THE MECHANISMS OF THE MUSCLE PARALYSING ACTIONS OF ANTIBIOTICS, AND THEIR INTERACTION WITH NEUROMUSCULAR BLOCKING AGENTS

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1. INTRODUCTION

The first major reports of adverse reactions to the use of antibiotics concerned their nephrotoxicity, ototoxicity, and neuropathological hypersensitivity and allergic reactions (for references, see /1/). Reports that the intravenous administration of large doses of streptomycin caused respiratory paralysis in mice /2/ and that the animals could be saved by artificial ventilation /1/, were ignored although these findings indicated that streptomycin may possess muscle paralysing properties.

Systematic investigation of the neuromuscular effects of streptomycin and other antibiotics /3-5/ started only after Pridgen (1956) /6/ reported the first clinical cases of prolonged respiratory depression following the intravenous administration of neomycin in conjunction with ether anaesthesia. Subsequently a large number of antibiotics were implicated in cases of prolonged apnoea, usually when the patients had also received muscle relaxants such as tubocurarine /7-9/, gallamine /10, 11/ or general anaesthetics /12, 13/.

Although the concurrent administration of relaxants and anaesthetics has been the most common and most important precipitant of antibiotic-induced paralysis, various other factors have also been involved:

a. the route of administration *e.g.* intraperitoneal or intravenous instillation, which may result in rapid absorption of the drug leading to a toxic blood concentration /14/.
b. accidental overdosage or a rate of administration greater than that recommended /15, 16/.
c. administration of therapeutic doses to patients with impaired renal function leading to accumulation of antibiotics in toxic concentrations /11, 17, 18/ and
d. use of antibiotics in patients with neuromuscular disorders, *e.g.* Eaton-Lambert syndrome (myasthenic syndrome) and myasthenia gravis /19-21/.

Experimental studies in animal preparations have not only confirmed the neuromuscular blocking properties of antibiotics that induce prolonged apnoea in man but have also demonstrated that many other antibiotics have paralysing actions. However, not all antibiotics have muscle paralysing properties even when used in conjunction with muscle relaxants or anaesthetics or in concentrations higher than clinical doses. For example, bacitracin /22/, chloramphenicol /23/, the penicillins /5, 24, 25/, the cephalosporins, and cephamycins /26, 27/ have been reported to lack neuromuscular blocking activity.

The antibiotics known to produce muscle paralysis may be classified chemically into four main groups:

a. aminoglycosides
b. polymyxins
c. tetracyclines
d. lincosamides.

This review will consider the mechanisms of action of these four groups of antibiotics and discuss ways to reverse antibiotic-induced muscle paralysis.

2. THE AMINOGLYCOSIDES

2.1. Neuromuscular Effects

Members of this group shown to possess neuromuscular blocking actions include amikacin /28, 29/, aminosidine /22/, dihydrostreptomycin /30/, gentamicin and kanamycin /31, 32/, neomycin /3, 6/, streptomycin /5/ and tetramycin /33/.

These compounds are all organic bases containing amino sugars linked to the hydroxyl groups of either streptidine or its chemical congener deoxystreptamine /34/. It is now well established that the streptidine and deoxystreptamine moieties are responsible for the neuromuscular paralysing actions of these antibiotics /35, 36/.

The antibiotics in this group appear to share similar pharmacological properties, such as inhibition of synaptic transmission at various neuroeffector junctions /37, 38/, ototoxicity /39/, nephrotoxicity /40/, depression of cardiovascular function /41, 42/ and relaxation of different types of smooth muscle /43, 44/.

2.2. Mechanism of Action

The aminoglycosides do not directly depress elicited muscle twitches /3, 45/ and only affect nervous conduction at concentrations many times higher than those producing muscle paralysis /46-48/, indicating that these antibiotics produce muscle paralysis primarily by interfering with neuromuscular transmission. The aminoglycosides produce a progressive flaccid paralysis of skeletal muscles with no initial facilitatory action /7, 30, 38, 45, 49, 50/. Thus, the actions of the aminoglycosides superficially resemble those of non-depolarizing neuromuscular blocking agents such as tubocurarine, gallamine, and pancuronium which are

known to act primarily by blocking postjunctional acetylcholine receptors. However, neuromuscular block produced by tubocurarine-like drugs is well reversed by anticholinesterase agents whereas that produced by aminoglycoside antibiotics is only gradually and partially reversed by anticholinesterase drugs /3, 7, 29, 50, 51/. In contrast aminoglycoside-induced neuromuscular block is well-reversed by calcium salts whereas calcium has very little effect on tubocurarine-induced block /3, 7, 29, 38, 51/. The observations on the reversibility of the aminoglycosides plus a comparison of the post-tetanic twitch facilitation during streptomycin-induced neuromuscular block with that seen during magnesium-induced block /52/ led Vital Brazil and Corrado (1957) /3/ to propose that streptomycin acted by a magnesium-like action.

2.2.1. Prejunctional Actions

The main action of magnesium on neuromuscular transmission is to reduce the release of acetylcholine in response to nerve stimulation /53/. Magnesium is thought to act at the nerve terminal membrane by competing with calcium ions which are essential for the synchronous release of acetylcholine /54-57/. Dodge and Rahaminoff (1967) /57/ have proposed that magnesium acts at the postulated "calcium site" but that magnesium has a higher dissociation constant than calcium and is ineffective in promoting the release of acetylcholine. The above theory is based on experimental evidence obtained from intracellular microelectrode recording studies on isolated nerve-muscle preparations. In this type of experiment magnesium is found to reduce the endplate potential (e.p.p.), *i.e.* the potential change in response to the evoked release of acetylcholine, below the level at which it can trigger a muscle action potential. In addition e.p.p.s fluctuate randomly in amplitude. Miniature endplate potentials (m.e.p.p.s), *i.e.* the potential changes in response to the spontaneous release of individual packets, or quanta, of acetylcholine, are reduced only slightly in both amplitude and frequency. Statistical analysis of these observations indicates that magnesium reduces the number of quanta of acetylcholine released by nerve stimulation and only slightly reduces postjunctional acetylcholine sensitivity /53, 55/. Similar experiments with aminoglycoside antibiotics have shown that neomycin, gentamicin /58/, streptomycin /36, 59/, amikacin /47/ and the aminoglycoside-like agent spectinomycin /60/ produce prejunctional effects similar to those of magnesium. In addi-

tion it was found that neomycin, like magnesium, inhibited the increase in m.e.p.p. frequency produced by increasing the extracellular potassium concentration /58/. The increase in m.e.p.p. frequency produced by potassium-induced depolarization is also dependent upon the influx of extracellular calcium ions into the nerve terminal and hence this finding is additional evidence that the aminoglycosides act by competing with calcium for prejunctional receptors involved in acetylcholine release.

Inhibitory effects of aminoglycoside antibiotics on acetylcholine release have also been demonstrated by collecting and assaying the acetylcholine released either spontaneously or by nerve stimulation. Neomycin and gentamicin have been shown to have no effect on spontaneous release measured in this way /32/ but neomycin /32, 61/, gentamicin /32/ and streptomycin /61/ all reduced the amount of acetylcholine released in response to nerve stimulation. At equiactive neuromuscular blocking doses neomycin was found to be more effective than streptomycin in reducing evoked release /61/.

Aminoglycoside antibiotics have essentially the same effects at sympathetic ganglia as their effects at the neuromuscular junction /62,63/ and the superior cervical ganglion of the cat has been used to confirm the inhibitory action of neomycin on the prejunctional functioning of calcium /61/. In these experiments preganglionic stimulation increased ^{45}Ca uptake by the ganglion, and this uptake was inhibited competitively by neomycin but not by the postjunctionally active tubocurarine.

2.2.2. Postjunctional Effects

In addition to the evidence described above for a prejunctional action of aminoglycoside antibiotics and magnesium there is also evidence that these agents have some postjunctional effects.

Neomycin and gentamicin both depress acetylcholine-induced contractures in the denervated rat hemidiaphragm and increasing concentrations produce rightwards shifts of the dose-response curve to the agonist /32,58/. In the innervated rat hemidiaphragm, equiactive neuromuscular blocking concentrations of neomycin, streptomycin and tubocurarine were found to depress responses to close intravenous injection of acetylcholine to differing degrees /61/. Thus the predominantly postjunctionally active tubocurarine was more effective than either of the antibiotics, but in addition streptomycin was significantly more effec-

tive than neomycin indicating that postjunctional blocking activity probably plays a greater part in the neuromuscular blocking action of streptomycin than in the action of neomycin.

The postjunctional actions of aminoglycosides including neomycin /58/, streptomycin /59, 64/, amikacin /29/ and spectinomycin /60/ have also been investigated in intracellular recording studies and the aminoglycosides have been shown to reduce the amplitude of m.e.p.p.s. and/or responses to iontophoretically applied acetylcholine.

Magnesium, in addition to its prejunctional actions, possesses some postjunctional blocking action at high concentrations. However, despite the similarities between the pre- and postjunctional blocking actions of magnesium and the aminoglycosides it is unlikely that the postjunctional actions of either are mediated by competition with calcium ions as calcium itself has postjunctional blocking actions /65/.

The relative contribution of the pre- and postjunctional components to the action of the aminoglycosides is difficult to assess, particularly in the clinical situation when the action of the antibiotics is likely to be superimposed on the action of other neuromuscular depressant drugs. Information from intracellular recording studies shows that postjunctional sensitivity tends to be reduced more than is evoked release at low antibiotic concentrations. However the concentration-inhibition curve for inhibition of release is steeper than that for postjunctional sensitivity so that inhibition of release becomes more dominant as the concentration is increased /47, 64/.

2.3. Chelation and Competitive Hypotheses

As described above there is convincing evidence that the aminoglycosides act like magnesium to compete with calcium at prejunctional sites and also to block at the postjunctional membrane. However the observation that neomycin could reduce the blood level of ionised calcium /66/ led Corrado (1963) /67/ to suggest that the mechanism of the neuromuscular blocking action of the antibiotics involved the reduction of extracellular calcium levels. Many of the indirect observations purporting to support the chelation hypothesis /67/ also support a mechanism involving competition with calcium. Moreover, direct measurements of calcium levels in physiological solutions or in human and rabbit sera using either the murexide technique or the calcium selective electrode technique have failed to show reduction of calcium levels by neomycin

or streptomycin /58, 61, 68/. However kanamycin has been shown to reduce ionised calcium levels of solutions of calcium in distilled water /69/.

In addition to the conflicting evidence on the ability of aminoglycosides to chelate calcium under physiological conditions, it is unlikely that the chelation hypothesis can explain the pharmacological actions of the aminoglycosides at the neuromuscular junction. Thus, reduction of extracellular ionised calcium levels would be expected to reduce acetylcholine release but would also be expected to produce an increase, rather than the observed decrease, in postjunctional excitability.

Evidence from another area supports the calcium competition mechanism of action of aminoglycosides. Neomycin and gentamicin have been shown to produce a negative inotropic effect in the hearts of anaesthetized monkeys, and this action has been shown to be due to a direct myocardial depressant action of the aminoglycosides /41, 42/. This action of the antibiotics was antagonized competitively by calcium /42/. Subsequently, gentamicin has been shown to inhibit the influx of calcium into the myocardial cells /70/.

3. THE POLYMYXINS

3.1. Neuromuscular Effects

The polymyxins consist of a series of chemically related cyclic compounds having a 7-membered polypeptide ring attached to a short polypeptide chain which terminates in a branched fatty acid, 6-methyloctanoic acid /71, 72/. The polypeptide chain and ring are made up of 2,4-diaminobutyric acid and various amino acids so that the molecular weight of these antibiotics is approximately a thousand. Polymyxins bind strongly to the phospholipid component of bacterial membranes destroying both membrane integrity and function and leading to death of the bacterium /73/. Besides the similarities in chemical structure, these compounds have been shown to possess pharmacological properties which are almost identical. They have very similar antimicrobial spectra, exhibit cross resistance and nephrotoxicity /74-76/.

Members of the polymyxin group include polymyxins A, B, C, D, E (colistin), -M and colistimethate (colistin methane sulphonate). Of these only polymyxin A /22/, polymyxin B /5, 77/, colistin /22/ and colistimethate /78, 79/ have been shown to produce neuromuscular block.

Unlike the aminoglycosides where the streptidine and deoxystreptamine moieties possess neuromuscular blocking properties, the intact polypeptide molecules of the polymyxins are necessary for activity as the individual components (amino acids, 2,4-diaminobutyric acid and 6-methyloctanoic acid) do not produce neuromuscular blockade /22/.

Polymyxins have been implicated in more than 30 reported cases of clinical paralysis /59, 80, 81/, most of which occurred when the drugs were used with muscle relaxants or were associated with cases of renal dysfunction. In experimental studies the neuromuscular blocking properties of polymyxins are readily demonstrated and polymyxin B is the most potent neuromuscular blocking agent of all the antibiotics in current clinical use /5, 22, 51, 82, 83/. The order of neuromuscular blocking potencies of the polymyxins, which is also the order of antimicrobial potency /84/, is polymyxin B > colistin > polymyxin A > colistemethate /22, 51, 83/.

The neuromuscular blockade produced by polymyxins is augmented by tubocurarine and succinylcholine /5, 78, 85/ and by pancuronium /85, 86/. Calcium can reverse polymyxin-induced paralysis partially /50, 87, 88/ or not at all /22, 51, 89/. The reported effects of anticholinesterases on polymyxin B-induced neuromuscular block also vary. In general the block is enhanced or prolonged /5, 51, 80, 81/ although Sabawala and Dillon /82/ observed slight antagonism. The recent observations of Lee *et al.* (1977) /88/ may reconcile this controversy. They showed that, in the cat, small doses of neostigmine, edrophonium or pyridostigmine antagonised polymyxin B-block but such reversal rarely exceeded 20% and lasted only 3 to 5 minutes. Larger doses of the anticholinesterases, however, enhanced the block but the enhancement was transient, lasting only 10 to 15 minutes.

3.2. Mechanism of Action

Unlike the aminoglycosides, the polymyxins have depressant effects on muscle contractility as well as on neuromuscular transmission. Thus, in the isolated rat phrenic nerve-hemidiaphragm a concentration of polymyxin B sufficient to reduce responses to nerve stimulation by 95% also reduced responses to direct muscle stimulation by 50% /83/. The difference between the effects on the responses to the two types of stimulation is mainly due to inhibition of neuromuscular transmission. In both rat and mouse hemidiaphragm preparations the depression of neuromuscular transmission was readily reversed by washing, but the

action on muscle contractility was incompletely reversed /51, 83/. Successive administrations of polymyxin B to the same preparation resulted in a gradual and irreversible reduction of muscle contractility. In the clinical situation it is not known whether the neuromuscular depressant action or the action to depress contractility is responsible for the muscle paralysing actions of the polymyxins.

As far as the neuromuscular depressant actions of the polymyxins are concerned there is evidence for both pre- and postjunctional actions.

3.2.1. Prejunctional Actions

Although colistimethate has been reported to depress e.p.p. quantal content and the size of the immediately available store of acetylcholine /79/, these results have been queried because of the known ineffectiveness of colistimethate which would not have been able to be changed into the active form, colistin, under the conditions of the experiment /83/. However, neuromuscular blocking concentrations of polymyxin B have been shown to reduce e.p.p. quantal content in both mouse and frog preparations /47, 59/, although the depression of release was much less than that measured in the presence of equiactive neuromuscular blocking concentrations of streptomycin, amikacin, spectinomycin or magnesium.

In contrast to the results from intracellular recording studies, results from experiments involving the collection and assay of acetylcholine from isolated rat hemidiaphragm preparations have failed to show effects of colistin or polymyxin B on acetylcholine release /83, 90/.

3.2.2. Postjunctional Actions

Colistin and polymyxin B have been shown to depress responses of the rat hemidiaphragm to close intravenous injection of acetylcholine /83, 90/, and polymyxin B was also shown to depress contractural responses of the denervated rat hemidiaphragm /77/. McQuillan and Engbaek (1975) /79/ found that colistimethate reduced m.e.p.p. amplitude and Singh *et al* (1979) /52/ found that muscle paralysing concentrations of polymyxin B completely abolished m.e.p.p. activity. Since Singh *et al* (1979) /59/ had found that polymyxin B also reduced quantal content, they concluded that polymyxin B blocked neuromuscular transmission by a mixture of pre- and postjunctional actions.

3.2.3. Local Anaesthetic Action

Studies on desheathed frog sciatic nerve have shown that polymyxin B and the local anaesthetic lignocaine in equimolar concentrations (1.8×10^{-4}M) had approximately equal effects on the extracellularly recorded gross nerve action potential /83/. At high pH (9.2) neither polymyxin B nor lignocaine had appreciable local anaesthetic activity indicating that, as for lignocaine /91/, the charged form of polymyxin B is responsible for the local anaesthetic activity. The local anaesthetic activity of polymyxin B has also been demonstrated on intracellularly recorded muscle action potentials in the frog sartorius muscle in which the rate of rise and fall of the action potential were both decreased /47/.

Wright and Collier (1976) /83/ postulate that the local anaesthetic action of polymyxin B plays a large part in the muscle paralysing action of the compound. However, the evidence for local anaesthetic action was derived from experiments in the frog which is peculiarly sensitive to the neuromuscular blocking actions of polymyxin B. Thus endplate currents and e.p.p.s are depressed by 10^{-6} to 10^{-5} M polymyxin B which is around 20 to 200 times less than the concentration required to produce local anaesthesia in this species /47, 92/. Hence, it is likely that the effects of polymyxin B on transmitter release and postjunctional acetylcholine sensitivity are at least as important factors as the local anaesthetic activity. Neverthless, the local anaesthetic activity of polymyxin B could contribute to the observed depression of muscle contractility.

4. THE TETRACYCLINES

4.1. Neuromuscular Effects

Members of this group of antibiotics, which are all derivatives of polycyclic naphthacene carboxamide, are both chemically and pharmacologically distinct from the aminoglycosides, the polymyxins and the lincosamides /93/. At present, only 4 are commonly used in chemotherapy: chlortetracycline, oxytetracycline, rolitetracycline and tetracycline.

The ability of the tetracyclines to produce neuromuscular paralysis in experimental animals is well established /51, 83, 94-96/ but the number of reported clinical cases of tetracycline-induced muscle paralysis is few. Rolitetracycline and oxytetracycline have produced neuromuscu-

lar paralysis, but only transiently, when given intravenously to patients suffering from myasthenia gravis /20, 21/.

The neuromuscular blocking action of tetracyclines in experimental animals was more pronounced during concomitant administration with tubocurarine /24, 95/, gallamine /97/ or magnesium ions /98/. The muscle paralysing actions of the tetracyclines were not consistently reversible by cholinesterase inhibitors or by calcium /21, 51, 95/.

4.2. Mechanism of Action

Although the tetracyclines have not been widely investigated, there is evidence to indicate that they might interfere with neuromuscular transmission. In the most comprehensive study of the muscle paralysing actions of the tetracyclines, Wright and Collier (1976) /83/ found that rolitetracycline reduced postjunctional receptor sensitivity but did not affect muscle contractility, nerve conduction or the release of ACh. Although the actions of rolitetracycline resembled those of tubocurarine, the experimental evidence did not allow Wright and Collier to conclude that the two drugs acted by identical mechanisms.

In the mouse hemidiaphragm preparation a complete reversal by calcium of the block of indirectly elicited twitches produced by oxytetracycline was observed but very large concentrations of calcium were required to produce only a partial reversal of tetracycline-induced neuromuscular block /51/. Intracellular recording studies showed that both tetracycline and oxytetracycline reduced e.p.p. quantal content and the amplitude of m.e.p.p.s. These results show that the tetracyclines possess a mixture of pre- and postjunctional blocking actions. The results also showed that the prejunctional action of oxytetracycline was slightly more dependent on calcium than was that of tetracycline /47/.

However, in the mouse hemidiaphragm preparation, tetracycline and oxytetracycline produced an effect on membrane excitability that seemed to be as important as any neuromuscular blocking properties they might have. Such an action was indicated by the observations that in neuromuscular blocking concentrations of the drugs, an initial augmentation of both directly and indirectly elicited twitches was followed by a blockade of both types of twitch and was also accompanied by a contracture which was aggravated by washout /51/. Although the local anaesthetic activity demonstrated for the tetracyclines may not be a

major determinant in their actions at the neuromuscular junction, it may still be contributory to their effects on contractility.

The known ability of tetracyclines to chelate divalent ions, especially calcium, has led Pittinger and Adamson (1972) /99/ to suggest that this action may explain the action of tetracyclines at the neuromuscular junction. Chelation would lower the extracellular calcium concentration and this in turn would lead to a reduction in acetylcholine release, as observed in the present study. However, as this hypothesis is at variance with the results of Wright and Collier (1976) /83/ it is, at the present time, impossible to draw overall conclusions concerning the mechanism of action of this group of antibiotics. Further work in the future using the calcium ion selective electrode might clarify the situation. There is also evidence to show that the actions of individual members of the group may be different from each other.

5. THE LINCOSAMIDES

5.1. Neuromuscular Effects

Lincomycin and its semi-synthetic derivative clindamycin (7-deoxy 7-chlorolincomycin) are the only two of the various chemical congeners of lincomycin which are at present used in clinical practice.

Despite an initial report to the contrary /23/, lincomycin-induced neuromuscular blockade has been demonstrated in various *in vivo* and *in vitro* experimental animal preparations /15, 51, 100-102/. In clinical use both lincomycin /103-106/ and clindamycin /107, 108/ have been reported to prolong neuromuscular paralysis in patients after anaesthesia or treatment with muscle relaxants. Fogdall and Miller (1974) /107/ reported that clindamycin phosphate given intravenously prolonged a pancuronium-induced neuromuscular blockade to about 20 hours in a patient who had previously shown normal sensitivity to pancuronium. Despite the close structural similarity between lincomycin and clindamycin there are distinct differences in their muscle paralysing actions and also clindamycin is more effective than lincomycin against a wider variety of microorganisms *in vitro* /109, 110/, and is much better absorbed from the gastrointestinal tract /111/.

5.2. Lincomycin

Early experimental studies on lincomycin showed that the compound produces a blockade superficially similar to a non-depolarizing type in that flaccid paralysis is produced in chickens and no initial stimulation is seen in rabbits /15, 101/. However, the blockade is not well antagonized by edrophonium, neostigmine or calcium /15, 51, 100, 101/. The possibility that the anticholinesterase- and calcium- irreversible block produced by lincomycin might be due to a reduction of muscle contractility has been examined. In the isolated rat hemidiaphragm muscle Wright and Collier (1976) /101/ showed that lincomycin reduced directly elicited muscle contractions in the same concentration range as that which reduced responses to nerve stimulation. These workers concluded that lincomycin reduced contractility but they were also able to show that in combinations with tubocurarine, low concentrations of lincomycin blocked neuromuscular transmission. In the isolated mouse hemidiaphragm, lincomycin appears to have very little inhibitory action on muscle contractility and its action is confined to the neuromuscular junction /51/. An initial increase in muscle contractility was found with lincomycin by Wright and Collier (1976b) /100/ but not by Singh *et al.* (1978b) /51/.

The mechanism of action of lincomycin at the neuromuscular junction has been further studied by intracellular recording and acetylcholine collection and assay techniques. Rubbo *et al.* (1977) /102/ found that subneuromuscular blocking concentrations of lincomycin increased the frequency of the spontaneous m.e.p.p.s. At neuromuscular blocking concentrations a depression of m.e.p.p. amplitude and of responses to iontophoretically applied acetylcholine was seen indicating a depression of postjunctional receptor sensitivity. Singh *et al.* (1979) /59/ also found that m.e.p.p. amplitude was depressed by lincomycin but, in addition, showed that e.p.p. quantal content, *i.e.* evoked acetylcholine release, was depressed. These latter results on acetylcholine release are substantiated by results from collection and assay of acetylcholine /102/. Thus it can be concluded from the results of Singh *et al.* (1979) /59/ and Rubbo *et al.* (1977) /102/ that lincomycin possesses a mixture of pre- and postjunctional blocking activities. Attempts to reverse the neuromuscular block by a mixture of neostigmine and calcium were unsuccessful and hence it must be concluded that the pre- and postjunctional mechanisms of action are different from those of magnesium and tubocurarine respectively.

5.3. Clindamycin

In isolated nerve-muscle preparations clindamycin produces a marked increase in twitch tension before muscle paralysis ensues /51, 100, 112/. Similar initial increases in twitch tension have also been reported for lincomycin /100, 113/, streptomycin /114/, tetracycline, rolitetracycline and oxytetracycline /51, 83/ and erythromycin /51/. With lincomycin /100/, clindamycin /51, 100/ and erythromycin /51/, both directly and indirectly elicited twitches are enhanced which suggests that these three compounds produce an initial increase in muscle contractility, although the mechanism underlying this action has not been studied.

In the case of clindamycin, following the initial increase in twitch tension there is a decrease in tension of both directly and indirectly stimulated preparations, indicating that the main cause of muscle paralysis is failure of contractility /51, 100/. An additional effect of clindamycin on neuromuscular transmission has been demonstrated /112, 115/ but in general the effects of clindamycin are not reversed by anticholinesterases or calcium, suggesting that the junctional effects of clindamycin are less important than its action on muscle contractility.

Intracellular recording studies of the action of clindamycin on neuromuscular transmission show that the drug markedly increases spontaneous m.e.p.p. frequency but at neuromuscular blocking concentrations also reduces their amplitude /59, 102/. Responses to iontophoretically applied acetylcholine are also reduced and this indicates that clindamycin possesses postjunctional receptor blocking activity /102/. Studies on the evoked release of acetylcholine have produced conflicting results: collection studies show that release is increased slightly /102/ whereas intracellular recording studies show that e.p.p. quantal content is reduced /59/. Singh *et al.* (1979) /59/ also found that e.p.p. activity was difficult to measure and concluded that clindamycin induced failure of nerve terminal activity. Wright and Collier (1976) /100/ have demonstrated that clindamycin possesses appreciable local anaesthetic activity and this action may be responsible for the failure of both nerve and muscle activity.

Recent results on the effects of lincomycin and clindamycin on the decay rate of miniature endplate currents (m.e.p.c.s) recorded under voltage clamp conditions show that low concentrations of clindamycin markedly accelerated the rate of decay of the m.e.p.c. and that lincomycin produced an initial acceleration followed by a slowing of the

decay rate /116/. These results indicate that, in addition to the actions already described, the lincosamides possess a blocking action at the level of the endplate postjunctional membrane channels responsible for the conduction of ionic current. The importance of this action in the overall muscle paralysing actions of the lincosamides remains to be elucidated.

Thus most of the evidence indicates that lincomycin acts mainly on neuromuscular transmission by a mixture of, as yet, unelucidated pre- and postjunctional actions, and that, although clindamycin shares the above actions with lincomycin, it also blocks nerve and muscle activity, probably by a local anaesthetic action.

6. REVERSIBILITY OF ANTIBIOTIC-INDUCED PARALYSIS

It should be clear from the preceding analysis of the known mechanisms of action of antibiotics which produce muscle paralysis that the different antibiotics do not share a common mechanism of action. Similarly, the resultant neuromuscular blockades are reversible to differing extents by the commonly available reversal agents, viz. anticholinesterases and calcium salts.

Reversibility tends to be unpredictable because the clinical problem usually arises from the concomitant use of antibiotics with neuromuscular blocking agents and/or general anaesthetics. Neuromuscular blockade associated with the use of depolarizing blockers such as suxamethonium or carbolinium is irreversible by available pharmacological agents and it may be predicted that blockade due to a combination of depolarizers and antibiotics would be equally irreversible.

Nondepolarizing neuromuscular blockers such as tubocurarine, pancuronium, gallamine, alcuronium and fazadinium act by competitively blocking acetylcholine receptors on the postsynaptic membrane. It is known that there is a high safety factor in neuromuscular transmission *i.e.* the amount of acetylcholine released is far in excess of that necessary to activate sufficient receptors to produce a contraction of the muscle fibre. Also the postjunctional membrane contains far more receptors than are needed to initiate a contraction /117, 118/. Thus acetylcholine release can be greatly reduced or a non-depolarizing neuromuscular blocking agent can occupy many of the receptors (up to 70 to 80%) without reducing twitch tension /118/, *i.e.* these treatments decrease the safety factor but effective transmission can continue. It can be predicted that different antibiotics will also reduce the safety

factor for neuromuscular transmission but at differing sites and by differing degrees according to their mechanism of action.

After apparently complete recovery from non-depolarizing neuromuscular block, as assessed by recovery of single twitches, many receptors will still be blocked, *i.e.* the safety factor will still be reduced. In this situation the injection of an antibiotic may cause the safety factor to be further reduced such that contraction strength begins to fall.

In the presence of non-depolarizing blockers the postjunctional receptor safety factor is reduced and this may be restored by anticholinesterase agents. With aminoglycoside antibiotics the predominant action appears to be a reduction of the prejunctional safety factor—this may be restored by calcium. With combinations of non-depolarizing blockers and aminoglycosides both pre- and postjunctional safety factors will be reduced but the relative reductions will be dependent on the plasma concentrations of the two agents. In the theoretical situation where a non-depolarizing blocker is occupying 75% of the receptors then twitch tension will be normal but any reduction of release by the aminoglycoside would be expected to lower the safety factor sufficiently to reduce twitch tension. In this situation an increase in the postjunctional safety factor from the injection of an anticholinesterase would be expected to reverse the resultant neuromuscular block. Such reversal has been demonstrated with combinations of tubocurarine and amikacin /51/. In effect, in this situation, the anticholinesterase is reversing an unseen neuromuscular blockade due to the non-depolarizer, the reduction of release due to the aminoglycoside being insufficient to reduce twitch tension on its own. However in the situation in which the reduction of the safety factor is largely due to the aminoglycoside, an anticholinesterase would be less effective, whereas calcium would be expected to reverse the block.

In the case of neuromuscular block due to combinations of a non-depolarizer and aminoglycoside, both of which are individually reversible, it would be expected that combinations of reversal agents would be effective. It has been shown in isolated preparations that block due to a mixture of tubocurarine and magnesium is reversible by a mixture of neostigmine and calcium /59/.

With antibiotics other than the aminoglycosides the situation is different in that the neuromuscular blockades produced by the polymyxins, tetracyclines and lincosamides are themselves not well reversible. In combination with non-depolarizers, if the block is mainly due to the non-depolarizer it may be possible with some antibiotics to reverse the

block with neostigmine. However, neostigmine actually increases the blocking action of some antibiotics including polymyxin B and colistin /51/ and hence the success of this procedure is not likely to be predictable.

In general, because of difficulties in assessing safety factors in neuromuscular transmission in the clinical situation, it would appear that with the possible exception of blockade due to the aminoglycosides, artificial respiration until recovery of respiratory function is likely to be safer and more effective than the use of presently available reversal agents.

Despite the resistance of polymyxin and lincosamide-induced muscle paralysis to reversal by anticholinesterases and calcium, there is evidence that the neuromuscular block produced by these agents is not totally irreversible. It has been shown that the neuromuscular blocks produced by polymyxin B, lincomycin and low concentrations of clindamycin are reversible by 3,4- and 4-aminopyridines /29, 106, 115/. Neuromuscular block produced by tetracycline was not reversed by 3,4-diaminopyridine, although the block produced by oxytetracycline was completely, but only temporarily, reversed /115/.

Aminopyridines prolong action potentials and the resultant increased influx of calcium into nerve terminals produces an increase in acetylcholine release /119/. This action is known to reverse neuromuscular block induced by non-depolarizing agents /120, 121/ and prejunctional block produced by aminoglycosides /115, 120/ and it is probable that the reversal of antibiotic induced paralysis is caused by such an increase in transmitter output.

As described previously, high concentrations of clindamycin reduce muscle contractility and this effect is not reversible by 3,4-diaminopyridine. However, block due to combinations of tubocurarine and low concentrations of clindamycin is well reversed /115/. Unfortunately, the action of aminopyridines is not confined to the neuromuscular junction and the compounds produce marked augmentation of chemical transmission in the autonomic /122-124/ and central /125/ nervous systems. The central actions of aminopyridines result in convulsive activity /125, 126/.

Nevertheless, the reversing actions of the aminopyridines indicate that the neuromuscular blockade produced by certain classes of antibiotics is not totally irreversible and it is possible that future research on neuromuscular facilitatory agents may produce a suitable reversal agent.

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