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DECAMETHONIUM IN DEPOLARIZED MUSCLE AND THE EFFECTS OF TUBOCURARINE

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SUMMARY

1. In rat diaphragms immersed in solution containing 5 mM potassium the maximum uptake of labelled decamethonium was found at the end-plate region. In muscles depolarized in solution containing potassium methyl sulphate the uptake was reduced but a peak concentration at the end-plate region was still demonstrated.

2. The uptake of labelled decamethonium increased steadily with time and was interpreted in terms of the entry of decamethonium into the fibres. The permeability at 10–100 μM was similar to that of sodium.

3. The uptake of decamethonium at the end-plate region was dependent on the concentration. At low values the uptake in depolarized muscle was uniform along the fibres. Increase in concentration produced a peak at the end-plate region. This was interpreted as a change in permeability such that half the maximum effect was present at a concentration of approximately 5 μM.

4. At high concentrations the influx showed saturation and carrier-like kinetics with a half-saturation concentration of 400 μM.

5. Tubocurarine inhibited the peak uptake in depolarized diaphragm. The results were consistent with competitive inhibition with an inhibitory constant of 0·07 μM.

6. Acetylcholine in high concentration also inhibited the uptake of decamethonium in the end-plate region of depolarized muscle.

INTRODUCTION

Decamethonium is a depolarizing compound which becomes concentrated in the region of the end-plate (Waser, 1967; Taylor, Creese, Nedergaard & Case, 1965), and evidence has also been obtained by autoradiography of frozen sections that labelled decamethonium enters the fibres of rat muscle shortly after injection (Creese & Maclagan, 1967, 1970).

The depolarization produced by decamethonium in single fibres has been studied by Thesleff (1955) and del Castillo & Katz (1955), and Paton & Rang (1965) have suggested that the entry of labelled decamethonium is a consequence of the depolarization produced by the drug. It was found that an increased uptake of labelled decamethonium in the junctional region as compared with the rest of the fibre could be demonstrated in muscles which were depolarized in solutions containing potassium methyl sulphate, and this gave a means of measuring the uptake in conditions in which the electrical field remained constant. In addition the action of tubocurarine and other compounds could be studied without the complications produced by changes in the resting potential. A preliminary account of some of the findings has been given by one of the authors (England, 1969).

METHODS

Saline. The solution with high potassium resembled that used by Jenkinson & Nicholls (1961), and had the following composition (mM): K^+ 149, Na^+ 1·5, Ca^{2+} 1·3, $CH_3SO_4^-$ 149, Cl^- 2·6, HCO_3^- 1·5. The calcium was freshly dissolved. The solution had a glucose content of 200 mg/100 ml. and was gassed with oxygen. The potassium methyl sulphate was recrystallized and was kept in an evacuated desiccator (see Jenkinson & Nicholls, 1961). When saline with low potassium (5 mM) was needed the composition was that used by Creese & Northover (1961) and the solution was gassed with 5 % (v/v) carbon dioxide and 95 % oxygen. For solutions containing 10 mM potassium and 25 mM potassium the solution used by Creese & Northover (1961) was modified by substitution of potassium methyl sulphate for sodium chloride. The temperature was maintained at 38° C.

In some experiments acetylcholine chloride (25 mM) was employed. Additional sucrose was added to the solution used for some of the controls to produce an equivalent increase in osmotic pressure. The value of the osmotic coefficient was taken as 1·856, being the interpolated value for NaCl at 0·154 M (see Harris, 1960). In practice the sucrose was added to give a concentration of 46·4 mM.

Handling of muscles. Albino rats of 80–140 g were used. The animals were stunned and decapitated and the left diaphragm and rib were rapidly removed, attached to a holder (Creese, 1968) and immersed in a tube containing 10 ml. saline. The muscles were soaked for 30 min in solution with high potassium and were then transferred to a solution to which radioactive decamethonium had been added. After immersion in labelled drug the muscle and holder were passed for 10 min through saline of the same composition but without radioactive drug, the saline being changed each min. This procedure was used to wash out the radioactivity in the extracellular fluid (Creese, Taylor & Tilton, 1963). When tubocurarine was used the muscle was soaked for 30 min in saline containing tubocurarine and then transferred to saline containing both tubocurarine and labelled decamethonium.

Freezing of diaphragms. The muscles were frozen on brass plates to which graph paper had been attached by means of transparent adhesive tape. Diaphragm muscles were removed and the ribs were secured to the metal plates by projecting pins. The muscle on the brass strip was stretched with forceps and frozen on a block of solid carbon dioxide. A plastic cover was used to minimize condensation of water vapour on the muscle. The position of the band of end-plates was found by the 'white line' which appeared as soon as freezing occurred (Creese, England & Taylor, 1969;

England, 1970). The muscle was trimmed with a razor blade and was sliced horizontally to produce a series of strips 1 mm wide, from the tendon end to the rib. The blade was washed after each slice to avoid contamination. The frozen strips were rapidly weighed and transferred to plastic vials which contained 0·5 ml. N-KOH in methanol. Diaphragm muscles which were properly aligned gave a rectangle of tissue which contained a band of end-plates which was horizontal; in some cases the white line was diagonal and strips were then cut parallel to this white line.

Radioactive compounds and measurements. [^{3}H-methyl]decamethonium dichloride with specific activity of 250 mc/m-mole was obtained from the Radiochemical Centre, Amersham, Buckinghamshire. For measurements at low concentrations labelled decamethonium with specific activity of 1·1 c/m-mole was used. The compound was analysed at Amersham by thin-layer chromatography on alumina in chloroform-methanol (80:20, v/v) and the radiochemical purity was 98 %.

Strips of muscle which had been immersed in solution containing radioactive decamethonium were added to plastic vials which contained 0·5 ml. N-KOH in methanol. The tissues were dissolved by heating for 30 min in a water-bath at 70° C, and after the vials were cooled 14 ml. scintillator was added (Creese & Taylor, 1967). In other cases, 0·1 ml. of the radioactive saline was added to a vial which contained 0·5 ml. N-KOH in methanol and scintillator was added as before. Background was measured in vials which contained 0·5 N-KOH in methanol, plus scintillator. The radioactivity was estimated by liquid scintillation in a two-channel counter at room temperature (Isotope Development Ltd.). The efficiency varied between 6 and 10 % and the background was 60 counts/min. For measurements of low activity an automatic refrigerated counter was used (Packard) with efficiency 25 % and background 15 counts/min.

Drugs. The following drugs were used: decamethonium dibromide (Burroughs Wellcome, Tuckahoe, N.Y., U.S.A.: mol. wt. 418); tubocurarine dichloride pentahydrate (Burroughs Wellcome, London: mol. wt. 786); acetylcholine chloride (Hopkins & Williams: mol. wt. 182). When acetylcholine was used the dried salt was rapidly weighed into tubes and solution was added to produce a concentration of 25 mM.

RESULTS

Decamethonium in normal diaphragms

The unfilled rectangles in Fig. 1 represent the uptake of decamethonium in a diaphragm in physiological saline containing 5 mM potassium. The muscle was immersed for 1 hr in the presence of labelled decamethonium (10 μM), and then passed for 10 min through tubes of inactive saline. Fig. 1 gives distribution of radioactivity along the muscle from the tendon to the rib, and the arrow indicates the slice which contained the line of end-plates whose position becomes apparent when the muscles are frozen (see above). The uptake is expressed as a clearance, being ml. g^{-1} or (radioactivity per mg muscle)/(radioactivity per μl. saline). If Gauss curves are fitted to the histograms the peaks occur at the end-plate region, and asymptotes can be fitted to the values along the fibres.

Uptake of decamethonium in high potassium concentration

The stippled area in Fig. 1 represents the uptake of decamethonium in a muscle depolarized in a solution which contained 149 mM potassium

methyl sulphate. The uptake is much reduced but a peak is still present, while the asymptote is not much affected.

The effect of increasing concentration of potassium is seen in Fig. 2. Increase of external potassium produced a progressive diminution in the peak, with a much smaller effect on the asymptote. Depolarization was apparently accompanied by a fall in the uptake of decamethonium. The effect was clear between the extremes of the range which was used, though there was considerable overlap at low concentrations of potassium.

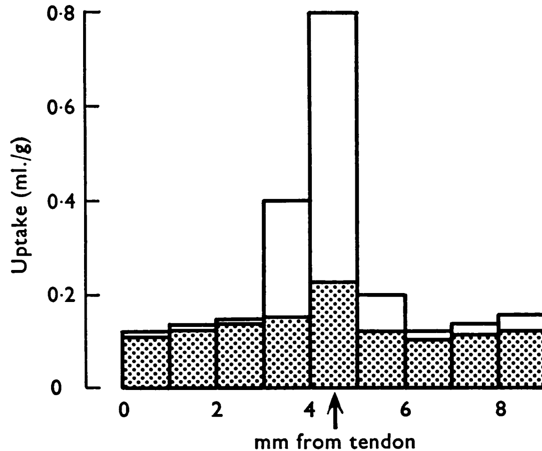

Fig. 1. Uptake of labelled decamethonium in diaphragm of rat (1 hr), in ml./g. Concentration 10 μM, with washout in inactive saline for 10 min. The arrow indicates the slice of muscle which contained the line of end-plates (see text). Histogram with clear area shows uptake in physiological saline (5 mM-K^+). Stippled area shows uptake in depolarized muscle (149 mM-K^+); a peak uptake in the region of the end-plates is still present.

Fig. 3 shows the peak uptake plotted against time for muscles in high potassium solution. The circles give the mean of at least four muscles and the limits give the S.D. The uptake showed a steady increase for the first 60 min which was linear with time, and the dashed line gives the regression through the origin.

In Fig. 3 the scatter increases with time. A weighting factor was required which was inversely proportional to the estimated variance (see Baker, 1941; Anderson & Bancroft, 1952). In practice a weighting factor was used which was the reciprocal of the square of the expected value of the regression. The dashed line in Fig. 3 has been calculated as though it passed through the origin, and in this case the regression from three cycles of iteration has a slope of 0·195 ml. g^{-1} hr^{-1}, the 95 % confidence limits being 0·165 and 0·204 (estimated from twenty muscles). If the true regression is used then the slope is 0·21 ml. g^{-1} hr^{-1} with a small (negative) origin at $-0{\cdot}006$ ml. g^{-1} which is considerably smaller than the estimated S.E. With the methods which were available it was not feasible to explore the uptake at shorter time intervals after

application of the drug. The points in Fig. 3 give the total uptake at the end-plate region; if the asymptote is subtracted the values would be approximately halved (see Fig. 4*b*).

The steady uptake seen in Fig. 3 is most easily interpreted in terms of the continuous entry of the labelled compound. In guinea-pig muscle the uptake of a decamethonium-like compound labelled with radioactive iodine

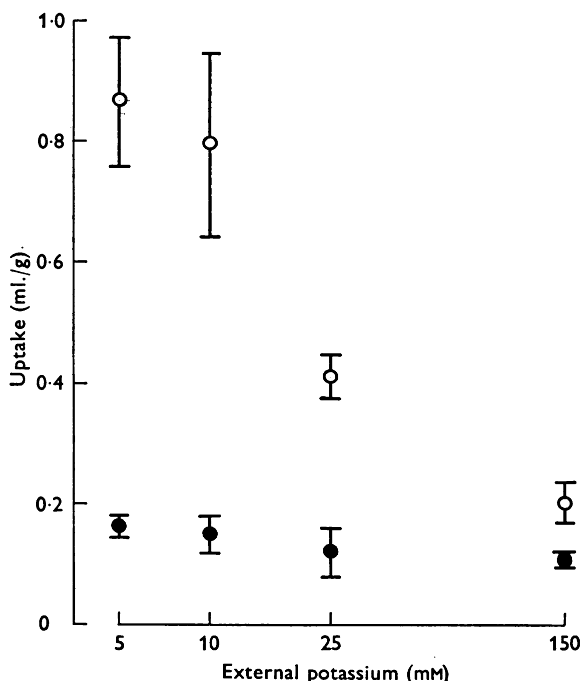

Fig. 2. Effect of potassium on peak uptake of labelled decamethonium (10 μM for 1 hr). Open circles show uptake (mg/g) in the end-plate region, filled circles show the uptake at the asymptote. Each point gives the mean of at least four muscles and the limits give ± S.D. Abscissa is on log scale. Increase in external potassium decreases the uptake of decamethonium at the end-plate region.

continues for at least 12 hr (Creese *et al.* 1963), and there is autoradiographic evidence for the entry of labelled decamethonium in rat muscle not only at the end-plate but also for several hundred microns on each side of the end-plate (Creese & Maclagan, 1970). The peak uptake seen in Fig. 1 can be used to give an estimate of the rate of entry in rat muscle as compared with that of inorganic cations.

From the results of Creese, El-Shafie & Vrbová (1968) and the formulas used by them, the permeability of decamethonium may be compared with that of sodium and potassium if these ions can be treated as though they moved independently down

electro-chemical gradients. The peak uptake in Fig. 1 after the extracellular drug has been washed out is 0·80 ml. g^{-1} in 1 hr. If the specific gravity is 1·07 and the extracellular space is 0·31 ml./ml. then the uptake is 0·856 ml. in 1 ml. of muscle or 0·69 ml. myoplasm. Hence the peak uptake is 1·24 ml./ml. myoplasm in 1 hr, so that the rate constant for inward movement is 1·24 hr^{-1}. No correction has here been made for the effects of diffusion through the interfaces. In the case of sodium, the rate for inward movement k_1 is $k_2\ S_i/S_0$ where k_2, the rate for outward movement, is

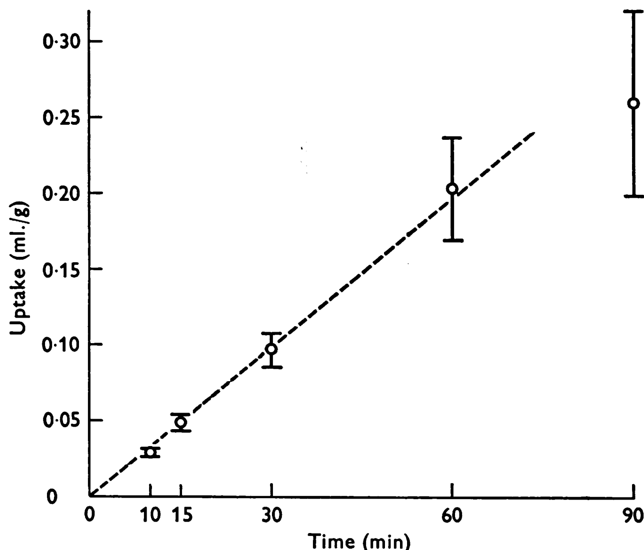

Fig. 3. Peak uptake of labelled decamethonium (10 μM) at the end-plate region in diaphragms depolarized by immersion in saline containing 149 mM K^+, with washout for 10 min in inactive saline. Circles show mean of four muscles and the limits give $\pm$ S.D.

0·137 min^{-1}; S_i the internal concentration of sodium is 9·0 μ-mole/ml. myoplasm, and S_0 the external sodium is 145 μ-mole/ml. Hence k_1 for sodium is 0·51 hr^{-1}.

The influx is given by the expression

$$\text{influx} = k_1 S_o \frac{V}{A}, \tag{1}$$

where V/A is the ratio of volume to area, and if a simplified treatment is permissible

$$\text{influx} = P \frac{nEF}{RT} \frac{S_o}{1 - \exp(-nEF/RT)}, \tag{2}$$

where E is the resting potential in mV, RT/F is 26·8 mV at 38° C and n is the valency of the ion. Hence the permeability P is

$$P = \frac{k_1}{n} \frac{V}{A} \frac{RT}{EF} \left[1 - \exp\left(-\frac{nEF}{RT}\right)\right]. \tag{3}$$

If E is 80 mV and n is 2, the term

$$\frac{RT}{EF}\left[1 - \exp\left(-\frac{nEF}{RT}\right)\right]$$

is 0·33. When E falls to 60 mV the above term is 0·44, and at 40 mV the term is 0·64. Comparison between sodium and decamethonium is complicated by uncertainty in E, but if changes in E may be neglected as a first approximation then P becomes proportion to k_1/n. For sodium, k_1/n is 0·51 hr^{-1} and for decamethonium the value is 1·24/2 or 0·62 hr^{-1}. Hence the permeability to decamethonium is at least as great as that of sodium in resting muscle. This is a minimum value for the slice of muscle which contained the band of end-plates and if allowance is made for depolarization

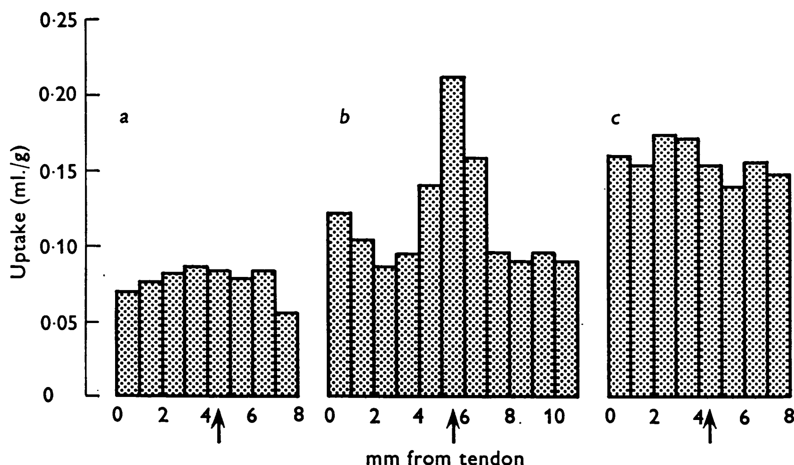

Fig. 4. (*a*) Uptake at low concentration of decamethonium in depolarized diaphragm (0·1 μM for 1 hr). (*b*) Uptake with 10 μM. Arrows indicate the slice of muscle which contained the line of end-plates (see text). (*c*) Uptake with 0·1 μM showing high value in all areas. A peak is present at 10 μM which is not seen at the low concentration.

the value would be somewhat increased. The comparison with the inorganic ions is only valid if the surface area for the movement of decamethnoium is similar. The evidence for the entry of decamethonium over a wide area on each side of the end-plate has been presented by Creese & Maclagan (1970).

The permeability of the end-plate region of the muscle to the organic ion decamethonium appears to be similar at this concentration (10 μM) to that of sodium ions. Exact comparison is not justified because of the simplifying assumptions which are necessary, but the results indicate the probable order of magnitude. The inward flux of decamethonium depends, however, on the external concentration, and in pharmacological doses the flux would be insignificant in comparison with that of the inorganic ions.

Effect of concentration on uptake of decamethonium

Fig. 4*b* shows the uptake in depolarized muscle at a concentration of 10 μM decamethonium. After immersion for 1 hr the muscle was removed and passed through a succession of tubes of inactive saline for 10 min. At this concentration there is a peak uptake in the end-plate region, the value being 0·21 ml. g^{-1}. The peak is absent or very small at low concentrations. Fig. 4*a* shows the uptake at 0·1 μM, and the uptake at the end-plate region is similar to that elsewhere along the fibres.

Fig. 4*c* shows another result obtained with 0·1 μM and in this case the uptake (in ml. g^{-1}) is high in all slices of the diaphragm but there is no clear evidence of a peak.

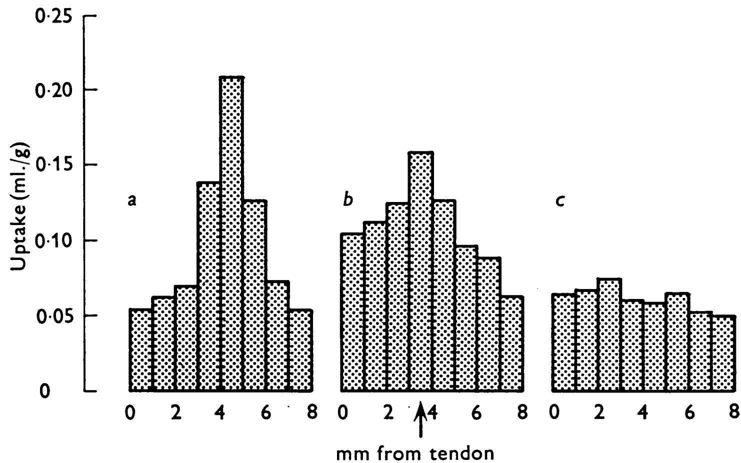

Fig. 5. Effect of high concentration of decamethonium in depolarized muscle (1 hr). (*a*) Uptake with 500 μM in ml./g. (*b*) Uptake with 3 mM. (*c*) Uptake with 10 mM; no peak is present at this concentration.

Fig. 5 shows that at high concentrations of decamethonium the peak is progressively diminished in depolarized muscle. The results are summarized in Fig. 6, in which the uptake at the end-plate minus that at the end of the fibre is plotted against the concentration of decamethonium. At low values there is no peak, and the uptake rises with increasing concentration to reach a maximum around 10–100 μM. This may be interpreted as a progressive increase in permeability with concentration. The influx is given by the uptake (in ml. g^{-1} hr^{-1}) multiplied by the concentration, and the results at concentrations above 100 μM are compatible with a saturable influx with carrier-like kinetics and a half-saturation concentration of approximately 400 μM.

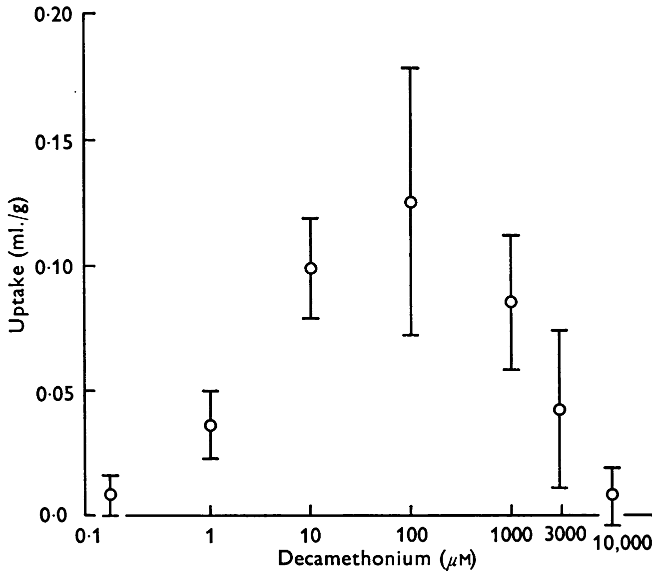

Fig. 6. Effect of concentration on uptake of decamethonium in depolarized muscle (1 hr.) Ordinate shows uptake at the end-plate (ml./g) minus that at the end of the fibre (see text). Each point gives the mean of at least four muscles. The largest entry is found at 10–100 μM. At low concentrations the peak is negligible. At high concentrations saturation occurs and the peak uptake is also reduced. Abscissa gives concentration on a log scale. Limits show ± S.D.

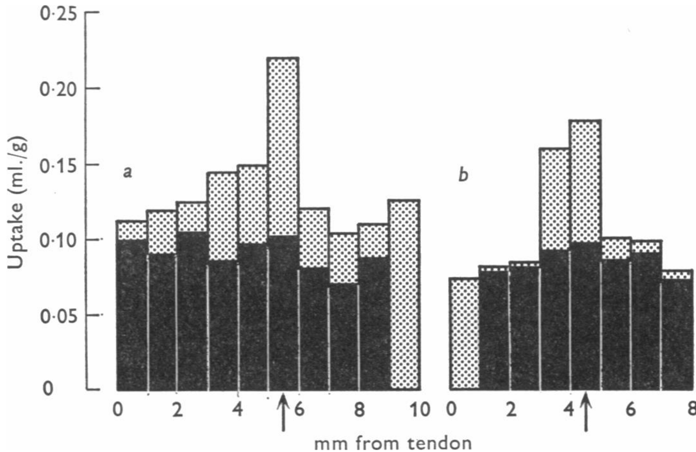

Fig. 7. Effect of tubocurarine on uptake of labelled decamethonium in depolarized diaphragm (1 hr.) Stippled area shows uptake in 10 μM decamethonium (*a*) and 1 mM decamethonium (*b*). Filled area shows uptake in the presence of 5 μM tubocurarine (*a*), and 200 μM tubocurarine (*b*). Arrows indicate the slice which contained the band of end-plates (see text).

Effect of tubocurarine

Tubocurarine was effective in preventing the uptake of labelled decamethonium in depolarized muscles immersed in solution containing a high content of potassium. The stippled area in Fig. 7*a* shows a control muscle with the usual uptake after immersion for 1 hr in 10 μM labelled decamethonium. In the presence of 5 μM tubocurarine the peak uptake at the region of the end-plate is abolished (filled area), while the effect at

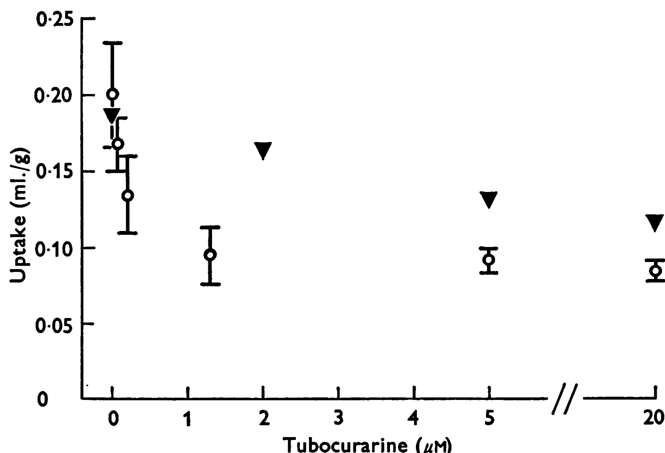

Fig. 8. Effect of tubocurarine on peak uptake of labelled decamethonium at the end-plate region of depolarized muscle. Circles show mean values with 10 μM decamethonium: limits give $\pm$ S.D. Triangles show mean values with 1000 μM decamethonium, and with this concentration the peak can be abolished with 200 μM tubocurarine (see Fig. 7).

the ends of the fibres is much smaller. In Fig. 7 *b* the concentration of decamethonium is 1000 μM, and in this case 200 μM tubocurarine was needed to abolish the peak.

Further results are shown in Fig. 8. The circles give the uptake of labelled decamethonium (10 μM) at the end-plate region in depolarized diaphragm. With increasing concentrations of tubocurarine the uptake at the end-plate becomes progressively reduced and appears to reach a steady value, so that the total uptake at the peak can be divided into a curarine-sensitive portion and a curarine-insensitive component. In Fig. 8 some results have also been included from muscles in the presence of 1000 μM decamethonium, and in this case considerably more tubocurarine is needed to reduce the peak uptake of decamethonium.

In Fig. 8 the results are similar to those obtained in other tissues. The effect of the inhibitor can be expressed quantitatively as the concentration required to halve the curarine-sensitive uptake, and for this purpose the curves in Fig. 8 should be rectified.

Creese & Taylor (1967) have treated such results in terms of the entry of depolarizing drug by a carrier-type mechanism which is competitively inhibited by tubocurarine. In this case the curve formed by the circles in Fig. 8 would be a hyperbola, and the appropriate rectifying plot would be the logit transform. The method of plotting may be illustrated as follows. From Fig. 8 the mean uptake for 10 μM decamethonium and zero tubocurarine is 0·200 ml. g^{-1} in 1 hour (mean of 9) and from a fitted curve the curarine-insensitive uptake is taken as 0·090 ml. g^{-1}. For 0·2 μM tubocurarine the mean uptake is 0·134 ml. g^{-1} (mean of 5), and the curarine-sensitive uptake is (0·134 − 0·090)/(0·200 − 0·090) or 0·40. The inhibitory effect of

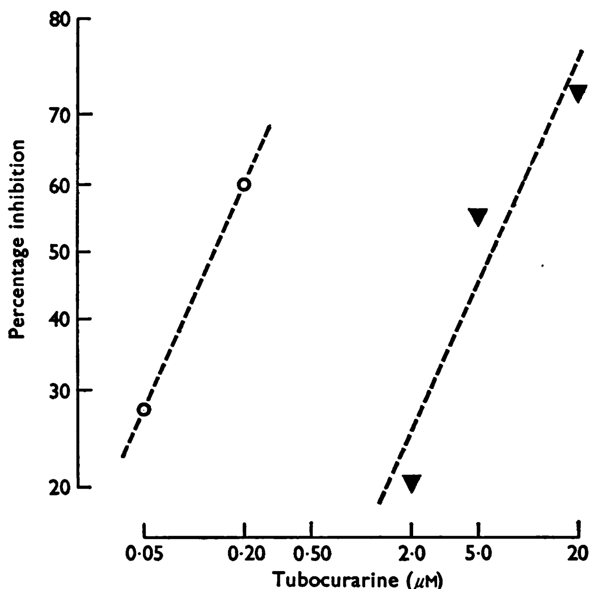

Fig. 9. Rectified plot from values shown in Fig. 8, showing effect of tubocurarine on peak uptake of decamethonium in depolarized muscle. Ordinate gives percentage inhibition on logit scale. Abscissa gives concentration of tubocurarine in μM on log scale. Circles: decamethonium 10 μM. Triangles: decamethonium 1000 μM.

tubocurarine is then (1 − 0·40) or 60 %. In Fig. 9 the values calculated in this manner are shown plotted on a logit scale (Finney, 1964). The circles give points for 10 μM decamethonium, and the slope on this scale is 1·08. The triangles give points for 1000 μM decamethonium, and the interrupted line is the weighted regression obtained by iteration (Finney, 1964), and has a slope of 1·13. For 10 μM decamethonium the concentration of tubocurarine which produces 50 % inhibition of the curarine-sensitive uptake (median effective dose, or M.E.D.) is 0·13 μM, and for 1000 μM decamethonium a concentration of 6·14 μM is needed. The mean slope of the regression is 1·11 logit units for a ten-fold change of concentration of tubocurarine, and this is close to the value of 1·15 which is expected if the points in Fig. 8 form a hyperbola (see Finney, 1964). For competitive inhibition the M.E.D. is related to the concentration of decamethonium by the expression used by Hunter & Downs (1945)

$$\text{M.E.D.} = K + \frac{K\,C}{\phi}, \tag{4}$$

where C is the concentration of decamethonium, K is the inhibitory constant of tubocurarine and ϕ is the concentration of decamethonium which produces half-saturation at the site where competition takes place with tubocurarine. This expression was originally derived from enzyme kinetics, but is also applicable to competitive inhibition at receptor sites or to competition for a carrier mechanism (see Creese & Taylor, 1967). From the two pairs of values M.E.D. and C the slope K/ϕ is 0·0061, and the value at the origin which gives the inhibitory constant K is 0·07 μM.

In Fig. 9 the inhibitory effect has been plotted against the concentrations of decamethonium, and a logit scale (Finney, 1964) has been used to obtain a linear relation. An increase in decamethonium requires a larger concentration of tubocurarine to produce 50 % inhibition of the curarine-sensitive uptake, and the results in Fig. 9 are compatible with competitive inhibition.

Effect of acetylcholine on the uptake of decamethonium

Acetylcholine in high concentration inhibited the uptake of decamethonium in depolarized muscle. In Fig. 10 the stippled area shows the radioactivity in the muscle after 2 hr in 2·5 μM decamethonium. Addition of acetylcholine (25 mM) produced marked inhibition. This result is unlikely to be due to osmotic effects, for the solution used for the control muscle contained additional sucrose so that the solutions were osmotically similar (see above). The result was not due to a change in the ionic strength, for in some experiments the control muscles were immersed in the usual saline containing 149 mM potassium methyl sulphate while for other muscles a solution containing K^+ 124 mM and acetylcholine 25 mM was used (four pairs). All the controls showed a peak uptake at the end-plate, while a total of six muscles in 25 mM acetylcholine showed low uptake and absence of peaks.

If decamethonium enters the muscle by a saturable carrier-like mechanism then other quaternary compounds might be expected to produce inhibition. In Fig. 10 the molar ratio between decamethonium and acetylcholine was 1:10,000. The inhibitory constant of acetylcholine was not measured, but it was found that a ratio of 1:1000 was largely ineffective in preventing the uptake of decamethonium at the end-plate. No anti-esterase was used in the experiment shown in Fig. 10.

DISCUSSION

The concept of surface receptors for depolarizing compounds has been confirmed by iontophoretic injection (del Castillo & Katz, 1955). However, the measurements made with labelled decamethonium are not explicable in terms of surface absorption alone, for entry of labelled decamethonium at

a large area in the junctional region of the fibre has been found soon after injection of a pharmacological dose in rats. Surface adsorption probably also occurs, but from autoradiograms of single fibres it has been concluded that the bulk of the labelled compound in the junctional region is within the fibre (Creese & Maclagan, 1970). When this drug has entered rat muscle *in vivo* it remains in the tissue for days or weeks after injection.

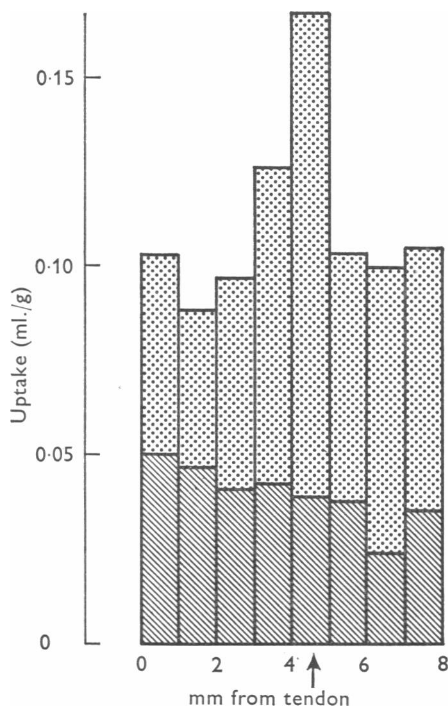

Fig. 10. Effect of acetylcholine on uptake of labelled decamethonium in depolarized diaphragm. Stippled histogram shows uptake in 2·5 μM decamethonium. Cross-hatched area shows uptake in presence of 25 mM acetylcholine. The ordinate gives the uptake (ml./g) obtained in 2 hr.

Presumably it becomes attached to some internal structure, for the rate of diffusion along the fibre is extremely slow (Taylor, Dixon, Creese & Case, 1967). Choline is also known to enter frog muscle at a rate which is similar to that of sodium, but choline is not localized at the end-plate nor affected by tubocurarine (Renkin, 1961; Alving & Renkin, 1964). When the uptake of decamethonium in rat diaphragm is measured *in vitro* the extracellular component can be washed out leaving a substantial portion of the cellular radioactivity, and although the uptake in depolarized muscle is low it is still possible to make measurements. Over a wide range of concentrations

there is a peak uptake at the end-plate region in depolarized muscle, as found also in normal muscles (Taylor *et al.* 1965). The uptake of decamethonium is consistently reduced as the external potassium is increased, and this is presumably related to the reduced driving force in muscles whose initital potential is progressively reduced by potassium.

These results were obtained in muscles which had been initially soaked in high-potassium solution for 15–30 min. The resting metabolism of such tissues is likely to be very high (Solandt, 1936; van der Kloot, 1969), and in most experiments the muscles were used for a maximum of 90 min after immersion in high-potassium solution. Jenkinson & Nicholls (1961) found that the resting potential in denervated diaphragm in similar solutions was negligible, and they did not detect any appreciable change when acetylcholine was applied. This is apparently in conflict with the results of Lüllman & Reis (1967) who reported a resting potential of −15 mV when the external potassium was raised to 140 mM in denervated diaphragm. These authors substituted potassium chloride for sodium chloride of the Tyrode solution, and it is known that an increase in external potassium at constant chloride concentration in frog muscle produces an initial depolarization followed by a slow drift towards the equilibrium value (Hodgkin & Horowicz, 1959). It is not clear how long the potential persisted in the experiments of Lüllman & Reis (1967). There appears to be no cause to dissent from the statement of Jenkinson & Nicholls (1961) that the possibility of depolarization by cholinomimetic agents in the presence of high concentration of potassium methyl sulphate has not been excluded but is unlikely.

It is characteristic of pharmacological action that the effect increases with concentration and reaches a maximum. Attempts have been made to demonstrate a saturable component with labelled decamethonium which could be related to the pharmacological response, but without success (see Waser, 1965). If compounds of high specific activity are used it is possible to work at low concentrations, and the present experiments have revealed a saturable component of influx which might be relevant. The uptake in diaphragm muscle may be divided into a portion which is found all along the fibre plus a component which forms a peak at the end-plate region. This peak is negligible at low concentrations but increases as the concentration is raised. If the results are interpreted as an alteration in permeability induced by decamethonium, then half the effect is achieved at a concentration of approximately 5 μM.

When unlabelled decamethonium is added to increase the total concentration to high values the peak is reduced and eventually abolished. This shows that there is an upper limit to the influx and that the unlabelled decamethonium can inhibit the movements of the labelled molecules.

Kinetics of this kind are explicable in terms of a carrier mechanism for the influx of decamethonium. Similar explanations have been advanced for the entry of choline in squid axons (Hodgkin & Martin, 1965), and for quaternary compounds in brain slices of the rat (Creese & Taylor, 1967). Hence there appear to be two saturable mechanisms which govern the movements of labelled decamethonium in depolarized rat muscle, a high-affinity system which probably initiates a change in permeability, plus a carrier-like mechanism with low affinity and half-saturation at 400 μM. More decisive evidence is needed before a carrier mechanism can be postulated with confidence, for transport through a chain of polar adsorption sites might be possible (Danielli, 1954) and this could give saturation kinetics (e.g. Bowyer, 1957.) If a carrier is present it need not be mobile, for transfer by a system of rotating molecules would equally well account for the findings (Danielli, 1954). A model of this kind would imply a receptor molecule with several sites which interact with each other, as postulated for regulatory enzymes and certain transport molecules (see Wyman, 1968; Changeux, Thiery, Tung & Kittel, 1967).

The uptake of labelled decamethonium and related compounds in skeletal muscle is largely prevented by tubocurarine (Creese *et al.* 1963; Taylor *et al.* 1965). It was at one time suggested that the entry of decamethonium was a secondary consequence of the depolarization which is produced by the drug, and that tubocurarine acted by preventing depolarization (Paton & Rang, 1965). This scheme in its orginal form seems inadequate to account for the results in depolarized muscle. A more plausible concept is that the non-specific increase in permeability which is produced by depolarizing compounds is responsible also for the entry of organic cations (see Cookson & Paton, 1969). There is evidence for an increase in the permeability to decamethonium as the concentration is increased for low values. However, the kinetics of entry of decamethonium are consistent with those of a saturable mechanism with carrier-like kinetics rather than those of a non-specific increase in permeability, and the question is still unclear. In the present experiments it has been shown that the movements of labelled decamethonium which can be measured in normal muscle can also be demonstrated in depolarized tissue, but the further significance of these results must await studies in which the pharmacological response can be estimated in conjunction with the movements of labelled agonists.

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