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PRE- AND POSTJUNCTIONAL BLOCKING EFFECTS OF AMINOGLYCOSIDE, POLYMYXIN, TETRACYCLINE AND LINCOSAMIDE ANTIBIOTICS

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SUMMARY

The effects of seven antibiotics (streptomycin, amikacin, polymyxin B, lincomycin, clindamycin, tetracycline and oxytetracycline) were compared with those of magnesium, tubocurarine and lignocaine in the frog sciatic nerve – sartorius muscle preparation, using intracellular recording techniques All compounds except tubocurarine decreased end-plate potential quantal content. The prejunctional effects of magnesium, streptomycin, amikacin, polymyxin B and oxytetracycline (but not the other drugs) were well reversed by increasing the calcium concentration At concentrations which depressed quantal content, only magnesium, tetracycline and oxytetracycline did not reduce postjunctional sensitivity. Further postjunctional effects of the drugs were revealed by alterations in the time-courses of end-plate potentials. All the drugs tested except magnesium, tubocurarine and lincomycin produced changes in muscle action potentials. None of the compounds had anticholinesterase activity. The results confirm that aminoglycoside, polymyxin, tetracycline and lincosamide antibiotics produce neuromuscular block by a combination of both pre- and postjunctional actions.

It has been known for more than 25 years that certain antibiotics can induce paralysis of skeletal muscle. Clinical and experimental studies have shown that muscle paralysis can be caused by four main classes of antibiotic: the aminoglycosides (streptomycin and neomycin), the polymyxins, the tetracyclines and the lincosamides (lincomycin and clindamycin). However, the underlying mechanisms of action of all these groups have not been fully elucidated (for reviews see Pittinger and Adamson, 1972; Sanders and Sanders, 1979; Singh, Marshall and Harvey, 1980; Sokoll and Gergis, 1981).

Neuromuscular mechanisms suggested to be involved include: (1) reduction of the amount of acetylcholine released from the nerve terminals in response to motor nerve stimulation, (2) reduction of the sensitivity of the postjunctional acetylcholine receptors by block of these recognition sites, and (3) reduction of end-plate ionic conductance by blockade of the receptor-activated ion channels. Any of these mechanisms, either alone or in combination, would reduce the end-plate potential (e.p.p.) below the level at which it can initiate a muscle action potential and hence muscle contraction would cease. Additionally, antibiotics may possess a local anaesthetic action which would prevent nerve action potentials from invading the motor nerve terminals, or block muscle action potentials and hence contraction, or have both these effects.

The purpose of the present study was to determine the effects of one or more antibiotics from the above four classes on the different physiological processes involved in neuromuscular transmission in the frog, using intracellular recording techniques, and hence establish more precisely their mechanism of muscle paralysis.

METHODS

Frog sciatic nerve–sartorius muscle preparation

The isolated sciatic nerve–sartorius muscle preparation from *Rana temporaria* (during summer) or *Rana pipiens* (during winter) was used as this muscle possesses large muscle fibres, allowing long periods of stable penetration with microelectrodes to be maintained through several changes of drug solution. No difference was found between results obtained on the two species. The preparations were pinned to a resin-coated 10-ml Perspex tissue bath and bathed in normal frog Ringer solution (sodium chloride 111 mmol litre^{-1}; potassium chloride 2 mmol litre^{-1}; sodium bicarbonate 2 mmol litre^{-1}; calcium chloride 2 mmol litre^{-1}; Tris 1 mmol litre^{-1}) or in high magnesium

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(8 mmol litre^{-1}) – low calcium (1 mmol litre^{-1}) Ringer solution ("high magnesium Ringer"). The pH of the solution was 7.3 and the experiments were conducted at room temperature (16–21 °C).

Intracellular recording

Membrane potentials were measured between an intracellular glass microelectrode filled with potassium chloride 3 mol litre^{-1} or potassium acetate 2 mol litre^{-1} (5–15 Ω resistance) and a silver–silver chloride reference electrode. A high impedance unity gain electrometer (WPI M4A or M701) amplified the signal, which was displayed on a storage oscilloscope and on an oscilloscope fitted with an oscilloscope camera. Filmed records were enlarged and measured by hand.

The preparations were observed at a magnification of 300 times with a binocular microscope fitted with a Leitz UM 20/0.33 long working distance objective. End-plate regions were localized by following nerve twigs and penetrating muscle fibres until spontaneously occurring miniature end-plate potentials (m.e.p.p.) with rise times of less than 1.5 ms could be recorded. Cells were rejected if the initial resting membrane potential was more positive than − 75 mV.

To study evoked transmitter release the sciatic nerve was stimulated at a frequency of 0.5 Hz through a bipolar platinum electrode by rectangular pulses of 0.2 ms duration and of a voltage greater than that required to produce end-plate potentials (e.p.p.). Only one concentration of test drug was used in each experiment and after each addition of drug or change of solution at least 20 min equilibration was allowed before recordings were made.

The effects of the antibiotics were studied on e.p.p. quantal content (that is, the number of packets of acetylcholine released by a single nerve impulse) to assess prejunctional activity; the amplitude of the spontaneously occurring miniature end-plate potentials (m.e.p.p.) to assess postjunctional activity; and end-plate potential time-course to provide an indication of possible activity on end-plate ion channel properties.

At least 50 m.e.p.p. and 150 e.p.p. were recorded from each end-plate region for each measurement. All values of m.e.p.p. and e.p.p. amplitudes were corrected to a standard membrane potential of − 80 mV (Katz and Thesleff, 1957) and e.p.p. were also corrected for non-linear summation (Martin, 1955), assuming a transmitter reversal potential of − 15 mV. Whenever the postjunctional blocking action of a compound under study was sufficiently low to allow m.e.p.p. to be recorded, e.p.p. quantal content was determined by the ratio of the mean amplitudes of e.p.p. and m.e.p.p. However, when m.e.p.p. were reduced to the noise level of the recording system, the method of variance (del Castillo and Katz, 1954) was used for estimation of quantal content, although this method overestimates quantal content at high levels of release (Miyamoto, 1975).

Two types of experiment to assess effects on quantal content were performed: in normal frog Ringer solution in which transmitter release was not impaired by other drugs, and in high magnesium Ringer solution in which evoked transmitter release had been reduced to a low level. In addition, it is known that aminoglycosides exert their prejunctional blocking action by competing with calcium ions for sites on the nerve terminal in a manner similar to magnesium ions (Elmqvist and Josefsson, 1962; Prado, Corrado and Marseillan, 1978; Maeno and Enomoto, 1980). Therefore, in the present study we have attempted to assess if the effects of the other three classes of antibiotic tested are a result of a similar competition with calcium ions by examining the effects of the antibiotics on end-plate potential quantal content at four different calcium concentrations. In preparations in which three changes of calcium concentration were made, penetrations were maintained for 2.5–3 h.

The rise times of m.e.p.p. and e.p.p. were measured from the first discernible depolarization to the peak of the response and the time to half decay was measured from the peak of the response to 50% repolarization. The m.e.p.p. frequency per second was calculated from the number of m.e.p.p. measured × 1000 divided by the number of oscilloscope sweeps × the sweep time (ms).

To assess local anaesthetic activity of the antibiotics, their effects were studied on the configuration of muscle action potentials. Muscle action potentials were elicited by inserting a second microelectrode into a fibre about 600 μm from the recording electrode and stimulating with a rectangular 0.2-ms pulse of strength sufficient to trigger an action potential.

Anticholinesterase activity

The anticholinesterase activity of the antibiotics was assessed using the colorimetric cholinesterase assay described by Ellman and others (1961). Homogenates of frog sartorius muscles (about 5 mg wet weight/ml) were used as the source of cholinesterase with acetylcholine iodide as substrate.

Statistics

The results are presented in the text as means ± SEM of experiments on at least five separate preparations for each drug concentration. Statistical comparisons of means shown in the tables were made by the Mann–Whitney U test, values of $P < 0.05$ being regarded as significant.

Multiple comparisons of slope values for log calcium concentration against log quantal content were made by linear regression analysis of covariance and the Newman–Keuls multiple range test (Zar, 1974). Comparison of single pairs of slope values was made by Student's t test.

Drugs

Drugs used were amikacin sulphate (Mead Johnson), lignocaine hydrochloride (Astra); acetylthiocholine iodide, oxytetracycline hydrochloride, streptomycin sulphate, tetracycline hydrochloride, tubocurarine chloride (all Sigma); clindamycin hydrochloride, lincomycin hydrochloride (both Upjohn) and polymyxin B sulphate (Wellcome or Sigma).

The drug solutions were freshly made before use by dissolving them in the appropriate physiological saline. Drugs were applied by total replacement of the tissue bath fluid or, in the studies on m.e.p.p., by local microperfusion to the end-plate region under investigation (Manthey, 1966).

RESULTS

Effects on m.e.p.p. amplitude and frequency

The effects of seven antibiotics (streptomycin, amikacin, polymyxin B, lincomycin, clindamycin, tetracycline and oxytetracycline) on the amplitude and frequency of m.e.p.p. were assessed in preparations paralysed by bathing in high magnesium Ringer. The effects of tubocurarine (used as a predominantly postjunctionally active drug), lignocaine (used as a local anaesthetic drug) and additional magnesium (used as a predominantly prejunctionally active agent) were also determined.

With all drugs tested there was a gradual decline in m.e.p.p. amplitude, indicating a postjunctional blocking action, starting from the time of application and reaching a constant mean amplitude after 15–20 min. With the exception of magnesium 4 mmol litre^{-1}, tetracycline 0.5 mmol litre^{-1} and oxytetracycline 0.5 mmol litre^{-1}, the decreased amplitude was significantly different from control (fig. 1).

With all drugs apart from lignocaine and clin-

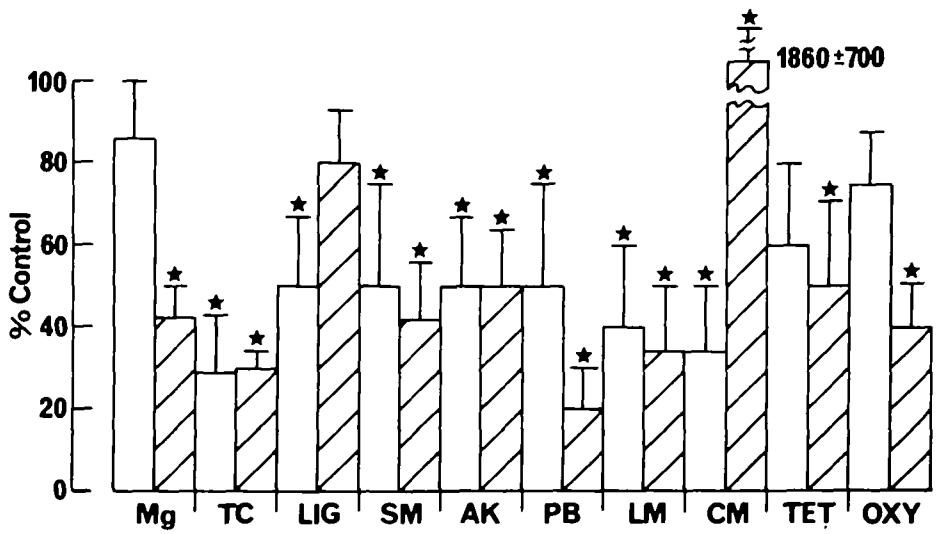

FIG 1. Effects of the 10 test drugs on amplitude (open columns) and frequency (hatched columns) of miniature end-plate potentials (m.e.p.p.) recorded in high-magnesium Ringer solution. Drug concentrations were: magnesium (Mg) 4.0 mmol litre^{-1}; tubocurarine (TC) 1.1 μmol litre^{-1}; lignocaine (LG) 0.35 mmol litre^{-1}; streptomycin (SM) 0.4 mmol litre^{-1}; amikacin (AK) 0.43 mmol litre^{-1}; polymyxin B (PB) 4.6 μmol litre^{-1}; lincomycin (LM) 3.2 mmol litre^{-1}; clindamycin (CM) 0.71 mmol litre^{-1}; tetracycline (TET) 0.5 mmol litre^{-1}; and oxytetracycline (OT) 0.5 mmol litre^{-1}. Values are expressed as % of predrug control values obtained in the same fibres. Control amplitudes ranged from 0.4 to 0.8 mV and control frequencies ranged from 0.5 to 2.3 m.e.p.p. s^{-1}. Each column represents the mean of experiments on at least six preparations; SEM are indicated by the vertical bars. *Significantly different ($P < 0.05$) from control values.

damycin, the decrease in m.e.p.p. amplitude was accompanied by a parallel decline in the frequency of m.e.p.p. (fig. 1). Spontaneous release stabilized at a new level 15–20 min after the start of drug application. In preparations treated with clindamycin 0.71 mmol litre^{-1}, m.e.p.p. frequency increased immediately after the onset of drug application. This increase in frequency was eight times control after 15 min and more than 17 times control after 25 min and was accompanied by a rapid diminution of amplitude so that it was difficult to distinguish small m.e.p.p. from baseline noise.

Effects on e.p.p. quantal content

The effects of seven antibiotics (streptomycin, amikacin, polymyxin B, lincomycin, clindamycin, tetracycline and oxytetracycline) and three control drugs (magnesium, tubocurarine and lignocaine) on e.p.p. quantal content were determined.

A control value of 296 ± 33 for e.p.p. quantal content in the absence of drugs was obtained from sartorius muscle preparations that had been cut to prevent twitching (Lambert et al., 1981). This represents the number of packets of acetylcholine normally released by a single nerve impulse.

Concentrations of paralysing drugs were chosen to be the least that consistently allowed intracellular recording, that is no muscle contraction in response to nerve stimulation, after 20–30 min equilibration time. In muscle paralysed by the predominantly prejunctional blocking substance magnesium (15 mmol litre^{-1}), the amplitudes of e.p.p. fluctuated randomly and the quantal content was 11 ± 2. In the presence of the predominantly postjunctional blocking drug tubocurarine (4 μmol litre^{-1}), the fluctuations of e.p.p. amplitude were slight and quantal content was 248 ± 50, which was not significantly different from the value obtained from the cut muscle preparations. E.p.p. quantal content was depressed by the local anaesthetic lignocaine (0.62 mmol litre^{-1}), but greater concentrations resulted in a sudden abolition of all e.p.p. activity. This effect was probably a result of a block of nerve conduction so that e.p.p. could not be elicited.

In the presence of streptomycin 0.61 mmol litre^{-1} or amikacin 1.7 mmol litre^{-1}, fluctuations of the amplitude of e.p.p. were similar to those recorded in the presence of magnesium and values for quantal contents were similar to those recorded in magnesium (table I). In the presence of the other antibiotics (polymyxin B 9 μmol litre^{-1}, lincomycin 9.7 mmol litre^{-1}, clindamycin 0.71 mmol litre^{-1}, tetracycline 2 mmol litre^{-1} and oxytetracycline 2 mmol litre^{-1}), the variance in amplitudes of e.p.p. was intermediate between that found in magnesium and in tubocurarine, and quantal content ranged from 35 to 106 (table I). E.p.p. could be recorded from only some end-plates in clindamycin-paralysed muscles, and with concentrations of clindamycin greater than 0.71 mmol litre^{-1} no e.p.p. activity could be measured. The pattern of clindamycin-induced block was similar to that seen with lig-

TABLE I. *Effects of the 10 test drugs on mean e.p.p. quantal content in normal and high-magnesium Ringer solution and the effects of different calcium concentrations on the quantal content in normal Ringer solution. Values represent the mean ± SEM of experiments on four to six preparations. *Significantly different (P < 0.05) from control; †slope value significantly different (P < 0.05) from that of magnesium; ‡Control quantal contents in high-magnesium Ringer solution ranged from 3.5 to 23.7*

	Normal Ringer solution			High-magnesium Ringer soln	
Test drug	Concentration	Mean e.p.p. quantal content	Slope of log quantal content: log [Ca] relationship	Concentration	% Reduction of quantal content‡
Control (cut muscle)		296 ± 33	—	—	—
Magnesium	15 mmol litre^{-1}	11 ± 2*	2.57 ± 0.49	4 mmol litre^{-1}	76 ± 4*
Tubocurarine	4 μmol litre^{-1}	248 ± 50	0.53 ± 0.04†	1.1 μmol litre^{-1}	0 ± 24
Lignocaine	0.62 mmol litre^{-1}	198 ± 20*	0.86†	0.35 mmol litre^{-1}	26 ± 13
Streptomycin	0.61 mmol litre^{-1}	16 ± 3*	2.35 ± 0.57	0.40 mmol litre^{-1}	54 ± 6*
Amikacin	1.7 mmol litre^{-1}	12 ± 2*	2.26	0.43 mmol litre^{-1}	42 ± 16*
Polymyxin B	9 μmol litre^{-1}	106 ± 14*	2.31 ± 0.35	4.6 μmol litre^{-1}	65 ± 8*
Lincomycin	9.7 mmol litre^{-1}	75 ± 5*	0.95 ± 0.10†	3.2 mmol litre^{-1}	35 ± 9*
Clindamycin	0.71 mmol litre^{-1}	74 ± 14*	1.25 ± 0.13†	0.71 mmol litre^{-1}	52 ± 25*
Tetracycline	2 mmol litre^{-1}	35 ± 3*	1.36 ± 0.09†	0.50 mmol litre^{-1}	60 ± 11*
Oxytetracycline	2 mmol litre^{-1}	56 ± 4*	1.81 ± 0.14	0.50 mmol litre^{-1}	37 ± 6*

nocaine.

In high-magnesium Ringer, lower concentrations of the drugs than those used in normal Ringer were used as, in most cases, the concentrations used in normal Ringer reduced e.p.p. amplitude to such an extent that accurate measurement of responses was not possible. The concentrations used were two to four times less than those in the normal Ringer experiments, but in the case of clindamycin it was possible to use the same concentration as in the normal Ringer experiments (table I). Smaller concentrations of clindamycin did not affect quantal content and greater concentrations blocked nerve conduction. Apart from tubocurarine and lignocaine, all drugs tested significantly reduced quantal content. The relative effectiveness of the antibiotics was similar to that found in normal Ringer solution.

With all drugs tested, an increase in the calcium concentration increased quantal content, but the relationship between calcium and quantal content differed with different drugs (fig. 2). Log–log slopes of quantal content against calcium concentration yielded straight lines (Dodge and Rahamimoff, 1967) with different slopes for different drugs (table I). Magnesium-paralysed preparations gave the largest value for the slope (2.57), whereas tubocurarine-paralysed preparations had the smallest value (0.53). Other values were between these two extremes (table I). The slopes were ranked and the slope for magnesium was compared with those of all the other drugs by linear regression analysis of covariance and Newman–Keuls multiple range test. This test is a ranking test that takes into account the scatter of the data for all the compounds tested when comparing the relative positions of a pair of compounds in the rank order. This reduces the probability of type 1 statistical errors. A large number of comparisons can be made using this test, but for the sake of clarity only the values related to the magnesium slope are included. The results are expressed as P values to indicate the level of probability of difference from magnesium and are as follows: tubocurarine, lignocaine and lincomycin ($P<0.05$); clindamycin and tetracycline ($P<0.2$); oxytetracycline ($P<0.5$); polymyxin B, streptomycin and amikacin ($P>0.5$). Individual pairs of slope values were also compared using Student's t test which disregards the data from other compounds tested in the same way. Using this test the slope for magnesium was not significantly different from those for streptomycin, amikacin, polymyxin B, or oxytetracycline, but was significantly greater than those of the remaining compounds ($P<0.05$). Other pairs of drugs tested showed that the slope for oxytetracycline was significantly greater than that for tetracycline, but that no difference existed between lincomycin and clindamycin, and between polymyxin B and oxytetracycline. Thus, in both statistical tests streptomycin, amikacin, polymyxin B and oxytetracycline were not significantly different from magnesium and hence it is likely that competition with calcium plays a part in the prejunctional action of these four agents.

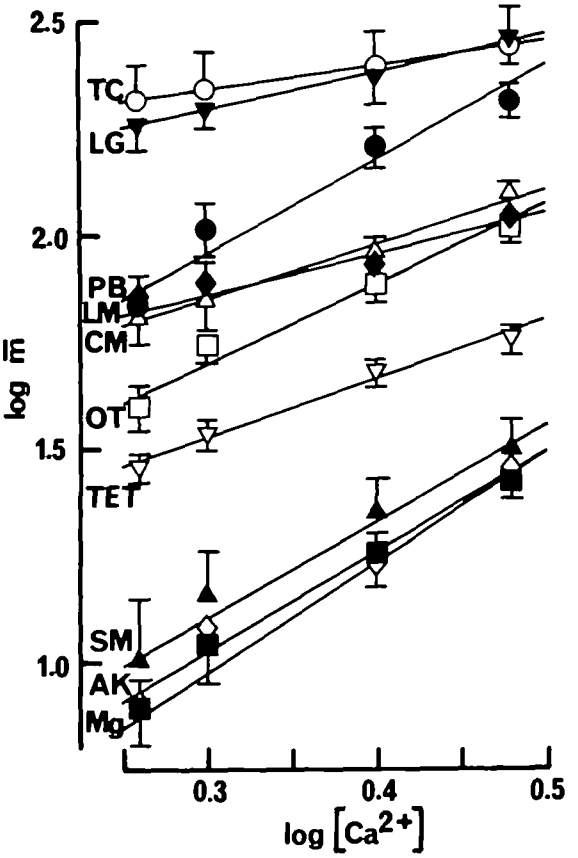

FIG 2. Log–log plot of the effect of different calcium concentrations in normal frog Ringer solution on mean e.p.p. quantal content ($\bar{m}$) recorded in preparations paralysed with tubocurarine 4 μmol litre^{-1} (TC, ○), lignocaine 0.62 mmol litre^{-1} (LG,▼), polymyxin B 9 μmol litre^{-1} (PB,●), lincomycin 9.7 mmol litre^{-1} (LM,◆), clindamycin 0.71 mmol litre^{-1} (CM,△), oxytetracycline 2 mmol litre^{-1} (OT, □), tetracycline 2 mmol litre^{-1} (TET,▽), streptomycin 0.61 mmol litre^{-1} (SM,▲), amikacin 1.7 mmol litre^{-1} (AK,◇) and Mg^{2+} 15 mmol litre^{-1} (Mg,■). Points represent the mean (± SEM) of experiments on four or five preparations. Lines through the points were derived by linear regression (slopes are given in table I).

Effects on time-course of e.p.p. and m.e.p.p. and on cholinesterase

The effects of the antibiotics and magnesium, tubocurarine and lignocaine on the time-course of m.e.p.p. and e.p.p. were determined in high-magnesium Ringer and in normal Ringer. The effects of the drugs on both e.p.p. and m.e.p.p. were qualitatively the same in both types of experiments. Figure 3 summarizes the effects on e.p.p. time-course in high-magnesium Ringer. Polymyxin B, clindamycin and lignocaine reduced both rise time and time to 50% decay, whereas the other drugs had no effect on rise time but increased time to 50% decay. Amikacin was not tested in this way.

Effects on time-course could not be ascribed to effects on cholinesterase as none of the compounds possessed significant anticholinesterase activity in concentration ranges which produced marked effects on the time-course of potentials.

Effects on muscle action potentials

With the exception of magnesium, tubocurarine and lincomycin, all compounds in concentration ranges similar to those inducing neuromuscular paralysis produced marked changes in muscle action potential parameters (fig. 4). Magnesium 15 mmol litre^{-1}, tubocurarine 4 μmol litre^{-1} and lincomycin 4–16 mmol litre^{-1} had no significant effect on overshoot, maximum rate of rise or maximum rate of fall of action potentials. Amikacin was not tested.

Tetracycline 2 mmol litre^{-1} and oxytetracycline 2 mmol litre^{-1} significantly reduced both maximum rate of rise and maximum rate of fall of action potentials. Overshoot was not significantly affected. The effects on rates of rise and fall developed progressively within the first 10–20 min of exposure and then remained constant during the remainder of the period of exposure.

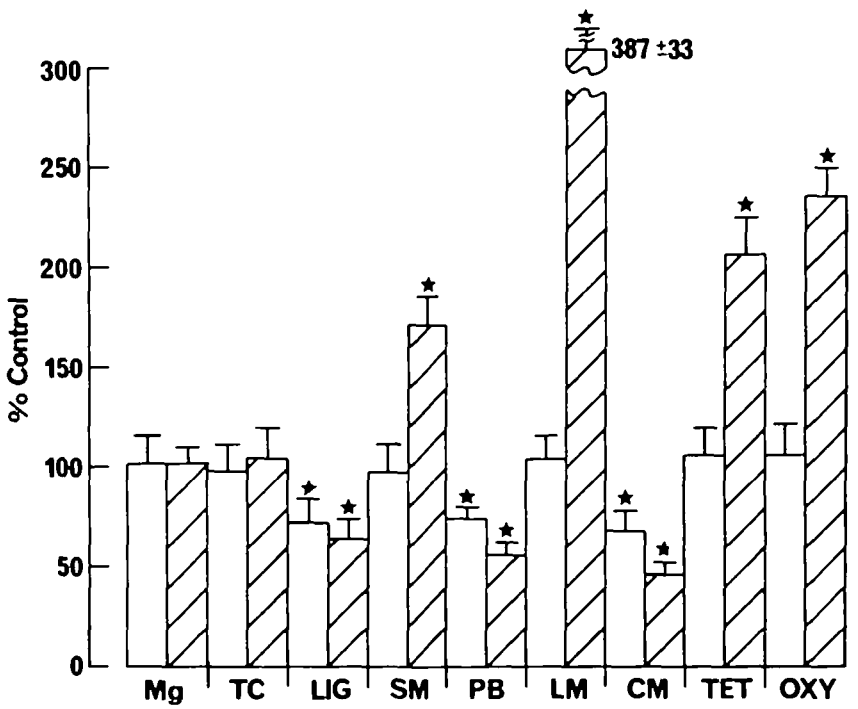

FIG. 3. Effects of nine test drugs on rise time (open columns) and time to 50% decay (hatched columns) of e.p.p. recorded in high-magnesium solution. Drugs used were as given in the legend to figure 1. Values are expressed as % of predrug control values obtained in the same fibres. Control rise times ranged from 1.31 to 1.37 ms and control times to 50% decay ranged from 2.66 to 3.05 ms. Each column represents the mean of experiments on at least six preparations; SEM are indicated by the vertical bars. *Significantly different ($P < 0.05$) from control values.

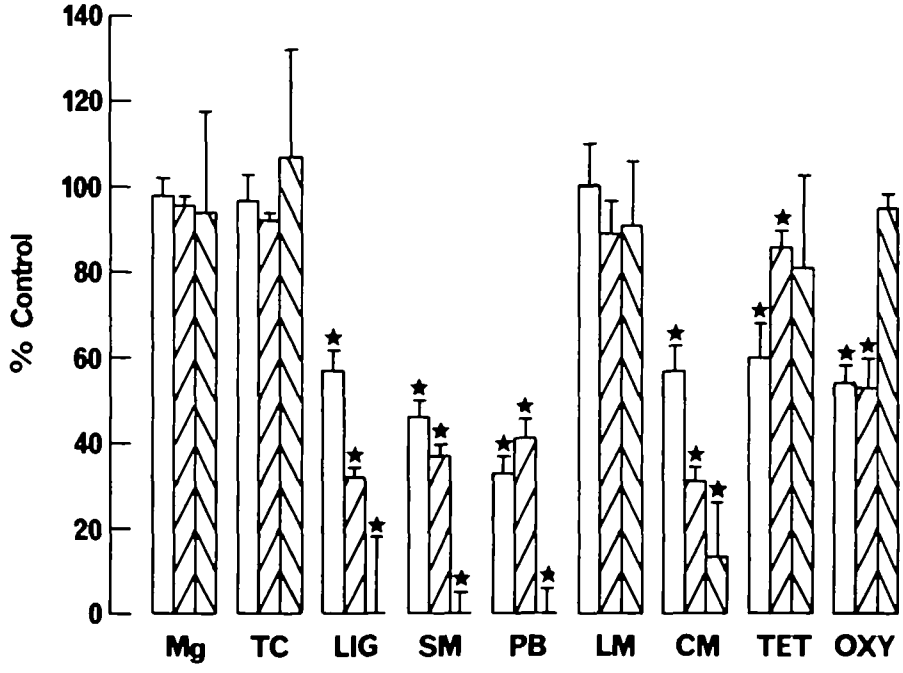

FIG 4. Effects of nine test drugs on overshoot, maximum rates of rise and of fall of muscle action potentials. Lefthand columns: maximum rate of rise; middle columns: maximum rate of fall; right hand columns: overshoot. Drug concentrations were: magnesium (Mg) 15 mmol litre^{-1}; tubocurarine (TC) 4 μmol litre^{-1}; lignocaine (LG) 1.4 mmol litre^{-1}; streptomycin (SM) 2.4 mmol litre^{-1}; polymyxin B (PB) 43 μmol litre^{-1}; lincomycin (LM) 16 mmol litre^{-1}; clindamycin (CM) 1.4 mmol litre; tetracycline (TET) 2 mmol litre^{-1}; and oxytetracycline (OT) 2 mmol litre^{-1}. Measurements were made after 60 min exposure to the test drugs and values are expressed as % of predrug control values obtained in the same fibres. Control values for maximum rate of rise ranged from 369 to 464 V s^{-1}; for maximum rate of fall from 106 to 133 V s^{-1}; and for overshoot from 28 to 38 mV. Each column represents the mean of experiments on at least six preparations, SEM are indicated by the vertical bars. *Significantly different ($P < 0.05$) from control values.

Clindamycin 2.8 mmol litre^{-1} produced a biphasic effect on action potential configuration. During the first 20–30 min of exposure, maximum rates of rise and fall were reduced and the overshoot became progressively smaller until the action potential failed to reach the zero potential at around 30 min. Further exposure to clindamycin resulted in a secondary increase in the maximum rate of rise from its depressed level until after 60 min the rate of rise was greater than that of control action potentials. There was no parallel secondary increase in the maximum rate of fall or overshoot, which remained depressed. After 60–90 min it became impossible to generate action potentials and at this time a stable membrane potential could not be recorded. At lower concentrations of clindamycin (0.7–1.4 mmol litre^{-1}) similar depressions of overshoot, maximum rates of rise and fall were observed without the secondary increase in the maximum rate of rise. Action potentials could still be generated 120 min after application of drug.

Streptomycin 1.2–2.4 mmol litre^{-1}, polymyxin B 0.043–0.086 mmol litre^{-1} and lignocaine 1.4 mmol litre^{-1} also decreased maximum rates of rise and fall and reduced the overshoot (fig. 4). Action potentials usually failed to reach zero potential at the greater concentrations of the drugs. With these drugs the reduction in action potential parameters progressed until no action potentials could be generated, and there was no secondary increase in the rates of rise.

Except with clindamycin, no changes in resting membrane potentials were noted with the compounds, even at the greatest concentrations used.

DISCUSSION

The results of the present study confirm and extend those from previous studies by showing that mem-

bers of the four classes of antibiotics tested possess relatively different effects on the various stages of neuromuscular transmission.

The concentrations used in the study were those that caused complete cessation of muscle twitching in response to nerve stimulation in normal solution. Concentrations two to four times smaller were used in the magnesium-paralysed preparations. In the main, these concentrations are greater than the "maximum therapeutic level" as recently used by Caputy, Kim and Sanders (1981). Nevertheless, it is important to determine mechanisms of side-effects of drugs at concentrations greater than those normally attained in the body. In this way an attempt is made to account for different susceptibilities of individual patients or different animal models to the drugs.

The neuromuscular blockade produced by the aminoglycosides streptomycin and amikacin was similar to that produced by a high concentration of Mg^{2+}, that is it was associated with a large significant decrease in evoked release of acetylcholine and with a smaller but still significant decrease in postjunctional receptor sensitivity and spontaneous release. These findings agree with results obtained in the mouse diaphragm with streptomycin (Singh, Marshall and Harvey, 1979) and amikacin (Singh, Marshall and Harvey, 1978a) and in the rat hemidiaphragm preparation with neomycin and gentamicin (Elmqvist and Josefsson, 1962; Caputy, Kim and Sanders, 1981). The effects of altering the external Ca^{2+} concentration on the neuromuscular blockade induced by the aminoglycosides were similar to the effects on Mg^{2+}-induced blockade, confirming the suggestion that the aminoglycosides reduce transmitter release by a mechanism similar to that of Mg^{2+} involving a competition for Ca^{2+} binding sites on the nerve terminal (Prado, Corrado and Marseillan, 1978; Maeno and Enomoto, 1980).

The effects of streptomycin on m.e.p.p. amplitude and on e.p.p. and m.e.p.p. time-course suggest that postjunctional effects could be attributable to either receptor or end-plate ion channel blockade. Channel blockade is usually measured from changes of the decay characteristics of end-plate currents measured under voltage clamp conditions, but changes in end-plate current time-course will be reflected to some extent in the time-course of end-plate potentials. Voltage clamp studies have shown that streptomycin possesses end-plate ion channel blocking activity in the mouse (Pennefather and Quastel, 1980), but not in the snake (Fiekers and Parsons, 1980). In the present experiments the aminoglycosides had no effect on muscle cholinesterase activity and hence the prolongation of e.p.p. and m.e.p.p. cannot be explained by such an action.

As previously found in other preparations (Sokoll and Diecke, 1969; Diecke, Westecker and Vogt, 1971) streptomycin altered the time-course of action potentials, indicating that it has blocking effects on electrically excitable membrane ion channels. The concentrations required were greater than those causing neuromuscular blockade. Although nerve terminals are probably more sensitive than muscle fibres to any local anaesthetic action, it is unlikely that such an action will contribute to the neuromuscular blocking action, as the characteristics of the neuromuscular block were quite different from that produced by the local anaesthetic lignocaine.

Paralysing concentrations of polymyxin B reduced quantal content, although not to as great extent as Mg^{2+} or the aminoglycosides. Previously, experiments involving collection and assay of acetylcholine from isolated rat diaphragm (Brownlee, 1957; Wright and Collier, 1976a) and frog gastrocnemius preparations (Dretchen et al., 1972) have failed to show any effects of polymyxin B or colistin (polymyxin E) on acetylcholine release. However, intracellular recording methods are probably more sensitive than transmitter collection experiments, and the finding of decreased acetylcholine release in the presence of polymyxin B is in accord with results obtained in the mouse diaphragm preparation (Singh, Marshall and Harvey, 1979) and in the cut frog cutaneous pectoris muscle (Durant and Lambert, 1981). The slope of the log Ca^{2+} concentration against log quantal content in polymyxin B was similar to that of magnesium and the aminoglycosides, suggesting a prejunctional competition with calcium. The fact that polymyxin B-induced blockade of muscle twitch is not well reversed by increased Ca^{2+} (Singh, Harvey and Marshall, 1978) may indicate that the prejunctional action of the compound on quantal content is less important than other actions in reducing twitch tension.

As shown in the rat diaphragm (Brownlee, 1957; Lüllmann and Reuter, 1960; Wright and Collier, 1976a; Caputy, Kim and Sanders, 1981) and polymyxin B reduced postjunctional sensitivity. Since polymyxin B markedly reduced e.p.p. and m.e.p.p. time-courses, it is probable that the drug at least partially decreases postjunctional sensitivity by blocking end-plate ion channel conductance, as has been shown in voltage-clamped snake and frog mus-

cle (Fiekers and Parson, 1980; Durant and Lambert, 1981; Fiekers, 1981).

Polymyxin B also produced effects on muscle action potentials, although this was seen only in concentrations greater than those needed to induce muscle paralysis. Wright and Collier (1976a) postulated that the local anaesthetic action of polymyxin B plays a large part in the muscle-paralysing actions of the antibiotic. However, the characteristics of the neuromuscular block produced by polymyxin B were quite different from those produced by the local anaesthetic lignocaine. Hence it is likely that the effects of polymyxin B on neuromuscular transmission are at least as important as the local anaesthetic activity of the compound.

The muscle-paralysing effects of tetracyclines have been studied less extensively than those of other types of antibiotics (Pittinger and Adamson, 1972). In the present study tetracycline and oxytetracycline decreased e.p.p. quantal content but did not significantly affect m.e.p.p. amplitude, suggesting that these antibiotics have predominantly prejunctional blocking actions. However, an action on muscle contractility may be as important as the neuromuscular blocking properties, since it was previously shown that tetracycline and oxytetracycline block both indirectly and directly stimulated mouse diaphragm preparations (Singh, Harvey, Marshall, 1978) and both drugs were found to depress muscle action potentials at the same concentration that decreased quantal content.

There were differences between the effects of the tetracyclines used in the present study and the primarily postjunctional effects previously reported for rolitetracycline (Wright and Collier, 1976a). In addition, there was a suggestion of differences in the

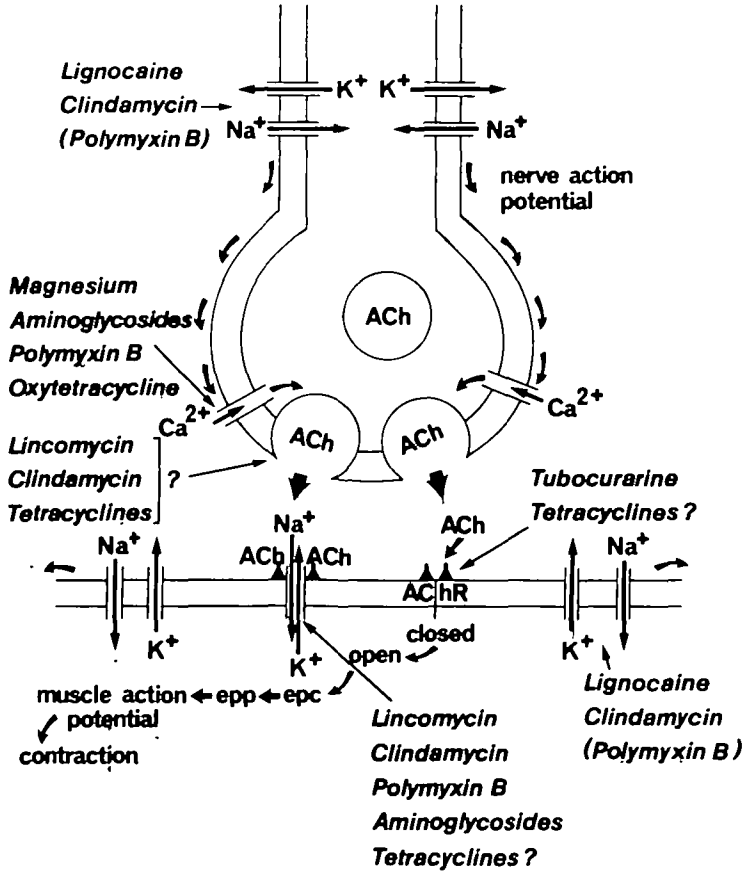

FIG 5. Schematic representation of neuromuscular transmission and suggested sites of action of some antibiotics.

effects of tetracycline and oxytetracycline. For example, the action of oxytetracycline on quantal content was as Ca^{2+}-sensitive as that of Mg^{2+}, whereas the action of tetracycline appeared to be less Ca^{2+}-sensitive than oxytetracycline. This suggests that tetracycline may act differently from Mg^{2+} at the nerve terminal, while oxytetracycline may act in a fashion somewhat similar to Mg^{2+}. Supportive evidence for this suggestion is that, in the isolated mouse phrenic nerve–hemidiaphragm preparation oxytetracycline, but not tetracycline, is reversed by doubling the concentration of Ca^{2+} (Singh, Harvey and Marshall, 1978) or by 3, 4-diaminopyridine (Singh, Marshall and Harvey, 1978b).

Despite the close chemical similarity of lincomycin and clindamycin there is evidence suggesting that these two lincosamides produce muscle paralysis by different mechanisms (Wright and Collier, 1976b; Rubbo, Gergis and Sokoll, 1977; Singh, Harvey and Marshall, 1978). In the present study, both drugs had pre- and postjunctional blocking actions. The prejunctional effects were not magnesium-like as they were significantly less affected than magnesium by alterations in external Ca^{2+} concentration. The prejunctional effects of clindamycin were detectable only in a very narrow concentration range and, as reported previously (Rubbo, Gergis and Sokoll, 1977), were accompanied by an increase in m.e.p.p. frequency, which was not seen with lincomycin.

The effects of clindamycin on the time-course of e.p.p. and m.e.p.p. were qualitatively similar to those of polymyxin B, suggesting, as has been shown by Fiekers, Marshall and Parsons (1979), that the compound blocks end-plate channel conductance. The prolongation of e.p.p. and m.e.p.p. by lincomycin was not explicable by an anticholinesterase action of the compound, but may be explained by the kinetics of the blocking action of the compound on channel conductance (Fiekers, Marshall and Parsons, 1979).

At neuromuscular blocking concentrations lincomycin had no detectable effect on muscle action potentials, whereas clindamycin had pronounced activity. Wright and Collier (1976b) have suggested that the local anaesthetic activity of clindamycin probably plays a major role in producing muscle paralysis and we have confirmed that the neuromuscular blocking action of clindamycin is similar to that of the local anaesthetic lignocaine.

Figure 5 shows a schematic representation of the potential sites of action of antibiotic drugs at the neuromuscular junction and surrounding areas, based on the present and previous data. It can be concluded that different antibiotics, often of the same chemical class, possess different mechanisms of action at the neuromuscular junction and hence caution is necessary when extrapolating data from a "typical" member of a class of antibiotics to any other antibiotic.

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EFFETS DE BLOCAGE PRE- ET POST-JONCTIONNELS DE DIVERS ANTIBIOTIQUES: AMINOSIDES, POLYMIXINE, TETRACYCLINE ET LINCOSAMIDE

RESUME

Les effets de sept antibiotiques (streptomycine, amikacine, polymixine B, lincomycine, clindamycine, tétracycline et oxytetracycline) ont été comparés à ceux du magnésium, de la tubocurarine et de la lignocaine sur une préparation de muscle de crapeau en utilisant des techniques d'enregistrement intracellulaires. Tous les composés, à l'exception de la tubocurarine, diminuaient le nombre de potentiels miniatures. Les effets présynaptiques du magnésium, de la streptomycine, de l'amikacine, de la polymyxine B et de l'oxytétracycline (mais pas des autres agents) étaient bien annulés en augmentant la concentration calcique. A des concentrations qui déprimaient le nombre de potentiels miniatures, seuls le magnésium, la tétracycline et l'oxytétracycline ne diminuaient pas la sensibilité post-synaptique. D'autres effets post-synaptiques des agents ont été révélés par des modifications du devenir en fonction du temps des potentiels post-synaptiques. Tous les agents testés, à l'exception du magnésium, de la tubocurarine et de la lincomycine, entraînaient des modifications des potentiels d'action musculaires. Aucun de ces composés n'avait d'activité anticholinestérasique. Les résultats confirment que l'aminoglycoside, la polymyxine, la tétracycline et la lincosamide entraînent un bloc neuromusculaire par une association d'actions à la fois pré- et post-synaptiques.

PRÄ- UND POSTSYNAPTISCHE HEMMWIRKUNG DER AMINOGLYKOSIDE, VON POLYMYXIN, TETRAZYKLIN UND LINCOSAMIDANTIBIOTIKA

ZUSAMMENFASSUNG

Die Wirkungen von sieben Antibiotika (Streptomycin, Amikacin, Poylymyxin B, Linkomycin, Klindamycin, Tetrazyklin und Oxytetrazyklin) wurden mit denen von Magnesium, Tubokurarin und Lidokain an einem N.-Sartorius Präparat aus dem Ischiasnerv des Frosches verglichen, indem man sich intrazellulärer Aufzeichnungstechniken bediente. Alle Mittel außer Tubokurarin setzten den Quanteninhalt der Endplattenpotentiale herab. Die präsynaptischen Wirkungen von Magnesium, Streptomycin, Amikacin, Polymixin B und Oxytetrazyklin (aber nicht die der anderen Mittel) wurde gut durch Erhöhung der Calzium-Konzentration aufgehoben. Bei Konzentrationen, die den Quanteninhalt herabsetzten, setzte nur Magnesium, Tetrazyklin und Oxytetrazyklin die postsynaptische Empfindlichkeit nicht herab. Weitere postsynaptische Wirkungen der Drogen zeigten sich durch Änderungen der Zeitverläufe der Endplattenpotentiale. Alle Mittel, außer Magnesium, Tubokurarin und Linkomycin riefen Veränderungen der Zeitverläufe der Endplattenpotentiale hervor. Keines der Mittel hatter eine anticholinesterase-Aktivitat. Die Ergebnisse bekräftigen, daß Aminoglykoside, Polymixin, Tetrazykline und Lincosamid-Antibiotika die neuromuskuläre Blockade durch eine Kombination von sowohl prä- als auch postganglionären Wirkungen entfalten.

EFECTOS DE BLOQUEO PRE- Y POST-JUNCIONAL DEL AMINOGLICOSIDO, DE LA POLIMIXINA, DE LA TETRACICLINA Y DE LOS ANTIBIOTICOS DE LINCOSAMIDO

SUMARIO

Se llevó a cabo una comparación de los efectos de siete antibióticos (estreptomicina, amiquacina, polimixina B, lincomicina, clindamicina, tetraciclina y oxitetraciclina) con los del magnesio, de la tubocurarina y de la lignocaina en preparaciones músculo sartorio–nervio sciático de rana, mediante técnicas de registro intracelular. Todos los compuestos salvo la tubocurarina hicieron bajar el contenido cuántico del potencial de placa-terminal. Al aumentar la concentración de calcio, se invertieron bastante los efectos pre-juncionales del magnesio, de la estreptomicina, de la amiquacina, de la polimixina B y de la oxitetraciclina (pero, no fué así para las demás substancias). Con concentraciones que deprimían el contenido cuántico, sólo el magnesio, la tetraciclina y la oxitetraciclina no pudieron reducir la sensibilidad post-juncional. Las alteraciones de las trayectorias-tiempo de los potenciales de placa-terminal revelaron efectos post-juncionales adicionales de las drogas. Todas las substancias ensayadas salvo el magnesio, la tubocurarina y la lincomicina, provocaron cambios de los potenciales de acción del músculo. Ninguno de los compuestos tuvo alguna actividad anticolinesterasa. Los resultados permiten confirmar que los antibióticos de aminoglicosido, polimixina, tetraciclina y lincosamido producen un bloqueo neuromuscular mediante una combinación de las acciones pre- y post-juncionales.