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The mechanism of the facilitatory action of edrophonium in cat skeletal muscle

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Summary

1. The effects of edrophonium have been observed in the transversely cut tenuissimus muscle of the cat.

2. Concentrations of edrophonium 10^{-7}M to 10^{-5}M increased the amplitude of the end-plate potential (e.p.p.) but produced no greater increase in time course than previously observed in curarized muscle.

3. When the e.p.p. and gross nerve action potential were recorded simultaneously, antidromic discharges were observed in the motor nerve concomitantly with repetitive e.p.ps in the presence of edrophonium.

4. Edrophonium produced no effect on the input resistance or equilibrium potential of the end-plate.

5. The fractional release of transmitter was significantly increased by edrophonium but there was no increase in the quantal release of transmitter in the transversely cut muscle preparation.

6. In curarized muscle edrophonium also increased the quantal release, the size of the available store of transmitter and the rate of refilling of the available store.

7. It is concluded that edrophonium facilitates transmission to skeletal muscle by inducing a repetitive antidromic discharge in the nerve, following orthodromic stimulation. The antidromic discharge propagates by axon reflex to other nerve terminals of the same motor unit producing repetitive e.p.ps. It is also suggested that edrophonium antagonizes tubocurarine by acting as a partial agonist at the motor nerve terminal.

Introduction

It has been proposed previously that edrophonium exerts an action on the motor nerve terminal in the skeletal muscle of the cat (Blaber & Bowman, 1959) producing repetitive antidromic discharges in response to an orthodromic stimulus (Blaber & Bowman, 1963a & b). It has also been shown that the amplitude of the end-plate potential (e.p.p.) was increased whilst the amplitude of the miniature end-plate potential (m.e.p.p.) was unaffected (Blaber & Christ, 1967). In the latter study it was concluded that edrophonium increased transmitter release in curarized cat skeletal muscle, since there was no change in the time course of the e.p.p. The question was raised of the mechanism of action whereby edrophonium produced repetitive muscle firing in non-curarized muscle, since it is usually assumed that

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drugs producing such repetitive firing do so by prolonging the end-plate potential beyond the refractory period of the muscle action potential (Eccles, Katz & Kuffler, 1942; Eccles & MacFarlane, 1949; Fatt & Katz, 1951).

It was suggested (Blaber & Christ, 1967) that this mechanism could still apply to edrophonium, since Eccles *et al.* (1942) had previously shown that tubocurarine would decrease the duration of the e.p.p. prolonged by the action of an anti-cholinesterase drug. Tubocurarine is also known to antagonize the presynaptic actions of edrophonium (Blaber & Bowman, 1963), and therefore it was possible that edrophonium could prolong the e.p.p. in non-curarized muscle, although no prolongation was observed in curarized muscle.

Barstad & Lilleheil (1968) introduced a transversely cut diaphragm preparation of the rat in which e.p.ps could be observed in the absence of any drug treatment, and this technique was modified by Blaber (1970) for use in the isolated tenuissimus muscle of the cat. In the present study the effects of edrophonium have been observed in this latter preparation and compared to the effects produced in curarized muscle.

Methods

The preparation of the isolated tenuissimus muscle for recording single e.p.ps and tetanic trains in curarized and cut-fibre preparations has been described previously (Blaber & Christ, 1967; Blaber, 1970). For recording single e.p.ps the nerve was stimulated at a rate of 0·77 Hz, for trains, at 200 Hz for 0.5 s each min, and during simultaneous recordings of e.p.p. and gross nerve action potential at 0·1 Hz. The gross nerve action potential was recorded across bipolar platinum electrodes, the nerve being submerged in liquid paraffin. In these experiments, approximately 6 cm of nerve was obtained by separating the tenuissimus nerve from the sciatic nerve. The nerve potentials were amplified using a Tektronix 122 pre-amplifier, displayed on a Tektronix type RM 565 Oscilloscope and recorded on 35 mm film. The e.p.ps were recorded using glass microelectrodes and amplified with a Grass P16 microelectrode D.C. amplifier. The membrane potential was monitored with a Devices oscilloscope type 3121 and Servoscribe chart recorder. The input resistance was measured by the method of Katz & Thesleff (1957) and equilibrium potential by the method of Fatt & Katz (1951) in which two micro-electrodes are used, the recording electrode being in the end-plate and the current electrode located extracellularly less than 50 μm from the recording electrode on the same muscle fibre. The recording electrodes had resistances of 10–15 MΩ and were filled with 3 M KCl; the current electrodes had resistances of 3–5 MΩ and were filled with 4 M NaCl. The method for calculating the effects of edrophonium on release, available store and refilling of the store of transmitter has been described previously (Blaber, 1970).

The drugs used were edrophonium chloride (Roche Products Ltd.) and (+)-tubocurarine chloride (Koch-Light Laboratories).

Results

Effects of edrophonium in the cut fibre preparation

In this series of experiments the amplitude and time course of twenty-five e.p.ps were measured before and after the addition of edrophonium to the solution irriga-

ting the muscle. The results from six experiments are shown in Table 1. These results are similar to those observed by Blaber & Christ (1967) in the curarized muscle, with the exceptions that, in curarized muscle, the rise time was unaffected by 10^{-6}M edrophonium and there was a large increase in e.p.p. amplitude produced by 10^{-5}M edrophonium. The amplitude of the e.p.ps is larger in the cut-fibre preparation and consequently the increase produced by edrophonium is smaller due to non-linear summation. The increase in amplitude produced by 10^{-5}M edrophonium is not significant because this concentration produced a decreased amplitude in some preparations. Decamethonium produces a similar desensitization at concentrations five to ten times lower than those producing end-plate depolarization (Blaber, 1970). The increase in time course produced by edrophonium was not more than twice and therefore is unlikely to account for the intense repetitive firing of the muscle produced by edrophonium *in vivo*.

TABLE 1. *Effects of edrophonium on end-plate potentials*

Concentration of edrophonium (M)	Amplitude (mV)	Rise-time (ms)	Half-decay (ms)
Control	13·8±2·2	0·8±0·1	1·0±0·1
10^{-7}	15·2±1·9*	0·9±0·1	0·9±0·1
10^{-6}	16·8±2·4*	1·0±0·1*	1·2±0·1*
10^{-5}	17·3±2·5	1·2±0·3*	2·0±0·3†

±S.E. * Difference statistically significant from control (P=0·05). † Difference statistically significant from control (P=0·01). n=6.

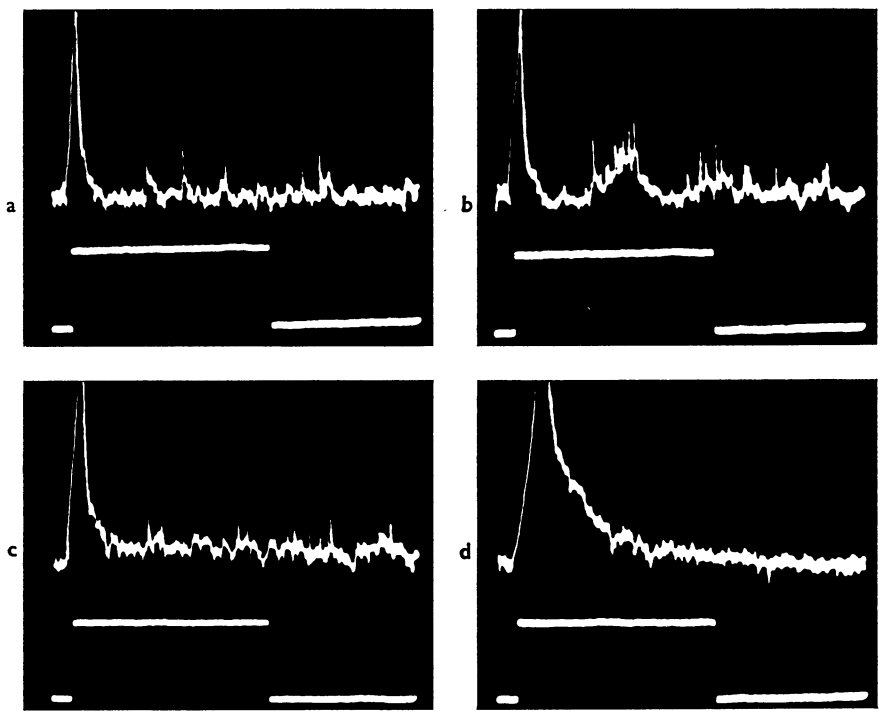

FIG. 1. The effect of cutting the muscle fibres on antidromic firing recorded in the motor nerve. (a) Control, (b) after edrophonium 10^{-7}M, (c) 1 min after cutting muscle fibres, (d) 4 h after cutting muscle fibres. Calibration 20 μV, 10 ms. Figure retouched.

Effects of edrophonium on end-plate potential and gross nerve action potential recorded simultaneously

Blaber & Bowman (1963a) observed that muscle repetitive firing produced by edrophonium was accompanied by antidromic discharges in the motor nerve. Antidromic discharges were observed in the motor nerve in the presence of 10^{-7}M edrophonium in the isolated tenuissimus nerve-muscle preparation before the muscle fibres were cut. Cutting the muscle fibres abolished the antidromic discharges for the duration of the experiment (Fig. 1). In the cut fibre preparation set-up to record e.p.ps no antidromic firing was observed in response to an orthodromic stimulus in the presence of edrophonium in concentrations of 10^{-7}M to 10^{-5}M.

Randić & Straughan (1964) observed that cutting the muscle fibres in the rat isolated phrenic nerve-diaphragm preparation caused the immediate abolition of antidromic firing in the presence of neostigmine. This was prevented by lowering the potassium and magnesium content of the Ringer solution.

Lowering the potassium and magnesium content of the Ringer solution irrigating the isolated tenuissimus muscle to 50% produced no effect on the resting potential or e.p.p. In twelve experiments in which the modified Ringer solution was used with the cut-fibre preparation, antidromic firing was observed in the motor nerve in the presence of 10^{-7}M edrophonium in five preparations. In three of these five preparations repetitive end-plate potentials were observed (Fig. 2). In these experiments there was also no change in the time course of the e.p.ps in the presence of edrophonium.

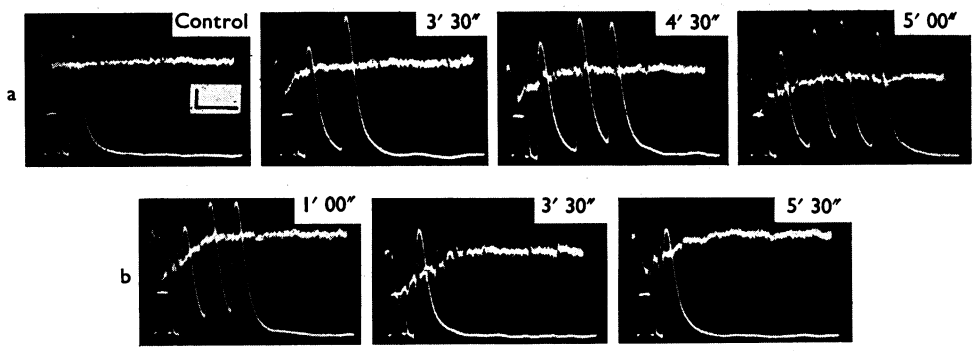

FIG. 2. Effect of edrophonium (10^{-7}M) on e.p.ps (lower trace) and motor nerve potentials (upper trace) recorded in the cut-fibre preparation in low potassium Ringer solution. (a) Time denotes period after irrigation with 10^{-7}M edrophonium. (b) Time denotes period after washing with Ringer solution. Calibration 20 μV, 5 ms for nerve recording, square wave recorded on e.p.p. sweep is 5 mV and 1 ms duration. Figure retouched.

TABLE 2. *Effect of edrophonium on equilibrium potential and input resistance*

	Equilibrium potential (mV)	Input resistance ($\Omega \times 10^3$)
Control	$-12{\cdot}65 \pm 2{\cdot}4$	$7{\cdot}72 \pm 0{\cdot}9$
Edrophonium 10^{-7} M (5 min)	$-12{\cdot}49 \pm 3{\cdot}5$	$7{\cdot}30 \pm 0{\cdot}7$
(10 min)	$-13{\cdot}73 \pm 3{\cdot}8$	$7{\cdot}10 \pm 0{\cdot}9$
(15 min)	$-13{\cdot}95 \pm 3{\cdot}6$	$7{\cdot}49 \pm 1{\cdot}0$

($\pm$S.E. $n=5$). Equilibrium potential ($n=27$)$=12{\cdot}78 \pm 1{\cdot}31$ mV. Input resistance ($n=30$)$=7{\cdot}13 \pm 0{\cdot}35$ KΩ.

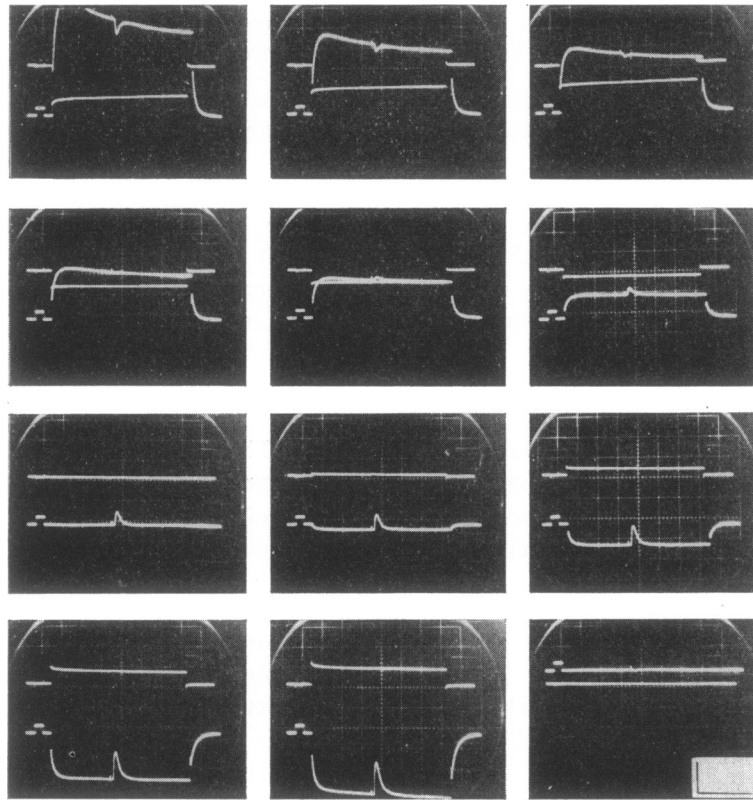

FIG. 3. Recording of equilibrium potential and input resistance. First panel, row two, shows recording at the equilibrium potential. First panel, row three, shows a recording at the resting potential. Last panel, row four, shows a recording with the recording electrode withdrawn from the cell. Calibration 4 μA, 10 ms. Square wave on e.p.p. sweep 5 mV, 2 ms.

TABLE 3. *Effect of edrophonium in the cut fibre muscle preparation*

	Quantal content of first e.p.p. (quanta)	Available store (quanta)	Fractional release	Quantum size (mV)	Rate of refilling of the available store (quanta/ms)
Control	230±39·9	3,071±635	0·08±0·009	0·12±0·03	19·1±3·7
Edrophonium	247±36·3	2,857±620	0·11±0·02*	0·13±0·04	17·7±3·4

$n=6$. * Difference statistically significant $P=0{\cdot}05$.

TABLE 4. *Effect of edrophonium in tubocurarine blocked muscle preparation*

	Quantal content of first e.p.p. (quanta)	Available store (quanta)	Fractional release	Quantum size (mV)	Rate of refilling of the available store (quanta/ms)
Control (Tubocurarine $3{\cdot}6\times10^{-6}$ M)	333±14·3	1,620±61·7	0·21±0·017	0·0098±0·002	14·9±0·21
Edrophonium (10^{-7} M)	412±43·2*	1,879±128·8*	0·22±0·015	0·0093±0·001	16·8±0·56*

$n=6$. * Difference statistically significant $P=0{\cdot}05$.

Effect of edrophonium on input resistance and equilibrium potential

The input resistance was measured by the ratio of voltage/current using current pulses of varying intensities. From Table 2 it may be seen that edrophonium 10^{-7}M produced no significant change in input resistance.

Figure 3 shows a typical experiment measuring equilibrium potential; the point at which the end-plate reversed was calculated from the linear regression, the regression coefficient in all experiments being greater than 0·997. The results are given in Table 2 and show that edrophonium 10^{-7}M produced no significant change in the equilibrium potential.

Effect of edrophonium on transmitter storage and release

Table 3 shows that edrophonium 10^{-7}M produced no change in quantum size which agrees with the observation of Blaber & Christ (1967) which showed no increase in the amplitude of m.e.p.ps. The only significant change produced in the present experiments was an increase in fractional release. There was a small but non-significant decrease in the refilling of the store and the size of the available store which may have offset the increase in fractional release, and which may account for there being no significant change in the quantal content of the first e.p.p.

In contrast to the effects in the cut fibre preparation, edrophonium (10^{-7}M) produced an increase in the quantal content of the first e.p.p. in curarized muscle. This was due to a significant increase in the rate of refilling of the available store producing an increased size of the available store.

Discussion

Comparison of the results of the present experiments, measuring the time course of e.p.ps in the cut-fibre preparation, with the results of Blaber & Christ (1967) in curarized muscle, shows that edrophonium produced no greater increase in time course in the cut fibre preparation than it did in curarized muscle. Therefore it is not possible, as speculated by Blaber & Christ, that the repetitive firing observed in *in vivo* muscle is due to prolongation of the e.p.p., unless the concentration of edrophonium *in vivo* is at least 10^{-5}M. The concentration of edrophonium at the end-plate which produces repetitive muscle firing *in vivo* is unknown; however, Blaber & Bowman (1959) showed that repetitive muscle firing was produced by a close-arterial dose one thousand times less than the dose that produced a muscle contraction by direct depolarization. Blaber & Christ (1967) observed that it required a concentration of 10^{-4}M edrophonium to depolarize the end-plate of the isolated tenuissimus muscle. These observations therefore suggest that a concentration of edrophonium which will produce repetitive muscle firing is of the order of 10^{-7}M. Blaber & Bowman (1963a) observed that the repetitive muscle firing produced by edrophonium was accompanied by antidromic discharges in the motor nerve; the present experiments have confirmed that a concentration of 10^{-7}M edrophonium will produce repetitive antidromic discharges in the nerve in the isolated nerve—uncut muscle preparation.

Cutting the muscle fibres prevents the antidromic firing in the motor nerve although orthodromic conduction still continues, since end-plate potentials can be observed following nerve stimulation. It is unlikely that injury currents from the

cut fibres, or potassium leakage, produced blockade by depolarizing the motor nerve terminal, since m.e.p.ps are no more frequent in the cut-fibre preparation than in uncut fibres (unpublished observation). However, the presence of potassium may, in some way, have prevented the generator potential for the antidromic firing, since antidromic firing could be observed in a proportion of the preparations irrigated with a low potassium Ringer solution.

In these preparations repetitive e.p.ps were still observed in the absence of any prolongation of time course of the e.p.ps. Werner (1960), Hubbard & Schmidt (1961, 1963), Blaber & Bowman (1963a) and Blaber & Goode (1968) have concluded that the antidromic firing produced by facilitatory drugs is a result of the augmentation and prolongation of the negative after-potential of the unmyelinated nerve terminal which repetitively stimulates the first node. The discharge generated in such a way would propagate not only into the ventral root but also, by an axon reflex, into the other nerve terminals of the same motor unit. Riker (1966) has shown that the nerve and muscle potentials occur in a one to one ratio when recordings are made from a single nerve fibre and motor unit.

The repetitive muscle firing induced in skeletal muscle by edrophonium is therefore due to repetitive e.p.ps produced as a result of repetitive nerve firing, and not due to prolongation of the e.p.p. Other facilitatory drugs also produce repetitive muscle firing associated with antidromic nerve impulses, an effect which cannot be correlated with acetylcholinesterase inhibition (Riker, Roberts, Standaert & Fujimori, 1957; Blaber & Bowman, 1963a). The evidence that any anticholinesterase produces repetitive firing in muscle by prolongation of the e.p.p. is not compelling, being based on the work of Eccles *et al.* (1943), Eccles & MacFarlane (1949) and Fatt & Katz (1951). The work of Eccles and co-workers was performed using external platinum electrodes recording grossly from the muscle, and repetitive e.p.ps, not in synchrony, would be recorded as a prolonged negativity. The experiment of Fatt & Katz (1951) was performed with a muscle blocked by reducing the sodium concentration in the Ringer solution. Prolongation of more than 2–3 times of the e.p.p. by anticholinesterases cannot be shown in muscles blocked by any other means (Fatt & Katz, 1951; Boyd & Martin, 1956; Blaber & Christ, 1967). This prolongation occurs usually at high concentrations of the anticholinesterases and even if these concentrations were to occur at the end-plate *in vivo*, a prolongation of the e.p.p. by 2–3 times would not account for the intense repetitive muscle firing which can be observed. It is, therefore, possible that all anticholinesterases produce repetitive firing in skeletal muscle by the same mechanism as edrophonium.

Tubocurarine prevents the antidromic firing in the motor nerve produced by edrophonium (Blaber & Bowman, 1963a) and antagonism of tubocurarine is due to the increase in e.p.p. amplitude. This has been attributed to increased transmitter release (Blaber & Christ, 1967). However, the possibility exists that edrophonium may change the membrane constants of the end-plate. Edrophonium did not change the input resistance or equilibrium potential in the cut-fibre preparation. This supports the conclusions of Blaber & Bowman (1959) that edrophonium has no direct sensitizing effect on the motor end-plate. The equilibrium potential is close to that reported for frog sartorius muscle (Takeuchi & Takeuchi, 1959). Cutting the muscle fibres reduced the input resistance one hundred fold over that reported by Boyd & Martin (1959) for cat tenuissimus muscle.

Measurement of transmitter release has shown that there was no increase in quantal content in the presence of 10^{-7}M edrophonium in the cut-fibre preparations, and no change in e.p.p. amplitude, in spite of the increase in amplitude observed when single e.p.ps were generated. This may be a consequence of the method of stimulation. In the present experiments it was observed that, when the stimulation was changed from single stimuli to tetanic stimuli, the amplitude of the first e.p.p. of the train was greater than that recorded using single stimuli, and it was necessary to allow the e.p.ps to equilibrate at the new level before the control readings were taken. It has been shown by Standaert (1963) that tetanic stimulation in the soleus muscle of the cat produced similar antidromic discharges to those produced by facilitatory drugs, and it may be that in the isolated tenuissimus the effect of the tetanus masks or reduces the effects of low concentrations of edrophonium.

The effects of higher concentrations of edrophonium on quantal release could not be calculated since the prolongation of time course would distort the recording and possibly prevent accurate measurement of e.p.p. amplitude. Nevertheless, it was observed that there was a significant increase in the fractional release and a non-significant decrease in the available store and the rate of refilling of the store. Assuming that these effects would become significant at higher concentrations, the presynaptic effects of edrophonium are similar to those reported for tubocurarine (Hubbard, Wilson & Miyamoto, 1969 ; Jacobs & Blaber, 1971), although edrophonium is considerably less potent. There was no change in quantum size, showing that edrophonium did not exert any postsynaptic effects at a concentration of 10^{-7}M.

In contrast to the effects in the cut-fibre preparation edrophonium (10^{-7}M) was found to increase transmitter release in the curarized preparation. In agreement with previous studies (Hubbard *et al.*, 1969 ; Jacobs & Blaber, 1971), tubocurarine was found to increase the fractional release and to decrease the size of the available store and the rate of refilling of the store. However, the blocking action of tubocurarine was entirely post-synaptic since there was no change in quantal content and a profound decrease in quantal size. Edrophonium (10^{-7}M) produced no further increase in the fractional release but produced a significant increase in the size of the available store and the rate of refilling of the store.

These results may be explained if edrophonium and tubocurarine combine with the same presynaptic receptor and edrophonium produces antagonism of the tubocurarine by acting as a partial agonist. Previous workers have shown that facilitatory drugs are ineffective against high concentrations of tubocurarine (Kuperman & Okamoto, 1964 ; Blaber & Christ, 1967). It is known that the rate of transmitter synthesis and mobilization can be changed very rapidly to maintain the available store during high frequency stimulation ; whereas the increase in fractional release produced by high frequency stimulation reverses more slowly (Elmquist & Quastel, 1965). Edrophonium may reverse the effect of tubocurarine on the store more rapidly than that on the fractional release thus producing an increase in the release of transmitter.

Edrophonium has been shown in *in vitro* experiments to be a rapidly reversible anticholinesterase (Wilson, 1955). The present experiments do not exclude the possibility that edrophonium inhibits the end-plate acetylcholinesterase. The high concentration of acetylcholine released from the nerve terminal into the junction would rapidly reverse the acetylcholinesterase inhibition and therefore only the

rising phase of the e.p.p. would be affected and not the time-course. However, this effect is probably of less significance than the presynaptic effect, since edrophonium produces no effect on the amplitude of m.e.p.ps in concentrations up to 10^{-5}M (Blaber & Christ, 1967).

The effects of edrophonium in non-curarized and curarized muscle are probably due to occupation of the same receptors. Tubocurarine in low concentrations is also known to produce an increase in transmitter release (Blaber, 1970) and to produce repetitive firing of the muscle fibres (Jones & Laity, 1965). It is only as the concentration of tubocurarine is increased that the increased fractional release is offset by a decrease in the available store.

An increase in the amplitude and duration of the negative after potential would explain not only the repetitive firing but also the increase in transmitter release, since Katz & Miledi (1967, 1971) have shown that an increase in the duration of the nerve terminal spike increases transmitter release. This mechanism could also explain the increase in time course of the e.p.p. at higher concentrations without affecting the time course of m.e.p.ps, since Katz & Miledi (1967) have additionally shown that the time course of transmitter release was affected by the duration of the nerve terminal spike. An attempt to observe the effects of edrophonium on the nerve terminal potential in the cut-fibre preparation was without success, because the focally recorded nerve terminal potential was almost completely obscured by the end-plate potential.

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