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Neuromuscular actions of edrophonium in the lateral segmental tail muscle of the rat *in vivo*

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The actions of edrophonium on neuromuscular transmission *in vivo* have been studied using the lateral segmental tail muscles of the rat. The drug produced an increase in amplitude of the miniature end-plate potentials (m.e.p.p.s) without an effect on their frequency and increased the amplitude of the end-plate potentials (e.p.p.s) without increasing quantal release of transmitter. It is concluded that the anticholinesterase action of this drug *in vivo* facilitates neuromuscular transmission, which confirms many of the findings from *in vitro* studies.

The mode of action of edrophonium on neuromuscular transmission is controversial. Several workers believe that its effects can be explained by its anticholinesterase action (Smith, Cohen & others, 1952; Nastuk & Alexander, 1954; Katz & Thesleff, 1957; Ferry & Marshall, 1971a). However, Blaber & Christ (1967) reported that edrophonium had both pre-junctional and post-junctional effects depending on the drug concentration, and concluded that its primary action was to increase the quantal release of acetylcholine from the motor nerve terminal.

Since previous electrophysiological studies of the actions of edrophonium on neuromuscular transmission have been confined to isolated nerve-muscle preparations, it was decided to study its actions *in vivo* to ascertain if they followed the same pattern as those *in vitro*.

METHODS

Male Wistar Albino rats, 150–250 g, were anaesthetized by an intraperitoneal injection of urethane (1·5 g kg^{-1}), a cannula was inserted into the trachea for artificial respiration when tubocurarine was used and injections were given through a cannula in a jugular vein. Steg's (1964) *in situ* nerve-muscle preparation of the segmental tail muscle was used, with the modifications of Roberts & Thesleff (1965) and the temperature of the preparation was maintained between 30–35°. The lateral nerve was stimulated by a Grass S8 stimulator and stimulus isolation unit SIU 4678, connected to two fine silver wires on the exposed surface of the nerve.

The usual techniques for intracellular recording were used (Fatt & Katz, 1951), with glass capillary microelectrodes filled with 3 M KCl and resistances 5–10 M ohms connected through a cathode follower to a Tektronix Type 502A dual beam oscilloscope. Membrane potentials, m.e.p.p.s and e.p.p.s were recorded both by photographing oscilloscope traces on 35 mm film and on paper using an ink-writer, Mingograf 34. Simultaneous dc recording was used at low amplification for membrane potentials and ac recording at higher amplification for m.e.p.p.s and e.p.p.s. The

frequency response of the recording system was flat from 5–500 Hz (−3db at 2 Hz and 700 Hz). One intravenous injection of atropine sulphate (1 mg kg^{-1}), administered at the start of the experiments before edrophonium injections was usually sufficient to prevent slowing of the heart.

Edrophonium was used as the chloride and doses quoted are in terms of this salt.

RESULTS

The effect of edrophonium on m.e.p.p.s

The electrode was inserted at an end-plate and when the membrane potential became stable at least 100 m.e.p.p.s were recorded as controls. With the electrode still in position edrophonium was injected during 1 min and m.e.p.p.s were recorded, sometimes both during and after the injection and at other times only after the injection was completed. The drug was injected no more than 3 times in the same rat at intervals of ½–1 h. In many preparations recording could be made for at least 5 min without depolarization and the time course of the drug effect was followed.

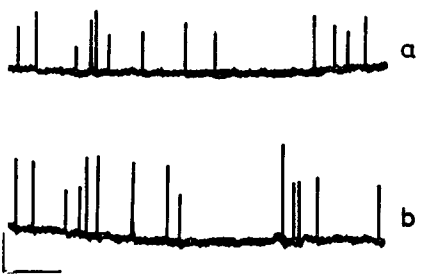

FIG. 1. The effect of edrophonium on m.e.p.p. amplitude. Intracellular recording from one cell (membrane potential 72 mV) of the lateral segmental tail muscles of the rat. (a) Control conditions; (b) immediately after edrophonium (50 μg, i.v. in 1 min). Calibrations: 1 mV and 1 s.

Edrophonium increased the amplitude of m.e.p.p.s which is illustrated in Fig. 1 and in histogram form in Fig. 2. There was no effect on the mean number of m.e.p.p.s recorded in 2 s periods (Fig. 3) and only slight reduction in the resting membrane potential (Table 1). The increase in amplitude of m.e.p.p.s was short lived and control values returned 4–6 min after the injection of the drug (Fig. 4). Table 1 shows the results from 6 experiments in which the mean % increases in amplitude of m.e.p.p.s were calculated after an injection of edrophonium, using Student's *t*-test

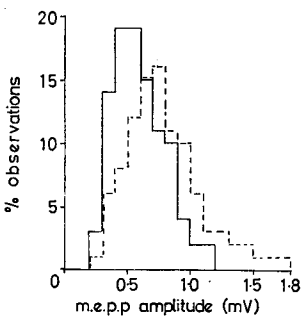

FIG. 2. Histograms of m.e.p.p. amplitudes. Intracellular recording from one cell (membrane potential 72 mV) of the lateral segmental tail muscles of the rat. Control conditions (full line) and after edrophonium (50 μg, i.v.), (broken line). Each histogram constructed from 100 m.e.p.p.s.

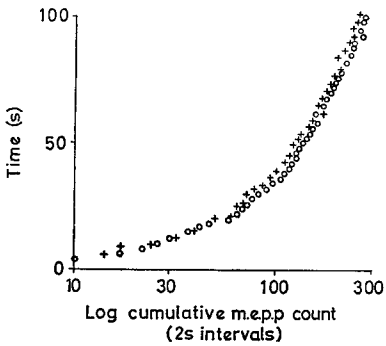

FIG. 3. Graph of log cumulative frequency of m.e.p.p.s (at successive 2 s intervals). Intracellular recording from one cell (membrane potential 78 mV) of the lateral segmental tail muscles of the rat. Control conditions (○) and after edrophonium (50 μg, i.v.), (×).

Table 1. *Effects of edrophonium* (50 μg, i.v.) *on amplitude of m.e.p.p.s and membrane potential.* Results from 3 end-plates, in each of 6 rats.

Experiment number	End-plate number	Mean % increase in amplitude of me.p.p.s* (with s.d.)	Membrane potential (mV) Before edrophonium	After edrophonium
1	1	34 (10)	65	63
	2	38 (14)	65	64
	3	26 (10)	65	64
2	1	20 (7)	74	71
	2	21 (7)	72	70
	3	16 (8)	73	70
3	1	21 (8)	83	80
	2	22 (8)	74	72
	3	27 (9)	84	82
4	1	37 (9)	70	68
	2	28 (7)	65	63
	3	16 (8)	73	70
5	1	36 (16)	70	67
	2	27 (10)	74	72
	3	16 (5)	70	68
6	1	21 (7)	80	77
	2	29 (9)	78	76
	3	16 (6)	78	76

*The results in this column represent statistically significant increases in m.e.p.p. amplitude ($P = 0{\cdot}01$).

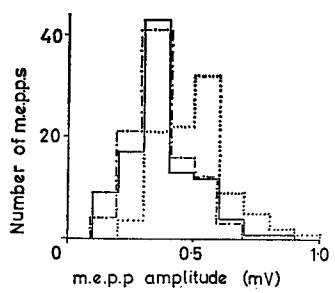

FIG. 4. Time course of the effect of edrophonium on m.e.p.p. amplitude. Intracellular recording from one cell (membrane potential 74 mV) of the lateral segmental tail muscles of the rat. Control conditions (—), 1 min after edrophonium (50 μg, i.v.), (. . .) and 4 min later (— · —).

as a test for significance ($P = 0{\cdot}01$). 100 m.e.p.p.s were measured before and after edrophonium in each experiment. The minimum dose which increased the amplitude of m.e.p.p.s was about 10 μg and increasing doses up to 50 μg further increased amplitude but did not alter the frequency of m.e.p.p.s.

The effect of edrophonium on e.p.p.s

Rats were atropinized, muscle twitches in response to nerve stimulation at 1 Hz abolished by intravenous injection of tubocurarine and at least 100 e.p.p.s recorded. Edrophonium was then injected intravenously and a similar number of e.p.p.s recorded from the same end-plate. The drug produced a marked increase in the amplitude of e.p.p.s (Fig. 5). Table 2 shows the mean % increase in amplitude of e.p.p.s recorded from a single end-plate in each of 6 rats. These increases in amplitude were much greater than the drug-induced increases in amplitude of m.e.p.p.s (Fig. 1 and Table 1), and also slight increases in amplitudes of e.p.p.s were produced by doses of edrophonium < 10 μg which did not affect the amplitude of m.e.p.p.s.

The effect of edrophonium on mean acetylcholine quantum content of e.p.p.s

The mean quantum contents of at least 100 e.p.p.s were determined before and after injection of edrophonium, by analysis of the variance in their amplitudes when

Table 2. *Effect of edrophonium* (50 μg i.v.) *on amplitude of e.p.p.s.* Results from 1 end-plate, in each of 6 rats.

Experiment number	Mean % increase in amplitude of e.p.p.s (with s.d.)	Resting membrane potential (mV)
1	72 (9)	80
2	63 (7)	74
3	75 (11)	82
4	83 (13)	78
5	59 (8)	71
6	64 (8)	72

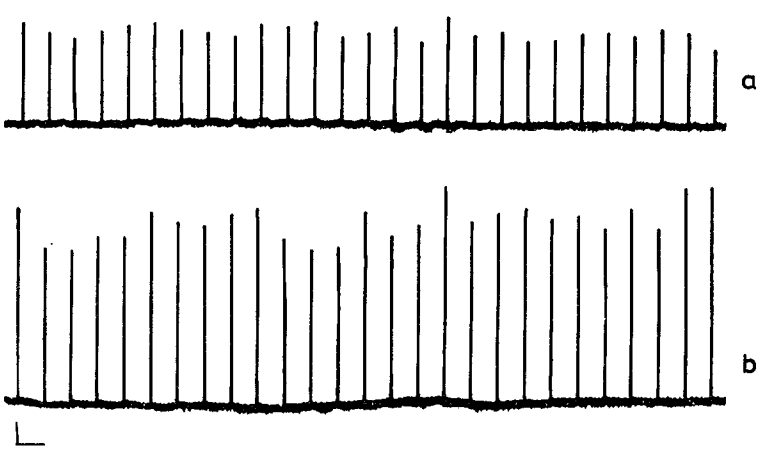

FIG. 5. The effect of edrophonium on e.p.p. amplitude. Intracellular recording from one cell (membrane potential 80 mV) of the lateral segmental tail muscles in the curarized rat, with nerve stimulation at 1 Hz. (a) Control conditions; (b) after edrophonium (50 μg, i.v.). Calibrations 1 mV and 1 s.

recorded from a single muscle fibre with nerve stimulation rate of 1 Hz. Two groups of rats (12 rats/group) were used. In the first group the control value (with s.d.) for the mean acetylcholine quantum content was 144 (99) and after edrophonium (25 μg, i.v.), 148 (71). The control value in the second group was 113 (64) and after edrophonium (50 μg, i.v.) 127 (45). There was no significant difference between control values and those after edrophonium injection ($P = 0{\cdot}01$).

DISCUSSION

The edrophonium-induced increase in the amplitude of m.e.p.p.s was of rapid onset (occurring within the first 30 s of a 1 min period of i.v. injection) and of similar duration (approximately 5 min) to that of the well known transient augmentation of indirectly elicited muscle twitches following intravenous administration of the drug in *in vivo* preparations. The increase in amplitude of m.e.p.p.s probably reflects a rapid but short lived inhibition of cholinesterase, although these studies do not provide information about any effect which the drug may have on acetylcholine quantum size. The greater increase in amplitude of e.p.p.s than m.e.p.p.s after edrophonium and the lower minimum effective dose needed to increase the amplitude of e.p.p.s may be due to greater importance of cholinesterase in removing acetylcholine released in response to the nerve impulse, than when much smaller amounts are released spontaneously. Ferry & Marshall (1971b) concluded from work with the isolated phrenic nerve-diaphragm preparation of the rat, in which time courses of e.p.p.s were measured, that when larger amounts of transmitter were released inhibition of cholinesterase activity led to marked prolongation of acetylcholine action. When smaller amounts are released spontaneously, diffusion of acetylcholine from the synapse may assume more importance in terminating its action. The slight potentiation of e.p.p.s. with doses of edrophonium < 10 μg which did not affect the amplitude of m.e.p.p.s could signify a pre-junctional action of the drug resulting in increased quantal release of acetylcholine from the motor nerve terminal. However, there was no increase in frequency of m.e.p.p.s throughout the dose range used in these experiments and moreover with larger doses, which produced marked increases in e.p.p. amplitude, there were no significant changes in mean acetylcholine quantum content of e.p.p.s.

It seems likely therefore that the effects of the drug reported here are explainable by inhibition of cholinesterase, by changes in muscle membrane resistance or by changes in unitary action of molecules of acetylcholine on the muscle cell.

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REFERENCES

BLABER, L. C. & CHRIST, D. D. (1967). *Int. J. Neuropharmac.*, **6**, 473–484.

FATT, P. & KATZ, B. (1951). *J. Physiol. Lond.*, **115**, 320–370.

FERRY, C. B. & MARSHALL, A. R. (1971a). *Ibid.*, **218**, 66P.

FERRY, C. B. & MARSHALL, A. R. (1971b). *Ibid.*, **219**, 33P.

KATZ, B. & THESLEFF, S. (1957). *Br. J. Pharmac. Chemother.*, **12**, 260–264.

NASTUK, W. L. & ALEXANDER, J. T. (1954). *J. Pharmac. exp. Ther.*, **111**, 302–328.

ROBERTS, D. V. & THESLEFF, S. (1965). *Acta anaesth. scand.*, **9**, 165–172.

SMITH, C. M., COHEN, H. L., PELIKAN, E. W. & UNNA, K. R. (1952). *J. Pharmac. exp. Ther.*, **105**, 391–399.

STEG, G. (1964). *Acta physiol. scand.*, **61**, *Suppl.* 225.