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Examination of the mechanisms involved in tetanic fade produced by vecuronium and related analogues in the rat diaphragm

A.J. Gibb[1] & I.G. Marshall

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, Scotland

1 The effects of vecuronium (Org NC45), Org 7678 and Org 7684 were examined on twitches and tetani recorded from rat isolated diaphragms

2 Org 7678 and Org 7684 exhibited approximately one tenth of the neuromuscular blocking potency of vecuronium.

3 At concentrations producing equivalent amounts of twitch block, Org 7684 produced significantly less tetanic fade than did vecuronium or Org 7678.

4 In cut muscles both vecuronium and Org 7684 reduced the endplate current (e.p.c.) amplitude ($I_{e.p.c.}$), reduced e.p.c. decay time constant ($\tau_{e.p.c.}$), and increased the e.p.c. train rundown.

5 The effects of vecuronium were not voltage-dependent and vecuronium did not change τ_{noise}.

6 The effect of Org 7684 on $I_{e.p.c.}$ and $\tau_{e.p.c.}$ became greater with hyperpolarization, but the effect on e.p.c. train rundown was not voltage-dependent.

7 It is concluded that both vecuronium and Org 7684 produce e.p.c. train rundown and tetanic fade by a prejunctional mechanism. However, whereas postjunctionally vecuronium blocks only the acetylcholine receptor, Org 7684 blocks both the receptor and its associated ion channel.

Introduction

Tetanic fade is characteristic of the effects of many nicotinic antagonists at the neuromuscular junction. There is now widespread agreement that the major effect of nicotinic antagonists is a competitive block of the postjunctional cholinoceptor (AChR) (Jenkinson, 1960; Colquhoun *et al.*, 1979). However, there is still considerable debate as to the importance of other effects, exerted either pre- or postjunctionally, which may contribute to the neuromuscular blocking action of a drug (Standaert, 1982; Dreyer, 1982) particularly at rates of stimulation sufficient to produce fused tetanic responses.

Evidence has accumulated for two main sites of action in addition to postjunctional AChR antagonism. These are: (1) a non-competitive block of the open AChR-activated ion channel (see Lambert *et al.*, 1983 for review), and (2) a prejunctional effect which results in a fall-off in transmitter release during repetitive stimulation (see Bowman, 1980 for review). Studies have centred on tubocurarine as the drug of choice (Colquhoun *et al.*, 1979; Gibb & Marshall, 1984) although in addition, pancuronium (Katz & Miledi, 1978), gallamine (Colquhoun & Sheridan, 1981) and some steroidal pancuronium analogues (Durant & Horn, 1984) have been tested for ion channel blocking activity at the frog neuromuscular junction.

In this study the recently introduced steroidal muscle relaxant vecuronium, a monoquaternary analogue of pancuronium, and three of its 17-desoxy analogues, Org 7684 (17-desoxy, 16N-allyl vecuronium bromide), Org 7678 (3-hydroxy, 17-desoxy, 16N-allyl vecuronium bromide) and Org 8764 (3,17-didesoxy vecuronium bromide) (Figure 1) have been tested. In experiments recording muscle tension, Org 7684 was found to produce significantly less tetanic fade than the other drugs studied and therefore, Org 7684 was chosen for a detailed electrophysiological study in conjunction with studies on vecuronium. These studies demonstrate that both vecuronium and Org 7684 produce tetanic fade by a prejunctional effect. However, postjunctionally vecuronium blocks only the AChR whereas Org 7684 also blocks the

[1]Present address: M.R.C. Receptor Mechanisms Group, Department of Pharmacology, University College London, Gower Street, London WC1E 6BT.

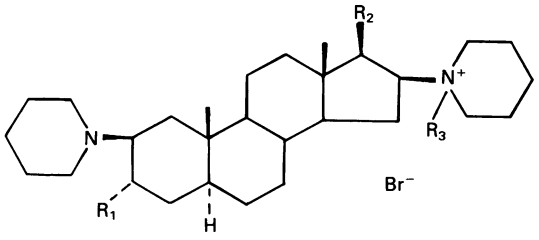

Figure 1 Chemical structures of vecuronium and three of its analogues. For vecuronium, R_1, R_2 = O-acetyl, R_3 = methyl. For Org 7678, R_1 = OH, R_2 = H, R_3 = allyl. For Org 7684, R_1 = O-acetyl, R_2 = H, R_3 = allyl. For Org 8764, R_1, R_2 = H, R_3 = methyl.

AChR-activated ion channel. Although channel block is important in the reduction of endplate current (e.p.c.) amplitude produced by Org 7684 it does not seem to contribute to e.p.c. train rundown. Thus, like vecuronium, Org 7684 seems to produce e.p.c. train rundown by a prejunctional effect.

Methods

Tension recording

Single twitches, trains of four twitches and tetani were recorded from hemidiaphragms isolated from male Sprague-Dawley rats (200–300 g), mounted at 32°C in Krebs solution of the following composition (mM): NaCl 118, KCl 5, $CaCl_2$ 2.5, $NaHCO_3$ 30, KH_2PO_4 1, $MgSO_4$ 1, glucose 11, and of pH 7.4 when aspirated with 95% and 5% CO_2. The phrenic nerve was stimulated with rectangular pulses of 0.2 ms duration and of a voltage greater than twice that required to produce a maximal twitch. Single twitches were elicited at 0.1 Hz, trains of four twitches at 2 Hz and tetani at 50 Hz for 1.9 s. Tension responses were recorded on a chart recorder via Grass FTO3C or FT10C semi-isometric force displacement tranducers.

Electrophysiological recordings

Endplate currents (e.p.cs), elicited by stimulation of the phrenic nerve, were recorded from voltage clamped endplate regions of cut rat diaphragm muscle fibres maintained at room temperature. Muscle contraction in response to nerve stimulation was prevented by cutting the muscle fibres as recently described (Gibb & Marshall, 1984). Endplates were voltage-clamped using a two-microelectrode voltage clamp system. Clamp gain was adjusted so that the membrane potential changed by less than 1% of the driving force (holding potential − reversal potential) during e.p.cs.

E.p.c.s. were elicited by rectangular pulses of 0.05 ms duration at 0.5 Hz when recording single e.p.cs. Trains of e.p.cs consisted of 20 e.p.cs elicited at 50 Hz. Ionophoretically-evoked e.p.cs with rise times of 2–3 ms were evoked by applying ACh to the endplate from ionophoretic microelectrodes of 30–60 MΩ resistance when filled with 2 M ACh. A Grass S88 stimulator supplied rectangular pulses of 10 μs duration and between 10 and 150 V amplitude to the ionophoretic electode via an isolation device (Grass SIU5 isolation unit and W.P. Instruments model LBB-1 breakaway unit). Endplate current noise was induced ionophoretically by applying ACh to the endplate for 5–10 s periods. All e.p.cs and noise were recorded on FM tape (Racal 4DS, bandwidth d.c. − 5kHz.) for subsequent computer analysis.

Endplate current analysis

E.p.cs were digitized and captured with 1 ms of pre-rising phase baseline by a laboratory minicomputer system (DEC PDP 11/23 and Cambridge Electronic Design 502 interface unit). Single e.p.cs were digitized at 25 kHz. Trains of e.p.cs and ionophoretically-evoked e.p.cs were digitized at 10 kHz. Computer analysis provided peak amplitude, rise-time and time constant of decay ($\tau_{e.p.c.}$) of e.p.cs.

ACh-induced noise was analysed as recently described (Gibb & Marshall, 1984). Briefly, 250 ms segments of current signal were digitized at 2 kHz after filtering (bandpass, 2 Hz–5 kHz, 34dB/octave roll-off, Butterworth characterisic). The mean amplitude of the ACh-induced current was measured before high-pass filtering. Spectra were calculated from successive 512 point data records after rejecting records containing miniature endplate currents (m.e.p.cs) or obvious artifacts. Spectra were calculated at 4 Hz resolution and the spectral density was averaged around 36 frequencies exponentially distributed between 4 Hz and 1 kHz. Net power spectra were obtained from the difference of the averaged spectra obtained from noise records recorded in the absence and in the presence of ACh. Spectra were fitted by non-linear least squares between 8 and 800 Hz with a single-sided Lorentzian function of the form

$$S(f) = \frac{S(O)}{1 + (2\pi f \tau_{noise})^2}$$

where S(f) is the power spectral density at frequency f, S(O) the d.c. power spectral density and $\tau_{noise} = (2\pi f_c)^{-1}$ where f_c is the spectral half power frequency. Single channel conductance (γ) was calculated from the relationship $\gamma = S(O)/4I(V_m - E_o)\tau_{noise}$ where I is the mean ACh-induced current, V_m is the holding potential and E_o and e.p.c. reversal potential. E_o was assumed to be −8mV in these experiments (Gibb & Marshall, 1984).

Drugs

Vecuronium (Organon), Org 7684, Org 7678 and Org 8764 were kindly supplied by Dr D.S. Savage (Organon Scientific Development Group, Newhouse, Scotland). Stock solutions of these monoquaternary compounds were made up in 10 mM citric acid of pH 4 for stability reasons. Neostigmine methylsulphate and acetylcholine chloride was obtained from Sigma.

Results

Twitches, trains of four twitches and tetanus experiments

Each drug was allowed to remain in contact with the preparation until submaximal neuromuscular block had reached a steady state. Typically, this occurred in 20–40 min. Representative records of the effect of Org 7684 (6.0×10^{-5} M) and Org 8764 (5×10^{-5} M) are shown in Figure 2. At these concentrations the most marked effect of each drug is the production of tetanic fade and train of four fade whereas single twitches are little affected. At the conclusion of some experiments neostigmine (3.3×10^{-7} M) was added to the bath in the continued presence of the blocker and allowed to remain in contact with the tissue for one hour. In the presence of vecuronium this always produced at least a partial reversal of tetanic fade. However, in the presence of Org 7684 and Org 7678, tetanic fade was increased by neostigmine. Higher concentrations of each of the drugs produced a reduction of single twitch tension. As with the tetani, twitch tension reduction produced by vecuronium was antagonized by neostigmine, but that produced by Org 7678 or Org 7684 was not reversed.

Concentration-effect relationships for the effects of vecuronium, Org 7684 and 7678 are shown in Figure 3. The most obvious differences between the drugs are that vecuronium is 10–12 times more potent than Org 7684 or Org 7678 and that while Org 7684 and Org 7678 are of similar potency, Org 7684 produces substantially less tetanic fade than either Org 7678 or vecuronium relative to twitch blocking potency. ED_{50} values for tension parameters are given in the legend to Figure 3. The twitch block to tetanic fade ratios reflect

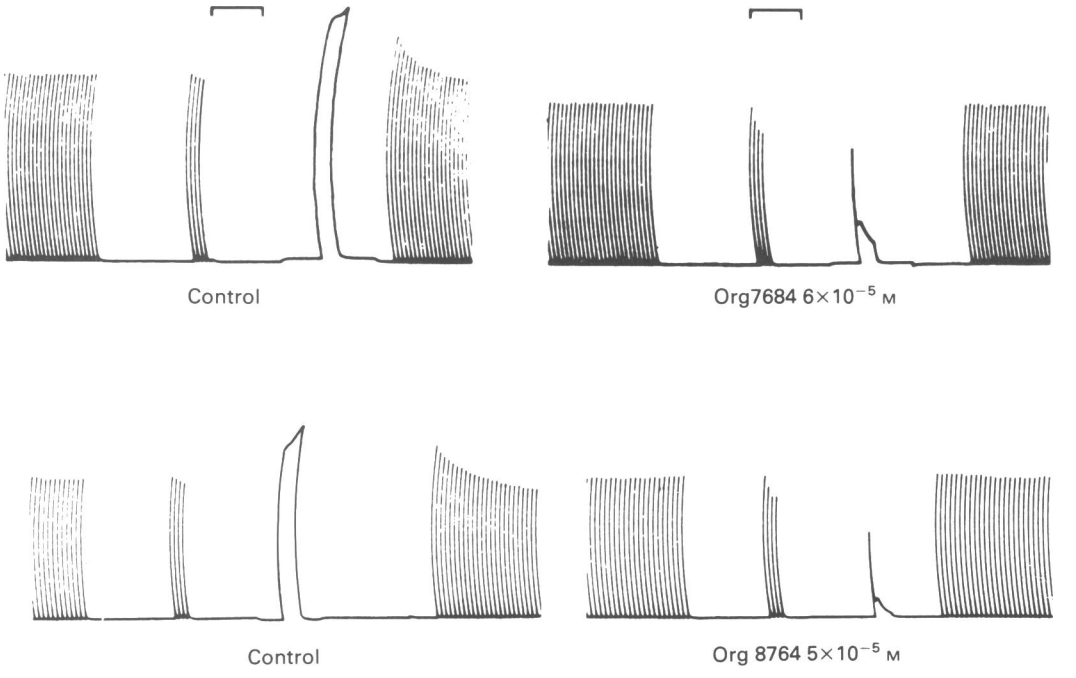

Figure 2 Representative records showing single twitches, trains of four twitches and tetani in control and in the presence of the indicated concentrations of Org 7684, and Org 8764. The scale bar represents 2 min when recording twitches and 4 s when recording trains of four twitches and tetani. The gain was reduced by a factor of 5 when recording tetani. Relative to block of the peak tetanic tension Org 7684 produced much less tetanic fade than did 8764. At higher drug concentrations train of four fade and twitch block became evident.

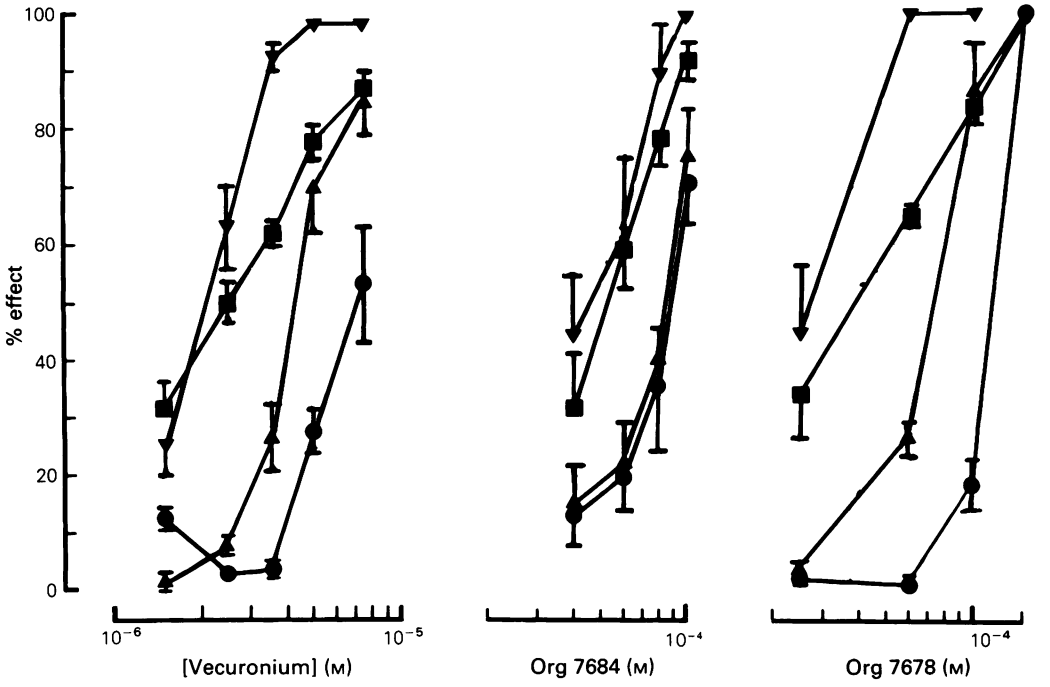

Figure 3 Concentration-effect relationships for twitch block (●), train of four fade (▲), block of the peak tetanic tension (■) and tetanic fade (▼) produced by (a) vecuronium, (b) Org 7684 and (c) Org 7678. Compared to vecuronium or Org 7678, Org 7684 produced much less fade relative to twitch block or block of the peak tetanic tension. ED_{50} values calculated by linear interpolation of the concentration-effect data were; tetanic fade: vecuronium 1.9 μM, Org 7684 52 μM, Org 7678 26 μM. Train of four fade: vecuronium 4.5 μM, Org 7684 87 μM, Org 7678 74 μM. Twitch block: vecuronium 7.5 μM, Org 7684 97 μM, Org 7678 109 μM. Twitch block to tetanic fade ratios: vecuronium 3.95, Org 7684 1.67, Org 7678 4.19.

the much lower propensity of Org 7684 to produce tetanic fade than either Org 7678 or vecuronium. On the basis of these results vecuronium and Org 7684 were chosen for study using electrophysiological techniques in order to assess mechanisms that might be responsible for the different effects measured.

Effects of vecuronium and Org 7684 on endplate currents

Figure 4 summarizes the effects of vecuronium (1.5×10^{-6} M) on the amplitude ($I_{e.p.c.}$) and decay time constant ($\tau_{e.p.c.}$) of e.p.cs evoked at 0.5 Hz and on ACh-induced e.p.c. noise. In cut rat diaphragms, as in other preparations, $I_{e.p.c.}$ is linearly related to membrane potential and $\tau_{e.p.c.}$ is exponentially related to membrane potential (Gibb & Marshall, 1984). Vecuronium reduced $I_{e.p.c.}$ to less than 10% of control amplitude (Figure 4a and b) independently of membrane potential. Vecuronium decreased $\tau_{e.p.c.}$ (Figure 4b and c) by around 20–30% at all holding potentials. However, unlike other positively charged compounds, which have been shown to block endplate ion channels, vecuronium did not reduce the voltage-dependence of $\tau_{e.p.c.}$. Rather there was a tendency for the normal voltage-dependency of $\tau_{e.p.c.}$ to be increased by vecuronium. Thus the change in membrane potential required to produce an e-fold change in $\tau_{e.p.c.}$ was reduced from 139 ± 10 mV in control to 87 ± 8 mV ($n = 5$) in the presence of vecuronium.

Vecuronium reduced the power spectral density of ACh-induced endplate noise at all constituent frequencies, but did not significantly change τ_{noise} or the estimated single channel conductance (Figure 4d).

Org 7684 (6×10^{-5} M) reduced both $I_{e.p.c.}$ and $\tau_{e.p.c.}$ in a voltage-dependent manner (Figure 5a,b and c). The effect of Org 7684 on $I_{e.p.c.}$ was especially marked at hyperpolarized potentials. The change in membrane potential required to produce an e-fold change in $\tau_{e.p.c.}$ was − 188 ± 37 mV in control and + 683 ± 477 mV ($n = 7$) in the presence of Org 7684. In preliminary experiments Org 8764 (5×10^{-5} M), like Org 7684 reduced $\tau_{e.p.c.}$ in a voltage-dependent manner but produced biexponentially decaying e.p.cs.

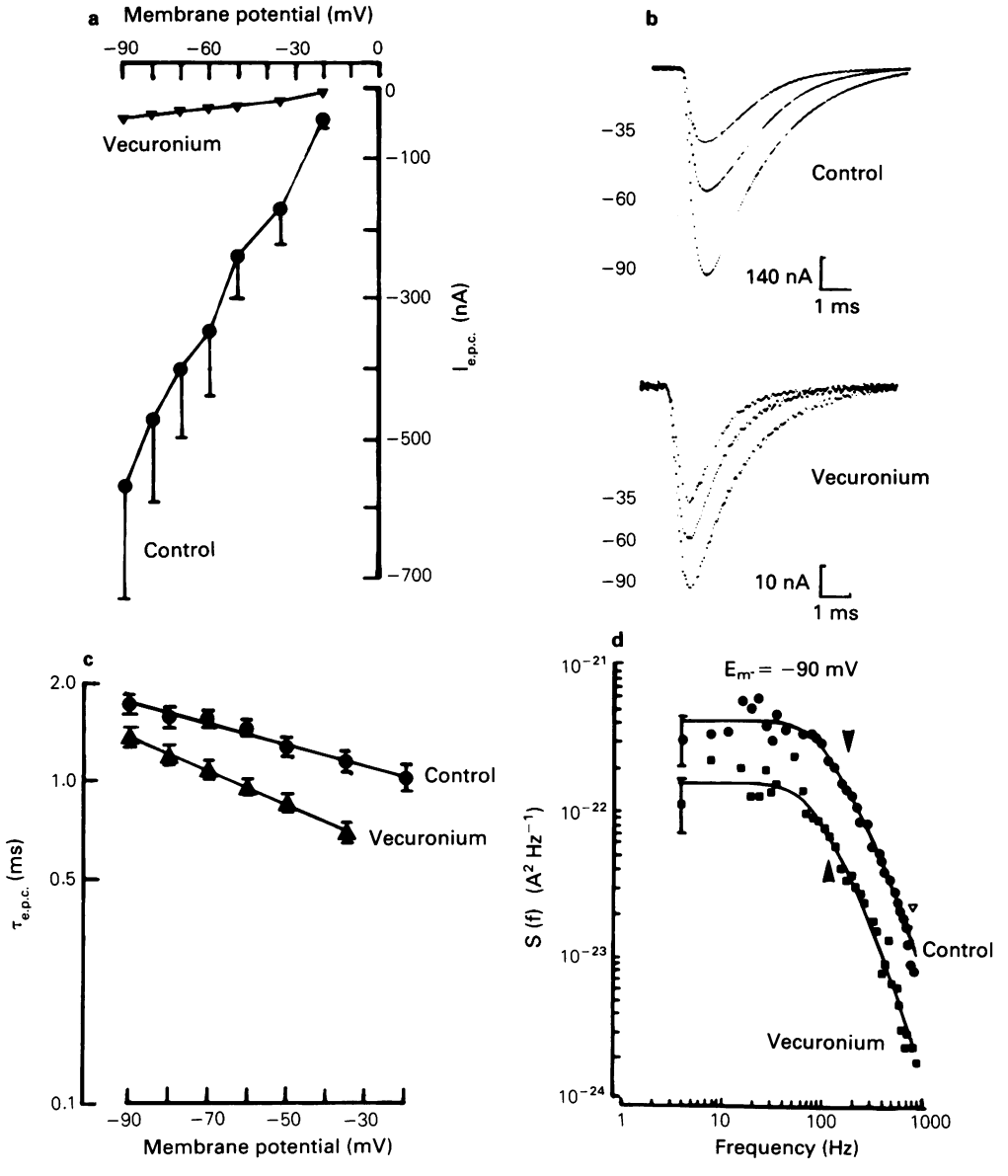

Figure 4 Effects of vecuronium (1.5×10^{-6} M) on single endplate currents (e.p.cs) and on endplate current noise. (a) Vecuronium (1.5×10^{-6} M) reduced e.p.c. amplitude ($I_{e.p.c.}$) independently of the membrane potential. In the presence of vecuronium the error bars are smaller than the symbols ($n = 5$). (b) Representative records of the effects of vecuronium on the amplitude and time course of e.p.cs. (c) Vecuronium (1.5×10^{-6} M) reduced e.p.c. decay time constant ($\tau_{e.p.c.}$) at all holding potentials studied probably by reading repetitive binding of acetylcholine (ACh) in the synaptic cleft. In addition, vecuronium slightly increased the voltage-dependence of $\tau_{e.p.c.}$. (d) Representative records of power spectral density (s(f)) of ACh-induced endplate current noise in control (●) and in the presence of vecuronium (■). The standard error bars at 4 Hz show the s.e.mean of 32 spectra derived from 32, 250 ms control segments of noise and 32, 250 ms segments of noise in the presence of vecuronium. The symbol (▽) shows the cut-off frequency (800 Hz) of the anti-aliasing filter used during data aquisition. The symbols ▼, and ▲ show the half power frequencies of the fitted Lorentzians. These were 123 Hz in control and 104 Hz in the presence of vecuronium corresponding to τ_{noise} of 1.29 ms and 1.53 ms respectively. In three experiments $\tau_{noise} = 1.72 \pm 0.28$ ms in control and 1.71 ± 0.52 ms in the presence of vecuronium. Single channel conductance was 41 ± 9.5 pS in control and 29 ± 2.6 pS in the presence of vecuronium.

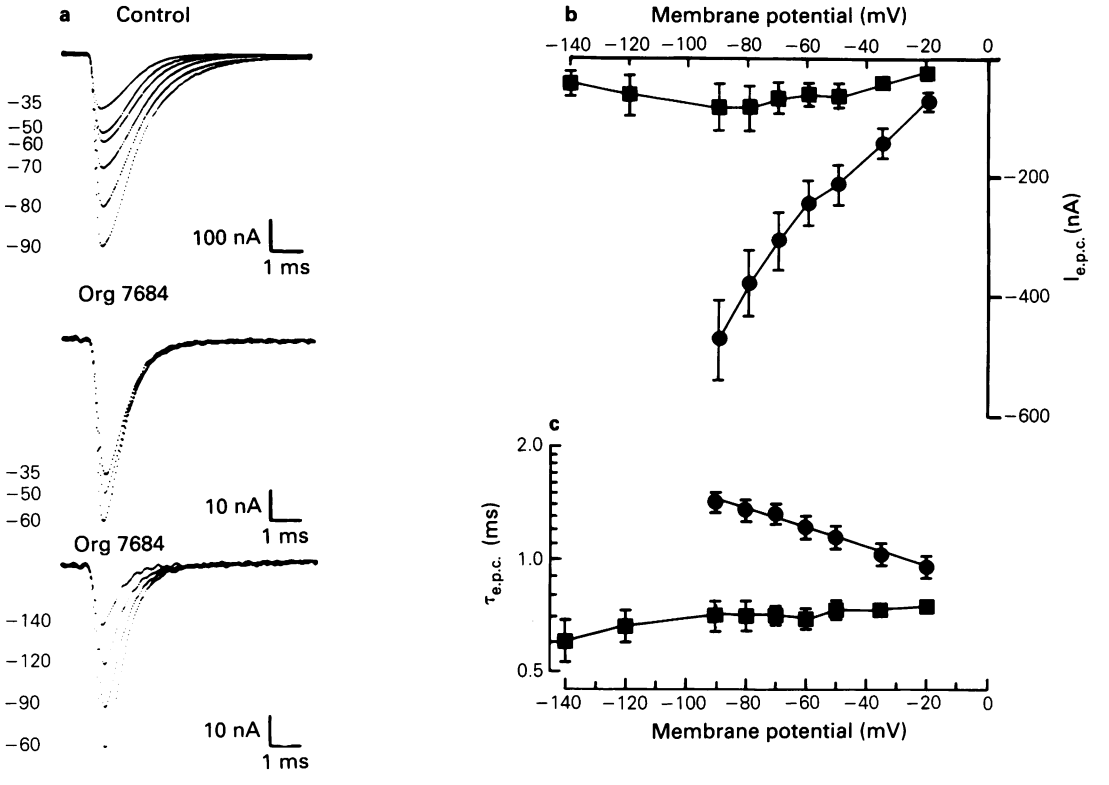

Figure 5 (a) Representative records of the effect of Org 7684 (6×10^{-5} M) on endplate current (e.p.c.) amplitude and time course. In the presence of Org 7684 the e.p.c. amplitude was no longer a linear function of membrane potential but was greatest around -60 mV to -80 mV and became smaller with increasing hyperpolarization. Concomitantly the e.p.c. decay became shorter. (b) The effect of Org 7684 (6×10^{-5} M) on the relationship betwen e.p.c. amplitude ($I_{e.p.c.}$) and membrane potential. In control (●) this relationship was approximately linear while in the presence of Org 7684 (■) the e.p.c.s became smaller with hyperpolarization beyond about -80 mV indicating a voltage-dependent component in the action of Org 7684. (c) Org 7684 reduced the e.p.c. decay time constant ($\tau_{e.p.c.}$) at all potentials studied. (●) Control responses, (■) responses in presence of Org 7684 (6×10^{-5} M). The reduction in $\tau_{e.p.c.}$ became greater with hyperpolarization demonstrating the voltage-dependence of the effect of Org 7684 on e.p.c. decay. In (b) and (c) $n = 7$ except for points shown at -120 mV and -140 mV which are the mean of 3 results.

Thus Org 7684 differs from vecuronium in that the effects of Org 7684 are voltage-dependent suggesting an open ion channel blocking mechanism of action. In contrast, there was no evidence that vecuronium produces channel block at an equi-effective concentration to produce a reduction of e.p.c. amplitude.

Effects of vecuronium and Org 7684 on endplate current trains

As shown previously (Gibb & Marshall, 1984), in the absence of neuromuscular blocking drugs the e.p.c. amplitude runs down during a train of stimulation at 50 Hz to reach a plateau level after 8 to 10 stimuli (Figure 6). This effect is not influenced by changes in membrane potential (Figure 6).

Both vecuronium (1.5×10^{-6} M) and Org 7684 (6×10^{-5} M) increased the percentage rundown of e.p.c. trains evoked by nerve stimulation at 50 Hz (Figures 6 and 7). This increase was not influenced by changes in membrane potential in the presence of either vecuronium or Org 7684. Preliminary experiments indicate that Org 8764 (5×10^{-5} M) also increases e.p.c. train rundown in a voltage-independent manner.

The effects of vecuronium were also tested on e.p.cs evoked ionophoretically by applying trains of ACh pulses to endplates voltage clamped at -90 mV in tetrodotoxin-paralysed preparations. In the absence of vecuronium the amplitude of ionophoretically-

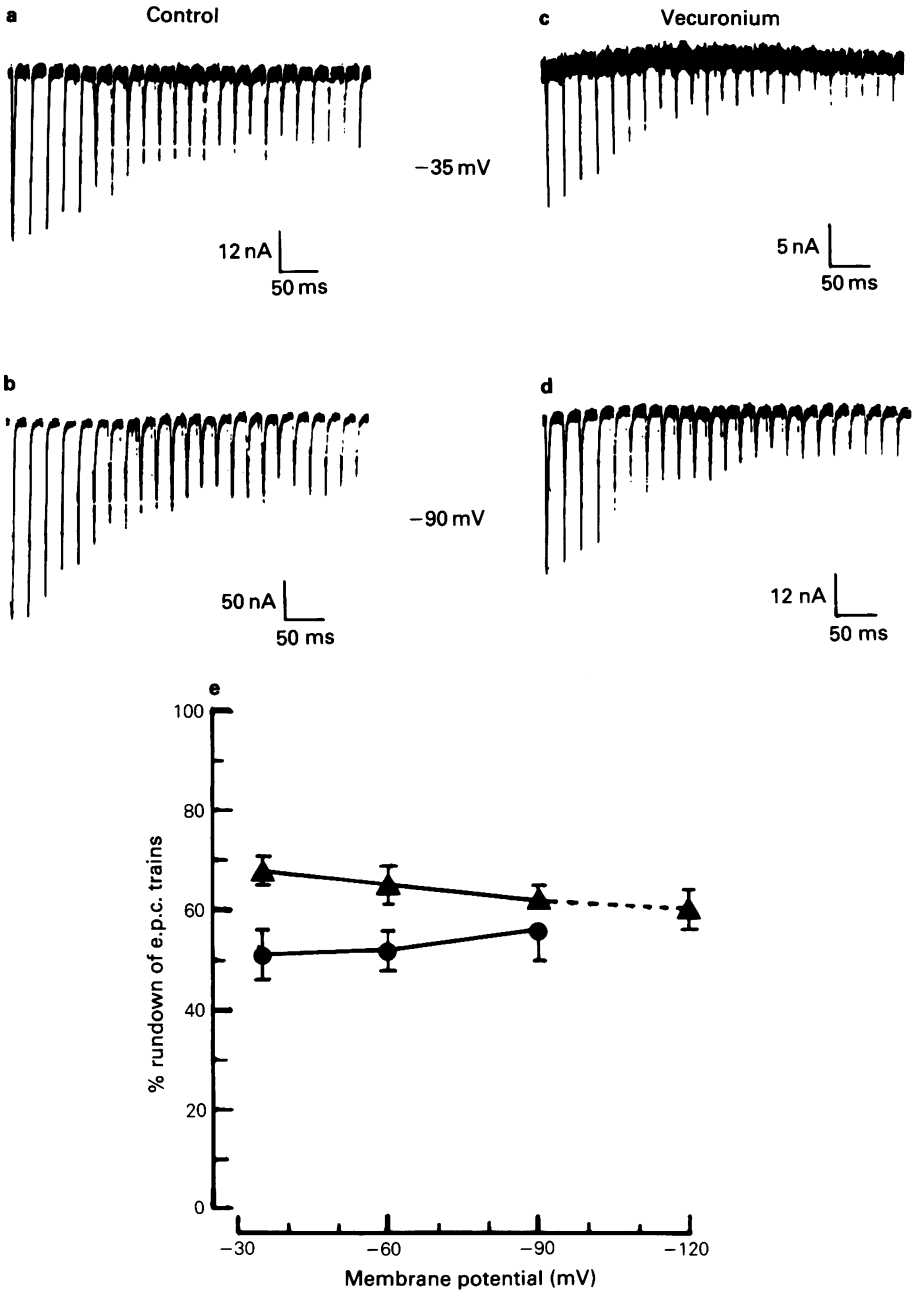

Figure 6 Representative records (c and d) of the effects of vecuronium on trains of neurally-evoked endplate currents (e.p.cs) at a frequency of 50 Hz at two holding potentials (a and b) Control responses. E.p.c train rundown was calculated by averaging the amplitude of the last 10 e.p.cs in the train and expressing the difference between this and the amplitude of first e.p.c. in the train as a percentage of the first e.p.c. amplitude. These values are shown in the graph (e). Vecuronium (▲, 1.5×10^{-6} M) significantly increased rundown relative to control (●) but this effect was not significantly voltage-dependent ($n = 5$). The point shown at −120 mV is the mean of 3 results.

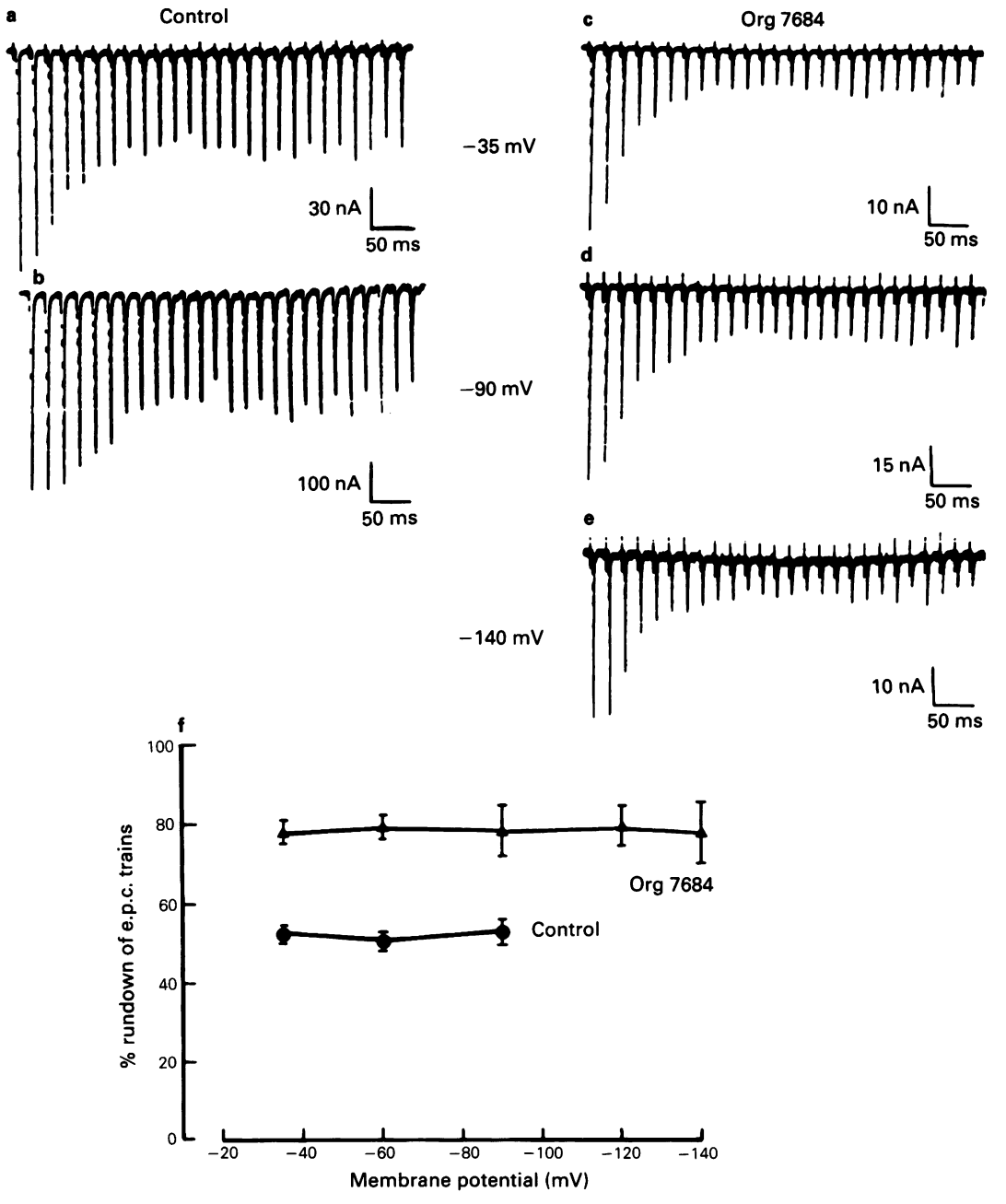

Figure 7 Representative records (c–e) showing the lack of voltage-dependence of the effect of Org 7684 (6×10^{-5} M) on trains of neurally evoked endplate currents (e.p.cs) ($n = 7$). (a and b) Control records. (f) The graph shows that rundown was significantly increased by Org 7684 (6×10^{-5} M) at all potentials but this effect was not voltage-dependent. The points at −120 mV and −140 mV are the mean of 3 results.

evoked e.p.cs increased for the first two or three responses evoked at 50 Hz and then remained constant in amplitude for the remainder of the train (Figure 8). Vecuronium (1.5×10^{-6} M) reduced the amplitude by $68 \pm 7\%$ but, in contrast to the effect of vecuronium on neurally-evoked e.p.cs, did not induce any run-down in ionophoretically evoked e.p.c. amplitude.

Discussion

The main conclusions to be drawn from the results presented in this paper concern the mechanisms involved in tetanic fade produced by nicotinic antagonists when acting at the rat neuromuscular junction. Both vecuronium and Org 7684 were found to produce tetanic fade by a prejunctional effect as evidenced by the lack of rundown of ionphoretically-evoked e.p.cs in the presence of vecuronium and the absence of any voltage-dependence in the rundown of neurally-evoked e.p.c. trains. Such effects have also been demonstrated for tubocurarine (Magleby *et al.*, 1981; Gibb & Marshall, 1984). We therefore conclude that this prejunctional effect may be generally applicable to the action of many nicotinic antagonists at the neuromuscular junction and is not an effect specific to tubocurarine.

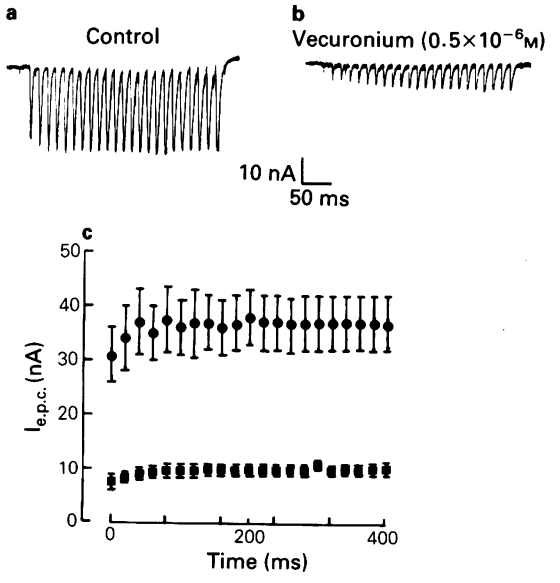

Figure 8 Representative records (top) of the effects of vecuronium (1.5×10^{-6} M, b) on trains of endplate currents (e.p.cs) evoked ionophoretically at 50 Hz from endplates voltage clamped at -90 mV. (a) Control record. Note that vecuronium reduced the ionophoretically-evoked e.p.c. amplitude considerably but did not produce any rundown. (c) The graph shows the results derived from 3 experiments. (●) Control; (■) $I_{e.p.c.}$ in presence of vecuronium (1.5×10^{-6} M).

Vecuronium and its 17-desoxy derivatives, Org 7684, Org 7678 and Org 8764 all produce tetanic fade. Org 7684, Org 7678 and Org 8764 all have approximately one tenth of the potency of vecuronium. However, relative to their ability to reduce peak tetanic tension, Org 7678 and Org 8764 are similar to vecuronium in their potency at producing tetanic fade whereas Org 7684 produces significantly less tetanic fade.

Org 7684 was therefore chosen for electrophysiological study in conjunction with vecuronium in order to determine if the differences between the two drugs would be manifest at the level of the single endplate. These studies were performed in cut hemidiaphragms where transmission can be studied without the use of other blocking agents to block muscle contraction. Problems due to non-linear summation of the endplate potential amplitude, at the low membrane potentials obtained in cut muscles, were avoided by voltage-clamping the endplate membrane and recording the underlying endplate current. This also allows any influence of the postjunctional membrane potential on drug action to be studied.

Both vecuronium and Org 7684 were found to reduce $\tau_{e.p.c.}$, an effect normally associated with open channel block. However, with vecuronium this effect was not influenced by membrane potential and, in addition, vecuronium did not change τ_{noise}, a measure of mean channel open time. The resolution of the noise spectra is such that a small change in τ_{noise} ($<20\%$) would not be detected particularly if this involved a change in the high frequency component of the spectra. However, these effects of vecuronium are very similar to those of the specific receptor antagonist erabutoxin-b (Gibb & Marshall, 1984). We therefore conclude that the shortening of $\tau_{e.p.c.}$ by vecuronium was not due to channel block but due to receptor block, reducing the number of repetitive bindings of ACh to its receptor in a manner similar to that previously described for tubocurarine at the frog neuromuscular junction (Katz & Miledi, 1973; 1978; Magleby & Terrar, 1975; Mallart & Molgo, 1978) and for α-bungarotoxin at frog (Katz & Miledi, 1973; 1978) and mouse (Pennefather & Quastel, 1981) neuromuscular junctions.

In contrast to the results with vecuronium, the reduction of $\tau_{e.p.c.}$ by Org 7684 was voltage-dependent and this was paralleled by the voltage-dependence of the reduction in peak e.p.c. amplitude produced by Org 7684. There is clear evidence that with other nicotinic antagonists, these characteristics are well described by an interaction with the ACh-activated ion channel (Colquhoun, *et al.*, 1979; Colquhoun & Sheridan, 1981). Our previous studies have shown that

timetaphan which, like Org 7684, produces voltage-dependent block, also produces a voltage-dependent rundown of trains of e.p.cs (Gibb & Marshall, 1984). It was therefore surprising to find no evidence for any voltage-dependence in the increased rundown of e.p.c. trains produced by Org 7684.

We conclude that Org 7684 must dissociate from the channel rapidly enough for there to be no accumulation of blocked channels at the endplate during stimulation at 50 Hz. However, in the presence of neostigmine tetanic fade of tension produced by Org 7684 and Org 8764 was increased. This seems to indicate that when receptor activation is greatly increased by blocking ACh hydrolysis, channel block becomes more important in determining the effects of these drugs. Neostigmine in higher concentrations than those used here, can itself cause tetanic fade (Chang *et al.*, 1986). Such an action could also contribute to the increased fade seen in the combined presence of Org 7684 or Org 8764 and the anticholinesterase.

As rundown in Org 7684 seems to be independent of the conditions imposed on the postjunctional membrane we conlude that, like vecuronium and tubocurarine, Org 7684 produces e.p.c. train rundown and tetanic fade by a prejunctional mechanism. As this effect appears not to be unique to tubocurarine but is shared by at least one other structurally unrelated nicotinic antagonist, this implies that there is a nicotinic receptor present on the nerve terminals at the neuromuscular junction, blockade of which produces rundown and tetanic fade. It has been proposed that this receptor is normally activated by transmitter ACh and that during rapid nerve stimulation this serves to stimulate transmitter mobilisation, maintaining release during high frequency stimulation (Bowman, 1980). Preliminary results indicate that exogenously applied agonists can partially reverse rundown produced by tubocurarine (Gibb *et al.*, 1984) and restore transmitter release previously depressed by tubocurarine (Hanalk *et al.*, 1985). Therefore, nicotinic antagonists may block two physiologically important nicotinic receptors at the neuromuscular junction. In order for ion channel block to produce rundown, fast blocking and slow unblocking kinetics are necessary. Thus, in many cases, prejunctional block may be found to be more important than channel block in producing rundown and tetanic fade.

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References

BOWMAN, W.C. (1980) Prejunctional and postjunctional cholinoceptors at the neuromuscular junction. *Anesth. Analg.*, **59**, 935–943.

CHANG, C.C., HONG, S.J. & KO, J-L. Mechanisms of the inhibition by neostigmine of tetanic contraction in the mouse diaphragm. *Br. J. Pharmac.*, **87**, 757–762.

COLQUHOUN, D., DREYER, F. & SHERIDAN, R.E. (1979). The actions of tubocurarine at the frog neuromuscular junction. *J. Physiol.*, **293**, 247–284.

COLQUHOUN, D. & SHERIDAN, R.E. (1981). The modes of action of gallamine. *Proc. R. Soc. Lond. B.*, **211**, 181–203.

DREYER, F. (1982). Acetylcholine receptor. *Br. J. Anaesth.*, **54**, 115–130.

DURANT, N.N. & HORN, R. (1984). Org 6368: A nondepolarising neuromuscular blocking agent with a novel effect on end-plate conductance. *J. Pharmac. exp. Ther.*, **228**, 567–572.

GIBB, A.J. & MARSHALL, I.G. (1984). Pre- and postjunctional effects of tubocurarine and other nicotinic antagonists during repetitive stimulation in the rat. *J. Physiol.*, **351**, 275–297.

GIBB, A.J., MARSHALL, I.G. & BOWMAN, W.C. (1984). Effect of ionophoretically-applied acetylcholine on tubocurarine induced end-plate current train rundown: evidence for a nicotinic feedback mechanism at the rat neuromuscular junction. *Proc. Aust, Physiol. & Pharmac. Soc.*, **15**, 86P.

HANALK, M., KILBINGER, H. & WESSLER, I. (1985). Facilitation by nicotinic auroreceptors of acetylcholine release from the rat phrenic nerve. *J. Physiol.*, **371**, 62P.

JENKINSON, D.H. (1960). The antagonism between tubocurarine and substances which depolarise the motor end-plate. *J. Physiol.*, **152**, 309–324.

KATZ, B. & MILDEI, R. (1973). The binding of acetylcholine to receptors and its removal from the synaptic cleft. *J. Physiol.*, **231**, 549–574.

KATZ, B. & MILEDI, R. (1978). A re-examination of curare action at the motor end-plate. *Proc. R. Soc. Lond. B.*, **203**, 119–133.

LAMBERT, J.J., DURANT, N.N. & HENDERSON, E.G. (1983). Drug-induced modification of ionic conductance at the neuromuscular junction. *A Rev. Pharmac. Tox.*, **23**, 505–539.

MAGLEBY, K.L., PALLOTTA, B.S. & TERRAR, D. (1981). The effect of d-tubocurarine on neuromuscular transmission during repetitive stimulation in the rat, mouse and frog. *J. Physiol.*, **312**, 97–113.

MAGLEBY, K.L. & TERRAR, D. (1975). Factors affecting the time course of decay of end-plate currents: a possible cooperative action of acetylcholine on receptors at the frog neuromuscular junction. *J. Physiol.*, **244**, 467–495.

MALLART, A. & MOLGO, J. (1978). The effects of pH and

curare on the time course of end-plate currents at the neuromuscular junction of the frog. *J. Physiol.*, **276,** 343–352.

PENNEFATHER, P. & QUASTEL, D.M.J. (1981). Relation between subsynaptic receptor blockade and response to quantal transmitter at the mouse neuromuscular junction. *J. gen. Physiol.*, **78,** 313–344.

STANDAERT, F.G. (1982). Release of transmitter at the neuromuscular junction. *Br. J. Anaesth.*, **54,** 131–145.

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