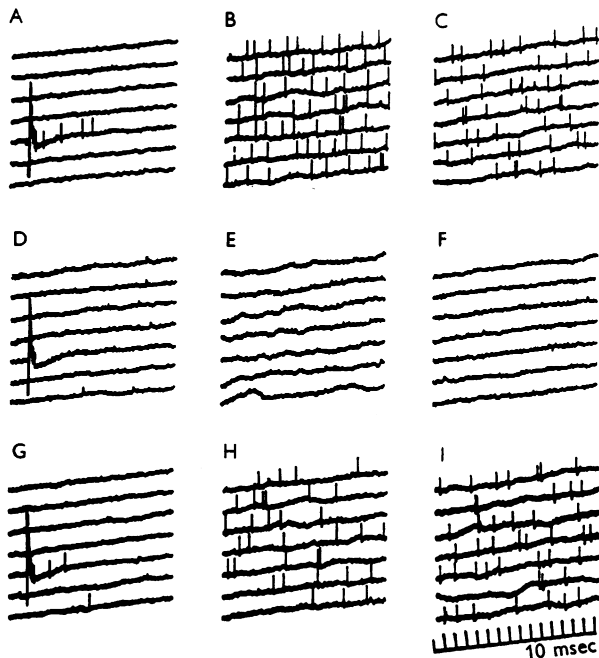

Fig. 1. Spike potentials recorded extracellularly from a Renshaw cell by means of the central barrel of a five-barrel electrode and evoked by a ventral root stimulus A, D and G; by iontophoretic application of acetylcholine, B, E and H; and by iontophoretic application of glutamic acid, C, F and I. The iontophoretic currents used in each of the two series of applications were constant throughout. After the control responses A–C, procaine was passed as a cation from another barrel of the electrode and the responses D, E and F were recorded during this application. G, H and I were recorded 2 min after the procaine application ceased. These responses were recorded on film moving parallel to the Y axis of the oscilloscope, there being less than 5 msec between sweeps. Time marker, 10 msec.

trode, and typically the spikes were repetitive (Fig. 2A). Following the application of procaine by means of a cationic current of 135 nA through the procaine-containing barrel of the electrode, the spikes were slowly reduced in size and number. The records (B) and (C) were taken 15 and 40 sec respectively after the commencement of this current. The effect was fully reversible, as is illustrated in Fig. 2D, which was recorded 20 sec after termination of the iontophoretic application. This action was not due to the flow of current

through the electrode, for the spike height of the cell was not altered by the passage of even greater currents through the NaCl-containing barrel, by means of which the responses were recorded, and currents in the reverse direction through the procaine-containing barrel did not increase the size or number of spikes. In some cells similar applications of procaine led to a reduction in the number of spikes without altering spike size. Usually, however, a diminution in spike size as well as number was observed.

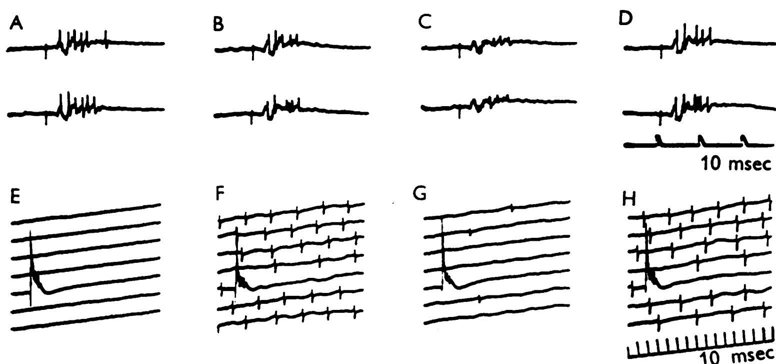

Fig. 2, A–D. Extracellular spike potentials of an interneurone located in the dorsal horn of the seventh lumbar segment and evoked by maximal stimulation of the peroneal nerve, recorded by means of one barrel of a double electrode. A, control responses; B and C, 15 and 40 sec after a current of 135 nA began to pass procaine from the other barrel of the electrode, this current flowing for 60 sec; D, 20 sec after the iontophoretic current ceased. Time marker, 10 msec.
E–H. Spikes of a similar cell but from another preparation, recorded in the same manner as those of Fig. 1. E, stimulation of the seventh lumbar dorsal root, this stimulus being repeated in F, G and H; F, application of glutamic acid as an anion; G, as for F but 48 sec after a current of 100 nA began to pass procaine from another barrel of the electrode; H, as for F but 12 min after the application of procaine ceased. Time marker, 10 msec.

The control responses of Fig. 2E and F were produced by stimulation of the seventh lumbar dorsal root and iontophoretic application of glutamate ion respectively. They were recorded by the central barrel of a five-barrel electrode. A cationic current of 100 nA then passed procaine from another barrel of the electrode, and Fig. 2G shows that not only was glutamate ion relatively ineffective thereafter, but also that the orthodromic spikes were reduced in number and size. Again full recovery was observed in Fig. 2H, these responses being recorded 12 min after the current applying procaine was terminated. These effects of procaine were observed upon spikes evoked by either aspartate, glutamate or cysteate ions and were not due to the interference between iontophoretic currents applying substances of opposite charge (cf. Curtis *et al.* 1959), since the observed action of

procaine often persisted for many minutes after the cationic current applying it had ceased.

In addition, procaine was passed iontophoretically around motoneurones, from which intracellular records were being obtained. Co-axial electrodes were used for these experiments and the results from several cells are illustrated in Figs. 3 and 4. Figure 3G represents the experimental situation with the inner barrel of the electrode in an intracellular position, whereas the outer barrel was located extracellularly with respect to the motoneurone (MN). In most cases no action was observed on the resting potential of the cell when procaine was applied iontophoretically, although in a few cells a small hyperpolarization of 1–2 mV was observed. In spite of this virtual absence of change in the resting potential, the spike potentials of motoneurones, whether elicited by synaptic, antidromic or direct excitation, were altered considerably. In all cells from which records were obtained the intracellular electrode was recording from the soma–dendritic region (cf. Coombs, Curtis & Eccles, 1957). Thus the full spike potential consisted of the spikes produced in a sequential fashion from both the initial segment (IS) and the soma–dendritic region (SD). In spite of resting potentials in the range – 50 to – 60 mV, the spikes were usually much less than 50 mV in height, owing to the distortion produced by the recording system. However, spike size was not an important consideration in the assessment of results. The responses of Fig. 3A–F were obtained from a motoneurone which was fired directly (ii) by a rectangular current pulse passed through the central barrel of the electrode, the size of this current pulse being indicated by the upper trace (i). In this manner the direct excitability of the membrane could be tested, the pulse size being altered under the varying conditions in order to straddle the threshold for excitation. Thus the size of the pulse indicated that current which was necessary to excite the cell in about half of 6–8 tests. Potentials generated by current flow through the electrode (see Fig. 3C, (ii)) were partially balanced out by a bridge circuit (Coombs, Curtis & Eccles, 1959). In addition, the axon of this cell was stimulated in the ventral root, the lower trace (iii) indicating the antidromic spike potential, which had an inflexion upon its rising phase due to the delay between transmission of the impulse from the IS to SD regions. Figure 3A is a control response; B and C were recorded 90 and 110 sec after a current of 400 nA commenced to pass procaine into the environment of the cell for a total duration of 115 sec. Following the cessation of this current and the re-application of a backing current of 200 nA in the reverse direction, the responses of Fig. 3D, E and F were recorded 25, 40 and 75 sec later. As indicated by the lower trace (iii), antidromic invasion of the SD segment was blocked (C and D) after a period in which invasion did not occur with every stimulus (B).

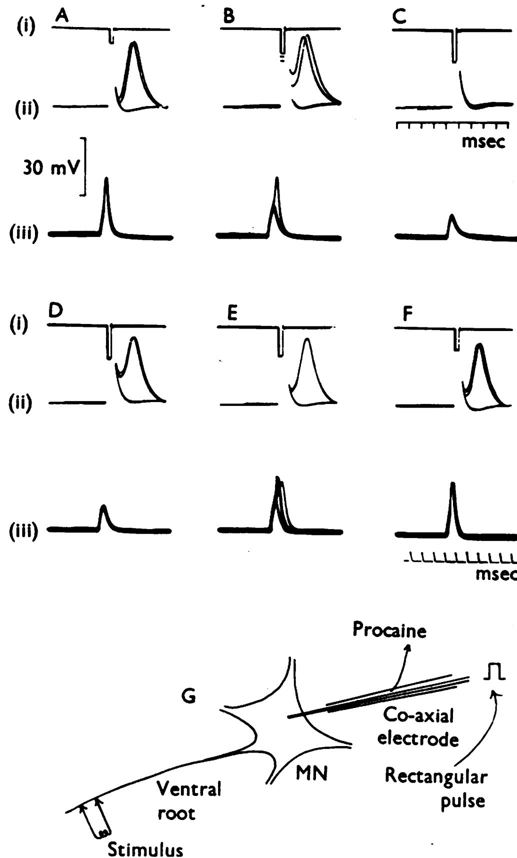

Fig. 3, A–F. Responses recorded from a motoneurone (MN) by means of the inner K_2SO_4-containing barrel of a co-axial electrode, as illustrated in G. The outer barrel contained a saturated solution of procaine hydrochloride from which procaine was applied as a cation for 115 sec after the control responses A were recorded. The resting potential of this cell was −56 mV and increased to −57 mV during the procaine application. Each record consists of six superimposed responses at a frequency of 1/sec, the antidromic and directly evoked spikes being recorded 500 msec apart.

A, control responses:

(i) Upper beam, relative magnitude of the current pulse which had a duration of 0·3 msec and evoked a spike in roughly half the tests.

(ii) Middle beam, spike potential evoked by a rectangular current pulse passed through the intracellular electrode.

(iii) Lower beam, spike potential evoked by stimulating the ventral root.

B–F, as for A except that the pulse size was altered in order to straddle the threshold for direct excitation. B, 90 sec; C, 110 sec after the commencement of the iontophoretic current; D, 25 sec; E, 40 sec and F, 75 sec after its termination. Time marker, msec for (i) and (ii) below middle beam of C; msec for the antidromic spike (iii) below F. Voltage, 30 mV for (ii) and (iii).

During recovery partial invasion of this segment occurred (E), but 75 sec after the procaine application had ceased, the SD segment was again being invaded by the spike potential (F). The direct threshold of the membrane was also increased by the action of procaine, as indicated by the size of the pulse (i) necessary to produce a full spike. In record C a pulse approximately 2·3 times the size of the control (A) failed to elicit a spike, but in F, after the cessation of the procaine, the spike was produced by a pulse 1·5 times the size of the control. Complete recovery occurred after a further period of about 30 sec. These changes were accompanied by a hyperpolarization of 2 mV, which is insufficient by itself to alter the direct excitability of the cell by a factor of 2·3.

Figure 4 illustrates responses from two other motoneurones to which procaine was applied iontophoretically. The cell of Fig. 4A–F was excited orthodromically, the just-threshold excitatory post-synaptic potential (EPSP) being shown in Fig. 4A and the truncated spike potential in Fig. 4B, C, E and F. The responses were evoked by stimulation of the gastrocnemius nerve, the intensity of the stimulus producing the potentials of Fig. 4A being doubled for the remaining records of the series. A current of 200 nA passed procaine from the outer barrel of the electrode for 75 sec and the subsequent responses C–F were recorded 48, 72, 95 and 135 sec after the commencement of this current. In records C and D, taken during the application, there was a progressive failure of spike generation, the EPSP of D being in fact larger than that of A, and yet completely failing to evoke a spike. Recovery occurred after the termination of the iontophoretic current, the EPSP's of Fig. 4E, however, producing spikes at slightly higher levels of membrane potential than those of A. Thereafter (F) a full spike only was produced. This cell had a comparatively low resting potential of − 50 mV and a low threshold for excitation, the control value of the just-threshold EPSP in Fig. 4A being 2–3 mV. However, the results were typical of those from five other motoneurones and clearly indicate that the orthodromic activation of motoneurones is blocked, not by an action of procaine on the production of excitatory post-synaptic potentials, but by a more direct effect upon the membrane. Observations of inhibitory post-synaptic potentials (IPSP's) indicated that they also were unaltered by procaine. Thus, for the cell illustrated in Fig. 4G–I, each of the pairs of IPSP's and antidromic spike potentials were recorded virtually simultaneously, Fig. 4G being the control responses and Figs. 4H and I the responses during and after the iontophoretic application of procaine. The procaine effectively blocked the antidromic invasion of the soma–dendritic membrane (H) but failed to alter the size of the IPSP. This is in contrast to the action of the depressant amino acids (Curtis *et al.* 1959), which, although having an action similar to procaine upon the

antidromic invasion of the cell, also reduced the size of both IPSP's and EPSP's. Direct measurement of the resistance of the cell membrane was not attempted in this series of experiments. However, the observation

Fig. 4, A–F. Monosynaptic excitatory post-synaptic potentials recorded, as in Fig. 3, but from another motoneurone, in response to orthodromic stimulation, the volley arriving at the dorsal surface of the spinal cord indicated in the lower beam. The cell had a resting potential of −50 mV. Each figure consists of 15–20 superimposed records at a frequency of 5/sec.
A, B, control responses with maximal stimulation in B, whilst in A the EPSP just straddled the threshold of the cell. C–F, as for B but during and after the application of procaine to the cell by a current of 200 nA for 75 sec: C, 48 sec; D, 72 sec; E, 95 sec and F, 135 sec after this current commenced. The horizontal arrows indicate the size of the threshold EPSP.
G–I. Potentials similarly recorded from another motoneurone having a resting potential of −55 mV. The inhibitory potential (upper beam) was evoked by stimulation of the peroneal nerve whereas the antidromic spike potential (lower beam) was produced by stimulation of the segmental ventral root. G, control response; H, 60 sec after a current of 330 nA had been passing procaine from the outer barrel of the electrode; I, 70 sec after this current ceased.

that post-synaptic potentials were unchanged indicates that this resistance was altered but little by procaine. If the membrane conductance had been increased by procaine, the subsynaptic currents generating post-synaptic potentials would have been partially shunted, thereby reducing the

magnitude of the resultant potentials (cf. Eccles, 1957). In addition, the failure to reduce the size of these potentials indicates that impulse transmission in the presynaptic terminals was not impaired by the concentrations of procaine obtained in these experiments.

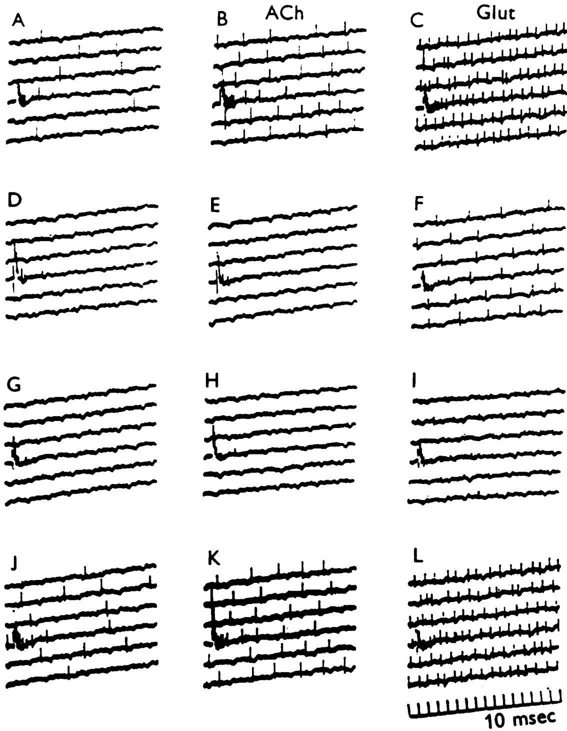

Fig. 5. Spike potentials recorded on moving film as in Fig. 1, from a single Renshaw cell and evoked by ventral-root stimulation (A, D, G and J), by iontophoretically applied acetylcholine (B, E, H and K) and by glutamate ions (C, F, I and L). A, B, C, control responses; D, E, F, 15, 7 and 12 sec respectively after a current of 100 nA began to pass atropine from one barrel of the five-barrel electrode; this current flowed for 18 sec; G, H and I, 5, 8 and 21 sec respectively after this current ceased; J, K, L, 3 min after the termination of the application of atropine. Time marker, 10 msec for all records.

Atropine

When administered intravenously, in comparatively large doses (2 mg/kg), atropine has but a slight depressant action upon the responses of Renshaw cells evoked by ventral root stimulation (Eccles, Fatt & Koketsu, 1954). A more powerful depression was observed when atropine was passed iontophoretically on to single Renshaw cells, the excitant action of iontophoretically applied acetylcholine also being blocked. In

addition, however, as with procaine, the repetitive activation of Renshaw cells by glutamate ions was blocked by atropine. This is illustrated in Fig. 5, which shows spike responses recorded from a Renshaw cell by means of the central barrel of a five-barrel electrode. The recording was made in a similar fashion to that of Fig. 1 and the control records A, B and C were evoked by a maximal ventral root volley, by acetylcholine and by glutamate ions respectively. In the latter two cases iontophoretic currents of 100 nA were used, this being cationic for acetylcholine and anionic for glutamate ions. An iontophoretic current of 100 nA then passed atropine from another barrel of the electrode for 18 sec, the responses D–F being recorded during this application, G–I shortly after the cessation of the iontophoretic current and J–L 3 min later, when complete recovery had occurred. The records D, G and J; E, H and K and F, I and L were evoked by the same stimuli as A, B and C respectively. This figure demonstrates that the synaptic activation of Renshaw cells as well as activation by acetylcholine and glutamate was reversibly suppressed by atropine, thus suggesting that this substance has a direct action upon the spike-potential generating mechanism. Alternatively, atropine could be blocking not only the cholinoceptive receptors but also those with which glutamic acid combines.

In view of the use of atropine in determining the cholinergic nature of transmission at junctional regions (Ambache, 1955) it was of interest to investigate if the cholinergic excitation of Renshaw cells was more susceptible to the action of this substance than that due to glutamate ion. The maximum frequencies at which applications of acetylcholine and glutamate ion fired a Renshaw cell are plotted in Fig. 6, with filled and open circles respectively. These substances were applied alternately at 8-sec intervals, with constant currents of 100 nA for periods of 4 sec. The magnitude of the current applying glutamate ion was chosen so that the frequency of firing was lower than that attained by acetylcholine. The constancy of the control responses to the left of the figure established that the interference between these applications was minimal. After the control responses a current of 15 nA passed atropine into the neighbourhood of the cell for 57 sec, and the alternating test applications of acetylcholine and glutamate continued during and after the passage of this drug. Throughout the series a ventral-root stimulus evoked responses from this cell and it was noted that when atropine blocked the responses to acetylcholine, these synaptically elicited spikes were reduced in number. However, there was very little alteration in the frequency of the firing produced by glutamate in this series, in spite of this substance being initially less effective in firing the cell than acetylcholine. With larger iontophoretic currents applying atropine, the spikes evoked by both glutamate and acetylcholine could be

blocked. Analyses such as these were performed upon several cells, and it was found that currents of from two to three times that necessary to block acetylcholine were necessary to suppress completely the excitation evoked by glutamate.

The responses of Fig. 7 show that atropine also suppressed the excitation of interneurones. These spike potentials were recorded extracellularly by means of the central barrel of a five-barrel electrode, those of Fig. 7A, B and C being evoked by a maximal dorsal root volley, whereas those of

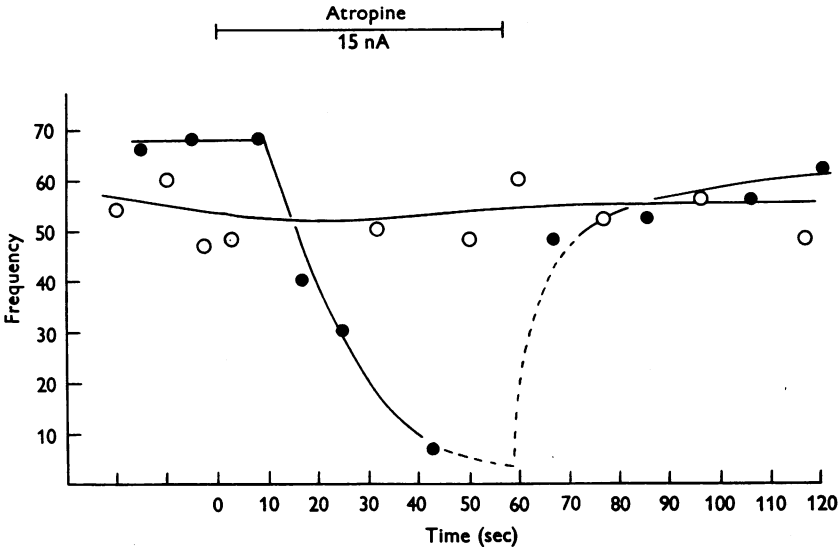

Fig. 6. Maximal frequencies of firing of a Renshaw cell evoked by applications of acetylcholine (●) and glutamate ions (○) passed iontophoretically from separate barrels of a five-barrel electrode, the central barrel of which was used for recording the responses. The applications were for periods of 4 sec, the substances being applied in turn approximately every 8 sec. During the time indicated by the horizontal bar, a current of 15 nA passed atropine from another barrel of the electrode. Ordinate, spikes per second.

Fig. 7D, E and F were produced by the iontophoretic application of glutamate ions. After the control responses A and D, a current of 250 nA was used to pass atropine out of the electrode for 30 sec and the traces of Fig. 7B, E and C, F were recorded 5 sec and 2 min after the cessation of this current, respectively. It is apparent that atropine depressed the production of both the synaptically and chemically evoked spikes of the interneurones. The observation that the excitant action of glutamate was blocked for some time after the cationic current applying atropine was terminated excludes the possibility that such action arose because of an interference between the currents applying these oppositely charged ions.

After applying atropine with currents of 100–200 nA for periods as long as 60 sec, the full recovery of both orthodromic and amino acid-evoked spikes of interneurones usually took 2–3 min. This action of atropine is very similar to that of procaine illustrated in Fig. 2. Although the ionto-phoretic method of applying drugs to neurones does not allow an accurate assessment to be made of relative potencies of action, the slower development of the blocking action of atropine with currents of 150–200 nA indicated that it was not as powerful a depressant as procaine.

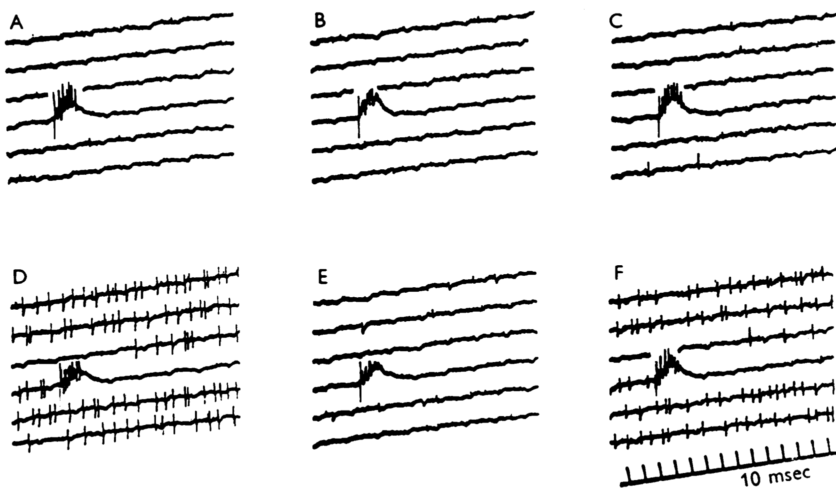

Fig. 7. Spike potentials recorded extracellularly from an interneurone of the L7 segment of the spinal cord by means of the central barrel of a five-barrel electrode. A, B and C, evoked by a dorsal root volley, this stimulus also being repeated in F, G and H; D, E and F, evoked by glutamate ion. In each case a current of 100 nA was used and the traces illustrated were recorded 3 sec after this current began. A, D, controls; B, E, 5 sec after the termination of a current of 250 nA passed atropine from the electrode; C, F, 2 min later than B, E; time marker, 10 msec.

In order to determine the action of atropine upon the membrane of motoneurones, it was applied extracellularly from the outer barrel of co-axial electrodes whilst intracellular records were obtained by means of the central barrel (cf. Fig. 3). The results were similar to those obtained with procaine, the records from one cell being illustrated in Fig. 8. This, a gastrocnemius motoneurone, had a resting potential of -50 to -58 mV for a period of 30 min and spike responses could be evoked by stimulation of the Group I gastrocnemius afferent fibres (A–F, (i)), the threshold of the spike being indicated by the horizontal arrow in each case. The volley arrival at the dorsal surface of the spinal cord was indicated by the tri-phasic potential of Fig. 8A–F, (ii). In addition, this cell could be excited

directly by a pulse of 0·3 msec duration which was passed through the intracellular electrode, as in Fig. 3. The size of this pulse (Fig. 8, A–F, (iii)) was varied in order to straddle the threshold of the cell, so producing spike potentials (Fig. 8A–F, (iv)) in approximately half of the six to eight tests. These tests were made 500 msec before the orthodromic responses, at 1 sec intervals.

In Fig. 8A (i) the spike potential was produced by a maximal Group I orthodromic volley, the threshold level of the EPSP for spike initiation being indicated by the horizontal arrow. Whilst the responses of Fig. 8B (i) were being recorded, the intensity of the stimulus applied to the gastrocnemius nerve was reduced in order to demonstrate the amplitude of the subthreshold, post-synaptic excitatory potentials; the threshold for excitation was about 3 mV. A current of 300 nA then passed atropine from the outer barrel of the co-axial electrode for 75 sec, the responses of C and D being recorded during this application and those of E and F after the termination of the current. Consideration of the orthodromic responses (i) shows that the threshold of the neuronal membrane was increased, the EPSP of Fig. 8D being 11 mV and yet failing to excite the cell, apart from one spike. After the current applying atropine ceased, the orthodromic activation of the cell recovered gradually, the threshold in Fig. 8F being approximately 3 mV. These observations were paralleled by the changes in the direct excitability of the membrane, a pulse of three times the size of the control (Fig. 8A) failing to evoke a spike in Fig. 8D. When connected to a direct-coupled amplifier, an intracellular electrode records not only the resting membrane potential of the impaled cell but also potentials due to the electrode itself. The 'resting' potential is determined as the difference between potentials recorded intracellularly and extracellularly, it being usual to assume that the 'electrode' potentials are identical under these circumstances. In many cells fluctuations are observed in the recorded potential which are not paralleled by changes in the excitability of the membrane. Consequently these changes in potential, of the order of 2–10 mV, are associated with the electrode. The potential of the cell from which the records of Fig. 8 were obtained had been steady during the 30 sec before the application of atropine. With the passage of iontophoretic current through the outer barrel of the co-axial electrode the recorded potential commenced to increase and continued to do so until at the cessation of current flow it was 8 mV greater than just before the beginning. The potential then remained constant at this new value for a further 140 sec. It might be considered that this 'hyperpolarization', seemingly associated with the action of atropine, accounts for the increase in threshold and decrease in membrane excitability of the cell. That such is not the case is demonstrated by the complete recovery of excitability

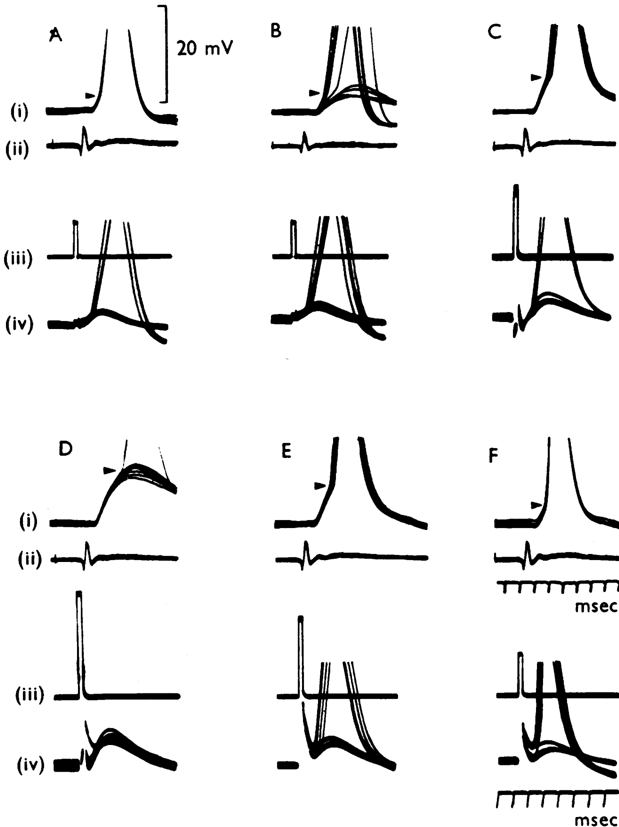

Fig. 8. Responses from a gastrocnemius motoneurone of resting potential −50 to −58 mV. All spike potentials are truncated and were recorded by the central barrel of a co-axial assembly.

(i) Orthodromic activation produced by stimulating the Group I afferent fibres of the gastrocnemius nerve, the arriving volley being recorded (ii) at the dorsal surface of the L7 segment of the cord. The threshold of the cell is marked by a horizontal arrow. The excitability of the cell was tested by means of a current pulse of 0·3 msec duration (iii) applied through the central barrel of the electrode. The size of this pulse was altered so that in approximately half the tests a spike potential was evoked (iv).

A, B, control responses, the size of the orthodromic volley being progressively decreased in B; C, D, 45 and 68 sec respectively after an iontophoretic current of 300 nA passed atropine from the outer barrel of the electrode: a current of 400 nA in the opposite direction had previously prevented this ion from leaving the electrode; E, F, 33 and 225 sec respectively after the atropine application was terminated. Each record consists of 4–8 responses at a frequency of 1/sec, the orthodromic and direct spikes being elicited 500 msec apart.

within 110 sec of the cessation of the current applying the atropine, in spite of the persisting 'hyperpolarization' (compare A, (i), (iii) and (iv) with F (i), (iii) and (iv)). The application of atropine to two other motoneurones with different co-axial electrodes resulted in similar changes of excitability without alteration in membrane potential. These results indicate, therefore, that atropine has a reversible depressant action upon the processes responsible for the rising phase of the spike potential of motoneurones.

DISCUSSION

Direct observations upon motoneurones of the lumbar segments of the cat indicate that procaine, in the absence of a change in membrane potential, raises the threshold of the neuronal membrane to direct, orthodromic and antidromic stimulation. There appears to be little or no change in either excitatory or inhibitory post-synaptic potentials or in the membrane resistance. Observations upon Renshaw cells and interneurones are in accordance with these findings, there being suppression of the spikes evoked either orthodromically or by excitant amino acids. In addition, it has been reported that the excitant action on neurones of compounds which chelate calcium ions is also prevented by procaine (Curtis, Perrin & Watkins, 1960). These results therefore indicate that procaine has a direct action upon the post-synaptic membrane of all of these types of neurone, independent of any effect it might have at the cholinoceptive receptors of Renshaw cells.

Thus, in common with the investigations upon squid axons (Shanes *et al.* 1959; Taylor, 1959), it can be concluded that procaine prevents the initiation of neuronal spikes, presumably by depressing the effectiveness with which depolarization of the membrane produces a high sodium permeability during the rising phase of the spike potential. Since the EPSP is unaltered and since there is probably an increased membrane permeability to sodium, as well as to other ions, during such a potential (Eccles, 1957), it can be concluded that the manner in which sodium ion passes through the membrane during the EPSP is different from that in which it does so during the spike. The progressive diminution in the size of the neuronal spikes produced by procaine can be accounted for by its blocking action upon areas of the neuronal membrane closest to the applying electrode, other portions of the cell continuing to be activated both chemically and synaptically. It is of interest that the membrane of motoneurones appears to be more susceptible to the action of procaine than that of the terminal afferent fibres having synaptic endings upon these cells. This conclusion is similar to that of Harmel & Malcolm (1958), who studied the action of procaine upon the motoneurones of the isolated frog's spinal cord.

Since extracellular records only were obtained from Renshaw cells, it could not be determined if procaine blocked the action of acetylcholine upon the post-synaptic receptors of these cells. However, since the end-plate potentials and potentials evoked by iontophoretic application of acetylcholine at the neuromuscular junction are reduced in size by this drug (del Castillo & Katz, 1957), it is very likely that procaine has a dual action upon Renshaw cells. Thus it is probable that procaine not only raises the threshold of the electrically excitable membrane of these cells, but also hinders the production of an EPSP by the synaptically released acetylcholine.

The results obtained in the present series of experiments indicate that atropine has actions similar to those of procaine, although it is probably not as effective a depressant of non-cholinoceptive neurones. The demonstration that this substance affects the electrical excitability of the motoneurone membrane renders unlikely the possibility that the blocking action of atropine upon the glutamate-induced spikes of Renshaw cells is due to this agent blocking the amino-acid receptor sites (p. 26). The actions of atropine upon cholinoceptive receptors and the sodium 'carrier' system are not surprising in view of the fact that both atropine and procaine are tertiary amino esters of aromatic acids and that atropine has approximately half the local anaesthetic potency of procaine (de Elio, 1948). In addition, many atropine analogues possess cocaine-like actions (Blicke & Kaplan, 1943). A variety of central actions of atropine have been described (Schweitzer & Wright, 1937; Bülbring & Burn, 1941; Calma & Wright, 1944; Feldberg, 1945; Rinaldi & Himwich, 1955; Bradley & Elkes, 1957; Feldberg, 1957) and many of these have been assumed to be due to the blocking action of this substance at cholinergic junctions, although Feldberg (1945) pointed out that atropine has a non-specific depressant action upon cells. The present results indicate that, since the concentration necessary to block the cholinergic excitation of Renshaw cells was lower than that which interferes with glutamate excitation by a factor of only two or three, care must be exercised when interpreting results obtained with this substance. Thus susceptibility to atropine may not of itself establish that synaptic transmission at a particular junctional region is cholinergic in nature.

Although direct evidence is lacking, atropine, being a tertiary amino compound, would be expected to penetrate the blood–brain barrier. Hence the relative ineffectiveness of this drug upon Renshaw cells following intravenous administration is presumably due to the lower local concentration attained by this method, compared with that obtained by iontophoretic application.

SUMMARY

1. Procaine and atropine have been applied iontophoretically to interneurones, motoneurones and Renshaw cells of the spinal cord of the cat, lightly anaesthetized with pentobarbital sodium.

2. The spike responses of such cells, whether elicited synaptically, antidromically, directly, or by the application of chemical excitants, were suppressed by these substances.

3. The post-synaptic and resting potentials of motoneurones were unaltered.

4. It is concluded that procaine and atropine, in addition to their blocking actions at cholinoceptive synapses, have a direct action on the post-synaptic membrane, presumably by preventing the activation of the sodium 'carrier'.

REFERENCES

AMBACHE, N. (1955). The use and limitations of atropine for pharmacological studies on autonomic effectors. *Pharmacol. Rev.* **7**, 467–494.

BLICKE, F. F. & KAPLAN, H. M. (1943). Synthetic mydriatics III. *J. Amer. chem. Soc.* **65**, 1967–1970.

BRADLEY, P. B. & ELKES, J. (1957). The distribution of cholinergic and non-cholinergic receptors in the brain: electrophysiological evidence. *Metabolism of the Nervous System*, ed. RICHTER, D., pp. 515–521. London: Pergamon Press.

BÜLBRING, E. & BURN, J. H. (1941). Observations bearing on synaptic transmission by acetylcholine in the spinal cord. *J. Physiol.* **100**, 337–368.

CALMA, I. & WRIGHT, S. (1944). Action of acetylcholine, atropine and eserine on the central nervous system of the decerebrate cat. *J. Physiol.* **103**, 93–102.

COOMBS, J. S., CURTIS, D. R. & ECCLES, J. C. (1957). The interpretation of spike potentials of motoneurones. *J. Physiol.* **139**, 198–231.

COOMBS, J. S., CURTIS, D. R. & ECCLES, J. C. (1959). The electrical constants of the motoneurone membrane. *J. Physiol.* **145**, 505–528.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955). The electrical properties of the motoneurone membrane. *J. Physiol.* **130**, 291–325.

CURTIS, D. R. & ECCLES, R. M. (1958*a*). The excitation of Renshaw cells by pharmacological agents applied electrophoretically. *J. Physiol.* **141**, 435–445.

CURTIS, D. R. & ECCLES, R. M. (1958*b*). The effect of diffusional barriers upon the pharmacology of cells within the central nervous system. *J. Physiol.* **141**, 446–463.

CURTIS, D. R., PERRIN, D. D. & WATKINS, J. C. (1960). The excitation of spinal neurones by the ionophoretic application of agents which chelate calcium. *J. Neurochem.* (in the Press).

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1959). The depression of spinal neurones by γ-amino-*n*-butyric acid and β-alanine. *J. Physiol.* **146**, 185–203.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1960). The chemical excitation of spinal neurones by certain acidic amino acids. *J. Physiol.* **150**, 656–682.

DE ELÍO, F. J. (1948). Acetylcholine antagonists: a comparison of their action in different tissues. *Brit. J. Pharmacol.* **3**, 108–112.

DEL CASTILLO, J. & KATZ, B. (1957). A study of curare action with an electrical micromethod. *Proc. Roy. Soc.* B, **146**, 339–356.

ECCLES, J. C. (1957). *The Physiology of Nerve Cells.* Baltimore: Johns Hopkins Press.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

FELDBERG, W. (1945). Present views on the mode of action of acetylcholine in the central nervous system. *Physiol. Rev.* **25**, 596–642.

FELDBERG, W. (1957). Acetylcholine. *Metabolism of the Nervous System*, ed. RICHTER, D., pp. 493–509. London: Pergamon Press.

FURUKAWA, T. (1957). Properties of the procaine end-plate potential. *Jap. J. Physiol.* **7**, 199–212.

HARMEL, M. & MALCOLM, J. L. (1958). The site of action of procaine on the isolated spinal cord of the frog. *J. Physiol.* **140**, 213–219.

HARVEY, A. M. (1939). The actions of procaine on neuromuscular transmission. *Johns Hopk. Hosp. Bull.* **65**, 223–238.

RINALDI, F. & HIMWICH, H. E. (1955). Alerting responses and actions of atropine and cholinergic drugs. *Arch. Neurol. Psychiat., Chicago*, **73**, 387–395.

SCHWEITZER, A. & WRIGHT, S. (1937). The action of eserine and related compounds and of acetylcholine on the central nervous system. *J. Physiol.* **89**, 165–197.

SHANES, A. M., FREYGANG, W. H., GRUNDFEST, H. & AMATNIEK, E. (1959). Anaesthetic and calcium action in the voltage clamped squid giant axon. *J. gen. Physiol.* **42**, 793–802.

TAYLOR, R. E. (1959). Effect of procaine on electrical properties of squid axon membrane. *Amer. J. Physiol.* **196**, 1071–1078.