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THE ACTION OF POLYMYXIN B AT THE FROG NEUROMUSCULAR JUNCTION

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1 The effects of polymyxin B at the neuromuscular junction of the frog were studied by conventional electrophysiological and voltage clamp techniques.

2 At a concentration of 2.5 μg/ml polymyxin B produced neuromuscular blockade in 10 to 15 min and endplate potentials (e.p.ps) could be recorded. Resting membrane potential was unaffected. The neuromuscular block was characterized by a depressed e.p.p. quantal content (28 ± 7), which was similar to that determined from endplates exposed to 13 mM magnesium (23 ± 3), and a low e.p.p. quantal size, which was similar to that determined from endplates exposed to 3 μM (+)-tubocurarine.

3 Polymyxin B (0.25 to 0.75 μg/ml) decreased mean miniature e.p.p. amplitude with little effect on frequency.

4 At a concentration of 5 μg/ml polymyxin B markedly shortened the duration of endplate currents (e.p.cs) and abolished the relationship between holding potential and the time to half-decay at negative potentials greater than −60 mV. This action is consistent with block of open acetylcholine activated ionic channels.

5 4-Aminopyridine (20 μM) antagonized the depressed e.p.p. quantal content produced by polymyxin B but did not alter the shortened e.p.c. duration.

6 It is concluded that polymyxin B decreases quantal release and produces some degree of postjunctional receptor blockade and a marked and persistent blockade of acetylcholine activated channels. The latter action may explain the difficulty of reversal of polymyxin B-induced neuromuscular blockade and its non-competitive nature.

Introduction

Neuromuscular block produced by a number of antibiotics has been widely documented in both man and experimental animals (Hokkanen, 1964; Pittinger, Eryasa & Adamson, 1970; Pittinger & Adamson, 1972; Chinyanga & Stoyka, 1974). Polymyxin B is amongst the most potent of the antibiotics in producing neuromuscular block and has been shown to depress the twitch responses of the indirectly stimulated mouse diaphragm in the same dose range as (+)-tubocurarine (Singh, Harvey & Marshall, 1978). The action of polymyxin B includes both a pre- and postjunctional component; a prejunctional component has been proposed by several workers (Elmqvist & Josefsson, 1962; Vital-Brazil & Prado-Francheschi, 1969; Singh, Marshall & Harvey, 1979) and is supported by the finding that polymyxin B-induced neuromuscular block is partially reversed by calcium but not by neostigmine both *in vitro* (Singh *et al.*, 1978) and *in vivo* (Lee, Chen & Nagel, 1977); however, the mechanism of action has still not been fully elucidated. Postjunctionally, polymyxin B has been shown to produce noncompetitive antagonism of acetylcholine on the frog rectus abdominus muscle (Viswanath & Jenkins, 1978). Recently, Singh, *et al.* (1979) have shown the postjunctional action of polymyxin B to be greater than the prejunctional action; however, the mechanism of action of polymyxin B was still not clear. The present study confirms the prejunctional action of polymyxin B and provides strong evidence for a separate postjunctional action of the antibiotic on the acetylcholine activated channels. We have demonstrated that polymyxin B causes a marked decrease of the conductance of acetylcholine activated channels which explains, in part, some aspects of the postjunctional block produced by the antibiotic.

Some of the initial results of this study have been described previously (Durant & Lambert, 1980).

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Methods

Frog (*Rana pipiens*) sartorius muscle-sciatic nerve and cutaneous pectoris preparations were pinned to the base of a small Sylgard coated tissue bath containing Ringer solution of the following composition (mM): NaCl 98.5, KCl 2.5, NaH_2PO_4 1.9, Na_2HPO_4 4.8, and $CaCl_2$ 2.0 (pH 7.4). When a pH of 9.2 was used the phosphate buffer of the Ringer solution was replaced with bicarbonate buffer to give the following composition (mM): NaCl 98.5, KCl 2.5, $NaCO_3$ 0.96, $NaHCO_3$ 4.06 and $CaCl_2$ 2.0. Muscle twitching was abolished by adding 2.5 to 5.0 μg/ml of polymyxin B, 13 mM $MgCl_2$ or 3 μM (+)-tubocurarine. In some experiments, muscle twitching was abolished by carefully cutting the muscle fibres 5 mm on either side of the nerve, and this preparation will be described in more detail elsewhere.

Glass microelectrodes (5 to 20 MΩ) filled with 2 M potassium acetate were used to record resting potential (Em), endplate potentials (e.p.ps) and miniature endplate potentials (m.e.p.ps). Standard recording systems were used and permanent records were made by photography or processed by a Zonic Technical Laboratories 200 kHz analog to digital converter and analysed by an on-line LSI-11 computer. The two microelectrode voltage clamp described by Deguchi & Narahashi (1971) was used to record endplate currents (e.p.cs). Two microelectrodes filled with 3 M KCl were placed in endplates with an interelectrode distance of 10 to 30 μm. One electrode was used to pass current for the membrane holding potential. The clamping circuit had a rise time of less than 20 μs and a clamping error of less than 5%. The membrane was clamped initially at −90 mV (−30 mV for cut muscle) and then stepped at 20 mV increments from −150 mV to −70 mV and in 10 mV increments to 0 mV.

Quantal content of e.p.ps was determined by calculating the variance (Elmqvist & Quastel, 1965) of the last 50 e.p.ps of a train of 80. The mean quantum size and mean amplitude of the train of e.p.ps was used to calculate the quantal content of individual e.p.ps. The reversal potential was taken as −15 mV and Martin's (1955) correction was used when appropriate. The analysis of variance assumes a Poisson distribution; however, when quantal content is large, there can be deviation from Poisson statistics (Del Castillo & Katz, 1954; Wernig, 1975). Since m.e.p.ps (which are required for calculating transmitter release described by binomial statistics) could only be recorded in the presence of magnesium, due to the decreased signal to noise ratio of m.e.p.ps recorded in neuromuscular blocking concentrations of either polymyxin B or (+)-tubocurarine, the analysis of variance was used for comparison, but this method only represents an approximate estimate of quantal release. Trains of stimuli were applied to the nerve at frequencies of 0.1, 1, 2, 10, 25 and 50 Hz. Statistical comparisons of unpaired data were made using the Mann-Whitney U-test, and of paired data using the Wilcoxon test, $P < 0.05$ being regarded as significant. Results are presented as the mean ± s.e. mean.

The drugs used were 4-aminopyridine (Baker, Phillipsburg, USA), (+)-tubocurarine (Squibb, Princeton, USA) and polymyxin B (Pfizer). The concentration of polymyxin B is expressed as μg/ml since the molecular weight cannot be accurately determined. The closest estimate of the molecular weight of polymyxin B is 1390 ± 50 (personal communication, Dr B. Petrick, Pfizer, New York, U.S.A.).

Results

General actions of polymyxin B

At a concentration or 2.5 μg/ml (1.8 ± 0.065 μM) polymyxin B produced neuromuscular block within 10 to 15 min. Polymyxin B (2.5 μg/ml) had no effect on resting membrane potential (Em) recorded at the endplate region. E.p.ps were recorded after 30 min of exposure to polymyxin B. The mean amplitude of e.p.ps recorded after 30 min of exposure to polymyxin B was not significantly different from the amplitude recorded after at least 2.5 h of exposure to the anti-

Table 1 Comparison of the actions of polymyxin B (2.5 μg/ml), magnesium (13 mM) and (+)-tubocurarine (3 μM) on quantal release and quantal size at a stimulation frequency of 0.1 Hz; the action of 0.5 μM 4-aminopyridine (4-AP) on the depressed e.p.p. quantal content due to polymyxin B is also shown

	n	*Mean e.p.p. quantal content*	*Mean e.p.p. amplitude* (mV)	*Quantal size* (mV)
Polymyxin B	14	33 ± 6	1.82 ± 0.36	0.057 ± 0.006
Polymyxin B + 4-AP	5	121 ± 29	5.30 ± 1.50	0.040 ± 0.010
Magnesium	9	23 ± 3	3.70 ± 0.63	0.160 ± 0.018
(+)-Tubocurarine	8	207 ± 37	4.30 ± 0.52	0.02 ± 0.003

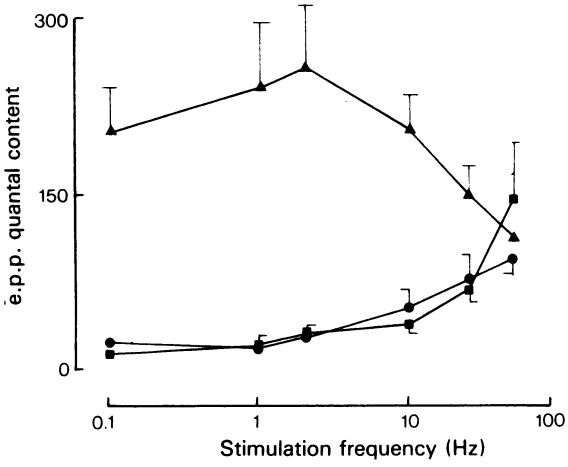

Figure 1 The mean e.p.p. quantal content calculated from the variance of the last 50 e.p.ps in a train of 80 plotted against the log of the stimulation frequency during exposure to 2.5 μg/ml polymyxin B (●), 13 mM magnesium (■) and 3 μM (+)-tubocurarine (▲). The vertical bars indicate the s.e. mean of at least five observations.

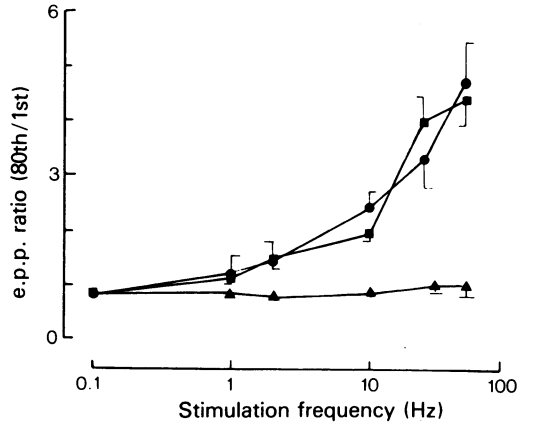

Figure 2 The facilitation of e.p.p. amplitude (taken as the ratio of the amplitude of the 80th e.p.p. to that of the 1st of a train of 80 stimuli) plotted against the log of the stimulation frequency during exposure to 2.5μg/ml polymyxin B (●), 13 mM magnesium (■) and 3 μM (+)-tubocurarine (▲). The vertical bars indicate the s.e. mean of at least five observations.

biotic. The polymyxin B-induced neuromuscular blockade could be antagonized by washing the preparation with normal Ringer solution for 5 to 10 min.

Prejunctional action of polymyxin B

At a frequency of nerve stimulation of 0.1 Hz the quantal content of e.p.ps recorded during exposure to polymyxin B is significantly ($P < 0.05$) less than those recorded during exposure to (+)-tubocurarine (3 μM), but is not significantly different from the quantal content of e.p.ps recorded in the presence of magnesium (13 μM) (Table 1), suggesting that the antibiotic may decrease the number of quanta released per impulse. 4-Aminopyridine (4-AP, 0.5 μM), an agent known to increase the number of quanta released from magnesium pretreated preparations (Horn, Lambert & Marshall, 1979), produced an increase of the e.p.p quantal content recorded from polymyxin B-treated preparations (Table 1). It is possible that the increased release of acetylcholine due to 4-AP may also antagonize a postjunctional blockade of acetylcholine receptors by polymyxin B although quantal size was not significantly altered (Table 1). Further evidence of a prejunctional action of the antibiotic is provided by the similarity of the actions of magnesium, an agent known to decrease evoked transmitter release (Del Castillo & Engbaek, 1954), and polymyxin B on trains of e.p.ps at different frequencies of nerve stimulation. E.p.ps recorded in the presence of polymyxin B or magnesium exhibited a similar frequency-dependent increase of mean e.p.p. quantal content in contrast to (+)-tubocurarine (3 μM) (Figure 1). Polymyxin B (2.5 μg/ml) and magnesium (13 mM) also produced a frequency-dependent facilitation of amplitude when the 1st and 80th e.p.p. amplitudes were compared, again unlike (+)-tubocurarine (3 μM) (Figure 2).

The determination of the effect of polymyxin B on m.e.p.p. frequency was complicated by the antibiotics postjunctional action of decreasing m.e.p.p. amplitude (*vide infra*) (Figure 3) which limited the dose range (0.25 to 0.75 μg/ml) which could be investigated. However, at a concentration of 0.25 μg/ml of polymyxin B no significant change of m.e.p.p. frequency was observed (1.1 ± 0.2 Hz control, vs 1.0 ± 0.3 Hz).

Postjunctional actions of polymyxin B

The quantal size determined from polymyxin B (2.5 μg/ml) treated preparations is similar to the value obtained from preparations treated with (+)-tubocurarine (3 μM) (Table 1) and as would be expected, polymyxin B decreased the mean m.e.p.p. amplitude in a dose-dependent manner confirming its postjunctional action (Figure 3).

In the presence of polymyxin B (5 μg/ml) the time to 50% decay of the e.p.c. ($T_{\frac{1}{2}}$) was very rapid when compared with e.p.cs recorded in magnesium (13 mM) or (+)-tubocurarine (3 μM)-treated preparations (Figure 4). Similarly, polymyxin B produced a marked shortening of the decay time of e.p.cs recorded from the endplates of the untreated cut

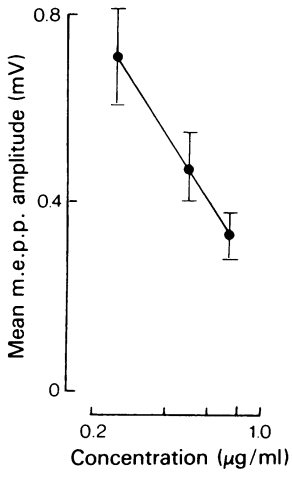

Figure 3 The log dose-response curve of the action of polymyxin B on mean m.e.p.p. amplitude. The vertical bars represent the s.e. mean of observations from four preparations with at least 50 m.e.p.ps recorded from each preparation at each concentration of polymyxin B.

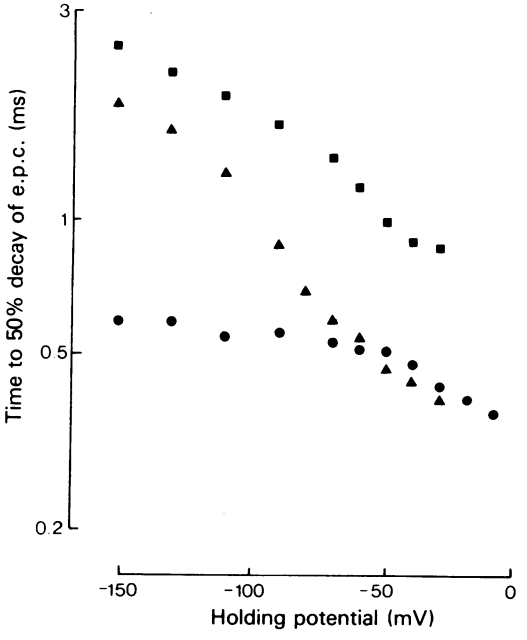

Figure 4 The log time to 50% decay ($T_{\frac{1}{2}}$) of e.p.cs versus holding potential recorded in the presence of 5 μg/ml polymyxin B (●), 13mM magnesium (■) and 3 μM (+)-tubocurarine (▲). Each point represents the mean of at least five observations and the s.e. mean is too small to be shown.

muscle preparation (Figure 5). These data are consistent with the antibiotic having an action to block open acetylcholine-activated ionic channels as has been suggested for procaine (Adams, 1977), atropine (Alder & Albuquerque, 1976), tetraethylammonium (Alder, Oliveira, Albuquerque, Mansour & Eldefrawi, 1979) and lobeline (Lambert, Reynolds, Volle & Henderson, 1979). The $T_{\frac{1}{2}}$s of e.p.cs recorded from endplates treated with glycerol (Magleby & Stevens, 1972), low concentrations of (+)-tubocurarine (Katz & Miledi, 1978) or alpha bungartoxin (Katz & Miledi, 1978) and the $T_{\frac{1}{2}}$ of untreated m.e.p.cs (Gage & McBurney, 1975) have been shown to vary with holding potential, becoming longer at more hyperpolarized potentials. In the presence of polymyxin B (5 μg/ml) the e.p.c. $T_{\frac{1}{2}}$ was independent of holding potential (Figure 4) between −150 mV and −60 mV, but between −50 mV and 0 mV the e.p.c. was related to holding potential in the normal manner. The $T_{\frac{1}{2}}$ of e.p.cs recorded from either magnesium-treated (13 mM) or (+)-tubocurarine-treated (3 μM) preparations was related to holding potential throughout the range of holding potentials studied. This result suggests that when the membrane is held at a potential between −50 mV and 0 mV the channel environment is not favorable for the binding of polymyxin B.

The action of polymyxin B on acetylcholine-activated ionic channels was investigated further using the cut cutaneous pectoris muscle preparation. On this preparation polymyxin B greatly shortened the decay phase of the e.p.c. compared to the untreated

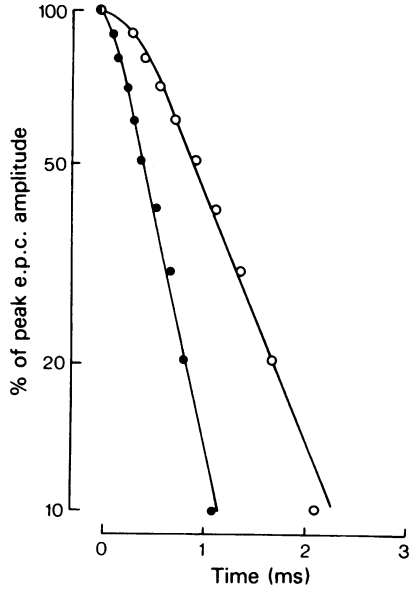

Figure 5 The effect of polymyxin B (5 μg/ml) on the e.p.c. decay phase in cut muscle. Control cell (○); same cell in polymyxin B (●); the membrane potential was held at −30 mV.

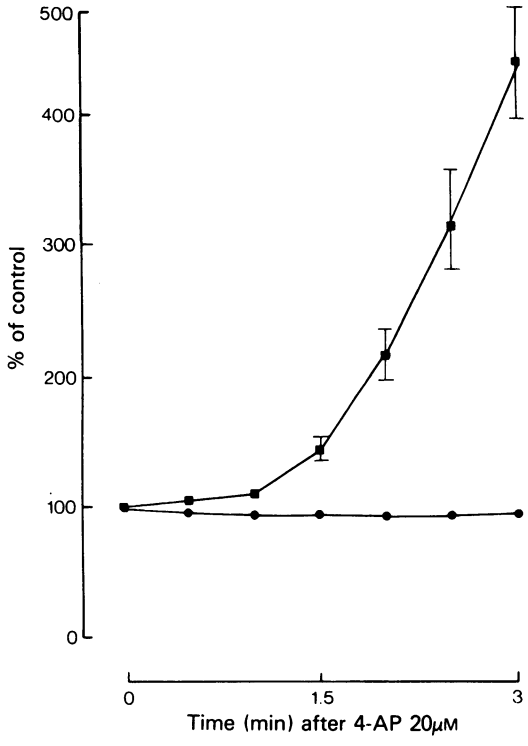

Figure 6 The effect of 20 μM 4-aminopyridine on the amplitude (■) and $T_{\frac{1}{2}}$ (●) of e.p.cs versus time of exposure, recorded in the presence of 5 μg/ml of polymyxin B from the cut muscle preparation at a holding potential of −30 mV. Results are presented as percentage of control, the vertical bars indicate the s.e. mean of four observations.

control (Figure 5). On the cut muscle preparation it was also found that polymyxin B produced its maximum effect on both e.p.c. amplitude and decay within less than 10 min (Table 2).

The five charged free amino groups which polymyxin B possesses are known to be involved in the neuromuscular blocking activity of the antibiotic since when these groups are protected by methanesulphonation, as in colistimethate sodium (colistin), the neuromuscular blocking activity is abolished (Wright & Collier, 1976). Indeed, McQuillen & Engbaek (1975) found that colistin does not produce neuromuscular block of the rat diaphragm at a concentration of 1 mg/ml. If the pH is increased to 9.2 the charge on the free amino groups of polymyxin B is decreased (Wright & Collier, 1976). However, in the present study we found, on the cut muscle, that the action of polymyxin B on e.p.c. amplitude and $T_{\frac{1}{2}}$ was slightly reduced at pH 9.4 compared to pH 7.2 (Table 2) but this was not significant ($P > 0.05$) which suggests that the charged amino groups on polymyxin B are not responsible for its action on the acetylcholine activated ionic channel.

4-AP (20 μM) greatly increased the amplitude of the e.p.cs recorded from polymyxin B (5 μg/ml)-pretreated preparations, an effect mainly attributable to the previously observed (Table 1) increase of e.p.p. quantal content, but had no effect on e.p.c. $T_{\frac{1}{2}}$ (Figure 6), suggesting that 4-AP did not antagonize polymyxin B's action on the acetycholine activated ionic channels, but did possibly antagonize the receptor blockade produced by polymyxin B, as suggested above.

Discussion

The results of the present study clearly demonstrate that polymyxin B has two distinct actions on neuromuscular transmission. First, an action on the nerve terminal decreasing quantal release in a manner similar to magnesium, and second, a postjunctional blocking action on acetylcholine-activated ionic channels which results in a decrease of conductance.

Trains of nerve stimulation at high frequencies have been shown by previous workers to produce a facilitation of e.p.p. amplitude in the presence of magnesium (Balnave & Gage, 1977). Such an action is explained by the accumulation of calcium in the nerve terminal which overcomes the magnesium-induced

Table 2 Comparison of the action of polymyxin B (5 μg/ml) on e.p.c. amplitude, time to peak and time to half decay ($T_{\frac{1}{2}}$) at pH 7.4 and pH 9.2 on the cut cutaneous pectoris preparation

	Amplitude (nA)	*Time to peak* (ms)	*Time to half decay* (ms)
pH 7.4 Control	171 ± 29	0.53 ± 0.12	1.38 ± 0.13
Polymyxin B	13 ± 7	0.47 ± 0.04	0.49 ± 0.11
pH 9.2 Control	329 ± 133	0.73 ± 0.08	1.24 ± 0.14
Polymyxin B	23 ± 6	0.63 ± 0.11	0.42 ± 0.14

Measurements of the action of polymyxin B were made after 10 min of exposure ($n = 4$).

depression of release. This effect was seen in our study with magnesium and also with polymyxin B, which suggests that, like magnesium, the prejunctional decrease of evoked transmitter release produced by polymyxin B may be antagonized by a raised intraterminal calcium level. Indeed, calcium has been shown to produce a partial reversal of polymyxin B-induced neuromuscular block (Lee *et al.*, 1977; Singh *et al.*, 1978). *In vivo*, 4-aminopyridine has been shown to reverse polymyxin B-induced block of the indirectly stimulated tibialis anterior muscle of the cat (Lee, de Silva & Katz, 1978). The antagonism by 4-aminopyridine of the polymyxin B-induced decrease of evoked transmitter release demonstrated in the present study can be explained by the known action of aminopyridines to increase evoked transmitter release by a prolongation of the nerve terminal action potential, which results in an increased calcium influx into the nerve terminal (Molgo, Lemeignan & Lechat, 1975; Harvey & Marshall, 1977).

Wright & Collier (1976) reported that polymyxin B had little action on acetycholine released in response to tetanic stimulation, when acetycholine was assayed biochemically. The discrepancy between the results of the present study and those of Wright & Collier (1976) may be explained by the fact that these workers applied a 20 Hz train of stimuli to the nerve lasting for 13 min to release acetylcholine. Our study suggests that such a frequency of nerve stimulation would be likely to cause facilitation of e.p.p. quantal content to near normal levels and as such it seems that the amount of acetycholine released in the presence of polymyxin B would not be greatly different from control.

The time to half decay of the e.p.c. ($T_{\frac{1}{2}}$) is well established as a measure of the rate of closing of the acetylcholine-activated ionic channels in frog skeletal muscle (Magleby & Stevens, 1972). Recently the antibiotics, lincomycin and clindamycin, have been shown to alter miniature e.p.c. decay in a manner consistent with the drugs interacting with the acetylcholine-activated ionic channel (Fiekers, Marshall & Parsons, 1979). We have clearly demonstrated that polymyxin B causes a marked decrease of the e.p.c. $T_{\frac{1}{2}}$ suggesting that polymyxin B blocks open acetylcholine-activated channels, as has been suggested for other agents (Adler & Albuquerque, 1976; Adams, 1977; Adler *et al.*, 1979; Lambert *et al.*, 1979). Blockade of acetylcholine-activated channels by polymyxin B may explain the non-competitive nature of the antagonism to acetylcholine by polymyxin B reported by Viswanath & Jenkins (1978).

We conclude that the prejunctional component of the action of polymyxin B at the neuromuscular junction is due to a magnesium-like action decreasing quantal release whilst the predominant postjunctional action is due to blockade of conductance through the acetylcholine-activated channels of the endplate. This study also emphasizes the clinical importance of choosing the correct agent to reverse neuromuscular block produced by polymyxin B. Neostigmine (or any other anticholinesterase) is likely to be of little use (Lee *et al.*, 1977) and although there is no ideal reversal agent it is suggested that 4-aminopyridine may be of some assistance.

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References

ADAMS, P.R. (1977). Voltage jump analysis of procaine action at frog end-plate. *J. Physiol.*, **268**, 291–318.

ADLER, M. & ALBUQUERQUE, E.X. (1976). An analysis of the action of atropine and scopolamine on the end-plate current of frog sartorius muscle. *J. Pharmac. exp. Ther.*, **196**, 360–372.

ADLER, M., CLIVEIRA, A.C., ALBUQUERQUE, E.X., MANSOUR, N.A. & ELDEFRAWI, A.T. (1979). Reaction of tetraethylammonium with the open and closed conformations of the acetylcholine receptor ionic channel complex. *J. gen. Physiol.*, **74**, 129–152.

BALNAVE, R.J. & GAGE, P.W. (1977). Facilitation of transmitter secretion from toad motor nerve terminals during brief trains of action potentials. *J. Physiol.*, **266**, 435–451.

DEL CASTILLO, J. & ENGBAEK, L. (1954). The nature of the neuromuscular block produced by magnesium. *J. Physiol.*, **124**, 370–384.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol.*, **124**, 560–573.

CHINYANGA, H.M. & STOYKA, W.W. (1974). The effect of colymycin M, gentamycin and kanamycin on depression of neuromuscular transmission induced by pancuronium bromide. *Can. Anaesth. Soc. J.*, **21**, 569–579.

DEGUCHI, T. & NARAHASHI, T. (1971). Effects of procaine on ionic conductances of end-plate membranes. *J. Pharmac. exp. Ther.*, **176**, 423–433.

DURANT, N.N. & LAMBERT, J.J (1980). The action of polymyxin B at the frog neuromuscular junction. *Fedn Proc.*, **39**, 609

ELMQVIST, D. & JOSEFSSON, J.O. (1962). The nature of the neuromuscular block produced by neomycin. *Acta physiol. scand.*, **54**, 105–110.

ELMQVIST, D. & QUASTEL, D.M.J. (1965). A quantitative

study of end-plate potentials in isolated human muscle. *J. Physiol.*, **178**, 505–529.

FIEKERS, J.F., MARSHALL, I.G. & PARSONS, R.L. (1979). Clindamycin and linocomycin alter miniature endplate current decay. *Nature Lond.*, **281**, 680–682.

GAGE, P.W. & MCBURNEY R.N. (1975). Effects of membrane potential, temperature and neostigmine on the conductance change caused by a quantum of acetylcholine at the toad neuromuscular junction. *J. Physiol.*, **244**, 385–407.

HOKKANEN, E. (1964). The aggravating effect of some antibiotics on the neuromuscular blockade in myasthenia gravis. *Acta neurol. scand.* **40**, 346–352.

HARVEY, A.L. & MARSHALL, I.G. (1977). The actions of three diaminopyridines on the chick biventer cervicis muscle. *Eur. J. Pharmac.*, **44**, 303–309.

HORN, A.S., LAMBERT, J.J. & MARSHALL, I.G. (1979). A comparison of the facilitatory actions of 4-aminopyridine methiodide and 4-aminopyridine on neuromuscular transmission. *Br. J. Pharmac.*, **65**, 53–62.

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

LAMBERT, J.J., REYNOLDS, L.S., VOLLE, R.L. & HENDERSON, E.G. (1979). The action of lobeline on the acetylcholine-activated ionic channel. *Soc. Neurosci. Abstr.*, **5**, P483.

LEE, C., CHEN, D. & NAGEL, E.L. (1977). Neuromuscular block by antibiotics: polymyxin B. *Anesth. Analg.*, **56**, 373–377.

LEE, C., DE SILVA, A.J.C. & KATZ, R.L. (1978). Antagonism of polymyxin B-induced neuromuscular and cardiovascular depression by 4-aminopyridine in the anesthetized cat. *Anesthesiology*, **49**, 256–259.

MCQUILLEN, M.P. & ENGBAEK, L. (1975). Mechanism of colistin-induced neuromuscular depression. *Arch. Neurol.*, **32**, 235–238.

MAGLEBY, K.L. & STEVENS, C.F. (1972). The effect of voltage on the time course of end-plate currents. *J. Physiol.*, **223**, 151–171.

MARTIN, A.R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol.*, **130**, 114–122.

MOLGO, J., LEMEIGNAN, M. & LECHAT, P. (1975) Modification de la libération du transmetteur à la jonction neuromusclaire de Grenouille sous l'action de l'amino-4-pyridine. *C. R. hebd. Séanc. Acad. Sci., Paris* (D), **281**, 1637–1639.

PITTINGER, C.B. & ADAMSON, R. (1972). Antibiotic blockade of neuromuscular function. *A. Rev. Pharmac.*, **12**, 169–184.

PITTINGER, C.B., ERYASA, Y. & ADAMSON, R. (1970). Antibiotic-induced paralysis. *Anesth. Analg.*, **49**, 487–501.

SINGH, Y.N., HARVEY, A.L. & MARSHALL, I.G. (1978). Antibiotic-induced paralysis of the mouse phrenic nerve-hemidiaphragm preparation, and reversibility by calcium by neostigmine. *Anesthesiology*, **48**, 418–424.

SINGH, Y.N., MARSHALL, I.G. & HARVEY, A.L. (1979). Depression of transmitter rleease and postjunctional sensitivity during neuromuscular block produced by antibiotics. *Br. J. Anaesth.*, **51**, 1027–1033.

VISWANATH, D.V. & JENKINS, H.J. (1978). Neuromuscular block of the polymyxin group of antibiotics. *J. Pharm. Sci.*, **67**, 1275–1280.

VITAL-BRAZIL, O. & PRADO-FRANCHESCHI, J. (1969). The nature of neuromuscular block produced by neomycin and gentamicin. *Archs int. Pharmacodyn. Ther.*, **179**, 78–85.

WERNIG, A. (1975). Estimates of statistical release parameters from crayfish and frog neuromuscular junctions. *J. Physiol.*, **244**, 207–221.

WRIGHT, J.M. & COLLIER, B. (1976). The site of the neuromuscular block produced by polymyxin B and rolitetracycline. *Can. J. Physiol. Pharmac.*, **54**, 926–936.

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