ANTIBIOTIC BLOCKADE OF NEUROMUSCULAR FUNCTION

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This review pertains to pharmacological actions of certain antibiotics associated with their depression of neuromuscular function. This effect is an acute, toxic phenomenon which has been implicated in respiratory and motor paralyses. Such adverse clinical consequences occurred most frequently as the result of interactions between offending antibiotics and ether, or various neuromuscular blocking agents employed in anesthesia. However, paralytic episodes have followed administrations of certain antibiotics to unanesthetized persons, especially those afflicted with myasthenia gravis (1–4). According to Koelle (5) the synergism between certain antibiotics and neuromuscular blocking agents is one of the most important interactions of the relaxants. Pittinger, Eryasa & Adamson (6) have recently reviewed clinical occurrences of antibiotic-induced paralysis. The present review concerns concepts of action of antibiotics at the neuromuscular junction and an analysis of proposed mechanisms in the light of recent developments.

SCOPE OF ANTIBIOTIC INVOLVEMENT

Since 1956 when the first cases of prolonged respiratory depression from neomycin-ether combinations were reported (7), various antibiotics, anesthetics, muscle relaxants, and routes of administration have been implicated as causative factors in clinical cases of prolonged apnea. Nine antibiotics have been associated with neuromuscular paralysis in patients. These include: colistin, colistin methanesulfonate, dihydrostreptomycin, kanamycin, neomycin, oxytetracycline, polymyxin B, rolitetracycline, and streptomycin (6).

Experimental studies utilizing in vivo and in vitro preparations have demonstrated the neuromuscular blocking activity of 18 antibiotics. Those producing experimental paralysis are: aminosidine (8, 9), colistin (10), colistin methanesulfonate (11), dihydrostreptomycin (12–14), gentamicin (15, 16), kanamycin (13, 14, 17, 18), lincomycin (19, 20), monomycin (probably paromomycin) (21), oxytetracycline and methylpyrrolidine tetracycline (22), neomycin (13–15, 23–27), polymyxin A (10), polymyxin B (10, 13,

14), paromomycin (28), rolitetracycline (29), streptomycin (12, 14, 22, 30–32), tetracycline (17, 33), and viomycin (10, 22, 34).

The following antibiotics were reported to be devoid of neuromuscular blocking activity: bacitracin (10, 14), cephalothin (35), chloramphenicol (35), erythromycin (14), hamycin (36), novobiocin (35), oleandomycin (14), penicillins (14, 17, 35, 37), rifamycin (8), ristocetin (10, 14), tyrothricin (10), and vancomycin (10).

Amphotericin A, amphotericin B, nystatin, and pimaricin, all antifungal antibiotics, did not exhibit curariform activity, but did produce contracture in the rat phrenic nerve-diaphragm preparations (36).

Structure Activity Relationships

An examination of the chemical structures of those antibiotics producing neuromuscular blockade reveals three distinct groups: (*a*) streptomycin and related compounds (38, 39); (*b*) the polymyxins, which are polypeptides with molecular weights of approximately 1000 (40, 41); (*c*) the tetracyclines (42, 43) (Table 1). In addition, lincomycin (40), which produces neuromuscular paralysis, is a monobasic substance chemically distinct from these other antibiotics.

The streptomycin group includes aminosidine, dihydrostreptomycin, gentamicin, kanamycin, neomycin, paromomycin, streptomycin, and viomycin. Except for viomycin, these drugs are organic bases (39) containing amino sugars linked to one of the hydroxyl groups of streptidine (1,3-diguanido, 2,4,5,6-tetrahydroxycyclohexane), or to the hydroxyl groups of deoxystreptamine (1,3-diamino, 4,5,6-trihydroxycyclohexane). The neuromuscular blocking activity of streptidine and streptamine has been established (31). The substitution of amino groups in streptamine by the guanido moiety as in streptidine, enhances neuromuscular paralysis. Viomycin is included with the streptomycin antibiotics because it is a strong base containing a guanidine group. A number of these antibiotics, including viomycin, have other pharmacological similarities such as poor enteric absorption, ganglionic blocking activity, smooth muscle depression, and ototoxicity (16, 34). In addition, the neuromuscular blockade of this group of compounds is reversible with calcium salts, and also with neostigmine if the blockade is incomplete.

The polymyxin group of antibiotics includes polymyxins A, B, C, D, E, colistin, and colistin methanesulfonate. Polymyxin E and colistin are very similar and may be identical (40). These agents are relatively simple basic polypeptides composed of various amino acids, +6-methyloctanoic acid and α,γ-diaminobutyric acid (Table 1). Polymyxin A, polymyxin B, colistin, and colistin methanesulfonate are known to possess neuromuscular blocking activity (10, 14). The intact polypeptide molecule is necessary for activity since individual components failed to produce neuromuscular blockade (10). These drugs have similar antibacterial spectra, exhibit cross resistance and are nephrotoxic. Chemically and pharmacologically these antibiotics com-

TABLE 1. CHEMICAL COMPOSITION OF ANTIBIOTICS PRODUCING NEUROMUSCULAR BLOCKADE

	Antibiotic	*Chemical Compositions*
Group I		
	Aminosidine	Oligosaccharide of empirical formula $C_{23}H_{45}O_{41}N_5$ chemically related to streptomycin
	Dihydrostreptomycin	Hydrogenated derivative of streptomycin
	Gentamicin	An aminoglycoside antibiotic whose structure is not fully elucidated but yields 2-deoxystreptamine upon degradation
	Kanamycin	Two amino sugars linked glycosidically to 2-deoxystreptamine
	Neomycin	Deoxystreptamine linked to 2,6-diamino-2,6-dideoxy-D glucose, and D-ribose linked to a diaminohexose
	Paromomycin	An aminoglycoside antibiotic which has a 2-deoxystreptamine nucleus
	Streptomycin	N-methyl-L-glucosaminidostreptosidostreptidine. Chemically it consists of 3 components: streptidine, streptose, and N-methyl-L-glucosamine
	Viomycin	Guanidino component, creatinine, L-serine, α-βdiaminopropionic acid
Group II		
	Polymyxin A	+6-methyloctanoic acid, D-leucine, L-threonine, L-α-γdiaminobutyric acid
	Polymyxin B	+6-methyloctanoic acid, D-leucine, L-threonine, D-phenylalanine, L-α-γ-diaminobutyric acid
	Colistin	+6-methyloctanoic acid, D-leucine, L-threonine, L-leucine, L-α-γ-diaminobutyrci acid
	Colistin methanesulfonate	Methanesulfonate derivative of colistin
Group III		
	Tetracyclines	Derivatives of polycyclic naphthacenecarboxamide
Others		
	Lincomycin	Derivative of the amino acid, trans-L-4-*n*-propylhygrinic acid, attached to an octose derivative plus a sulfur atom

prise a homogeneous group. In experimental studies paralysis from polymyxin A and polymyxin B was not reversed by calcium salts or neostigmine (10, 14, 44). Their effectiveness as antagonists of clinical paralysis induced by polymyxin B and colistin methansulfonate is discrepant (6).

The tetracyclines are chemically and pharmacologically distinct from the streptomycin and polymyxin groups. They are derivatives of polycyclic naphthacenecarboxamide. Some therapeutic and other pharmacological properties of the tetracyclines have been attributed to their metal-binding capacity (43). Experimental studies of methyl-pyrrolidine tetracycline, rolitetracycline, and tetracycline have demonstrated their neuromuscular blocking potentialities (17, 22, 29, 33). In addition, oxytetracycline, tetracycline, and rolitetracycline potentiate the neuromuscular effects of d-tubocurarine and gallamine (17, 29, 37). Evidence of the antagonistic effect of anticholinesterases toward the tetracyclines is conflicting (17, 29, 33).

Lincomycin is a derivative of the amino acid, *trans*-L-4-*n*-propylhygrinic acid. It does not conform chemically to the other three categories of antibiotics (Table 1). Its neuromuscular blockade is not consistently reversed by calcium chloride or anticholinesterases (19, 20, 45).

According to Tang & Schroeder (20), neostigmine produced variable results ranging from reversal to enhancement of the blockade from lincomycin in rabbits. However, edrophonium produced consistent partial antagonism of blockade induced by this drug. The flaccid paralysis produced in chickens and the lack of initial stimulation in rabbits suggest a nondepolarizing type of blockade. However, the deviation from parallelism of the dose-response lines for lincomycin and neomycin derived from studies in rabbits suggests different mechanisms of action for these two antibiotics.

Mechanism of Neuromuscular Blockade

Early recognition of streptomycin (30) and neomycin (26, 27, 46) as neuromuscular blocking agents and the similarities of their actions in this regard (23) account for their mutual roles in the development of concepts of mechanisms of action underlying their paralytic potentialities and those of related antibiotics. On the basis of structure-activity relationships (Table 1), pharmacological studies, and clinical observations (6), similar mechanisms of action seem likely for the following antibiotics: aminosidine, dihydrostreptomycin, gentamicin, kanamycin, neomycin, paromomycin, streptomycin, and viomycin. This list of antibiotics should not be construed to indicate that all "mycins" are neuromuscular blocking agents. Our first consideration relates to concepts of action regarding the streptomycin-neomycin group. The neuromuscular blocking activities of tetracyclines and polymyxins are discussed subsequently.

Certain features of the paralytic activity of streptomycin and neomycin are consistent with the effects of competitive neuromuscular blocking agents. In dogs (23, 24, 26, 27, 30), rabbits (26, 27, 47), fowl (27, 30), cats (23), and mice (26) they produce a progressive, flaccid paralysis of striated

muscle without evidence of an initial excitatory effect. Relaxation is enhanced by ether (24, 26, 27), d-tubocurarine, and other neuromuscular blocking agents (24, 27) and antagonized by neostigmine (24, 26, 27) and calcium salts (24, 26, 27). Myographic tracings invoked by indirect stimulation during administration of neomycin (23, 24, 26, 27) or streptomycin (30) resemble the pattern obtained with d-tubocurarine. Contracture is not evident. There is no diminished response to direct stimulation (30).

The effect of neomycin (6, 7, 27) and streptomycin (6) in man is also characterized by a progressive, flaccid paralysis. Most instances of paralytic complications from antibiotics have occurred during anesthesia involving ether or other neuromuscular blocking drugs employed for relaxation. Myasthenia gravis apparently causes a sensitization toward the paralyzing action of such antibiotics (1, 2). Anticholinesterases and calcium salts have been employed clinically as antagonists with varying degrees of success (6).

However, certain features of the neuromuscular effects of streptomycin and neomycin differ from those of competitive (nondepolarizing) agents. The divergencies outlined below invoke broader considerations of interference with neuromuscular transmission (5, 48).

Magnesium relationship.—In 1956, Keller & associates (49) reported similarities between the effect from intravenously administered streptomycin sulfate in rabbits and the apparent narcosis from magnesium sulfate. The antagonistic action of calcium gluconate toward both drugs and an additive relationship between their acute toxicities were noted. A subsequent study indicated comparable anticoagulant effects from streptomycin sulfate and magnesium sulfate (50). The anticoagulant effect of magnesium was attributed to displacement of calcium ions from erythrocyte receptor sites; the inferred mechanism for the antibiotic was its chelation with calcium.

Keller and associates did not suggest neuromuscular blockade as a possible cause of respiratory paralysis in acute streptomycin toxicity. This implication and its relationship with the neuromuscular effects of magnesium ions were first reported by Vital Brazil & Corrado (30). These investigators presented a comparison of the effects of streptomycin, d-tubocurarine, decamethonium, and magnesium ions, which is outlined in the following paragraphs.

When isolated volleys of indirect electrical impulses are blocked by streptomycin, indirect stimulation with tetanizing frequencies still evokes a tetanus. While this effect is consistent with that reported for magnesium (51), d-tubocurarine and decamethonium cause an initial twitch and no contraction respectively (30).

The nature of post-tetanic facilitation during streptomycin paralysis more closely resembles that during blockade from magnesium than from d-tubocurarine (30). With d-tubocurarine, post-tetanic twitches are initially weak but gradually increase in intensity; ionized magnesium causes twitches that are maximal immediately after tetanus; with streptomycin

twitches are at a maximum immediately after tetanus or after only a brief delay.

Muscle response to indirect tetanic stimulation during partial blockade from d-tubocurarine is characterized by a poorly sustained contraction as compared with the well sustained one observed with decamethonium (52, 53). With magnesium ions, the initial abrupt rise in muscle tension is followed by a secondary rise surpassing the first (54). The response to indirect stimulation during partial blockades from streptomycin or neomycin resembles that produced by magnesium ions or decamethonium (30).

The antagonistic effect of neostigmine toward streptomycin and neomycin blockades differs from that toward d-tubocurarine. In the latter instance it is predictable and effective. Antagonism of these antibiotics by anticholinesterases may not be complete and is predictable only when partial blockade prevails (6, 23, 27, 30). Contrariwise, the partial effectiveness of calcium salts as antagonists toward d-tubocurarine blockade (55) contrasts with their complete reversal of the neuromuscular effects of magnesium (56), streptomycin (30) and neomycin (27).

The similarity of depressions of ganglionic transmission by magnesium ions and streptomycin and the antagonism of this effect by calcium salts were also reported. Ganglionic blockade produced by hexamethonium or d-tubocurarine is not effectively antagonized by calcium salts (30).

Results of studies of neomycin (23, 26, 27), kanamycin (18), and dihydrostreptomycin (14, 57) allowed their inclusion in the concept of a relationship with magnesium ions. Corrado & Ramos (18) speculated that the antibiotics, like magnesium ions (58, 59), inhibited the release of acetylcholine and also competed with the chemical mediator at the end plate.

Two mechanisms of action were eventually postulated to explain the magnesium-like effect of these antibiotics with their pre- and post-junctional implications. The first, that of Corrado defined in 1963 (60), we designate the "chelation hypothesis"; the second mechanism of action, the "competitive hypothesis" of Vital Brazil & Prado-Franceschi was formalized in 1969 (15). Both concepts involved an explanation of the magnesium-like effects of the antibiotics in terms of their molecular relationships with calcium ions.

The chelation hypothesis—Corrado (60) attributed the mechanism of neuromuscular blockade from streptomycin, neomycin, and kanamycin to their ability to reduce the level of calcium in blood. He supported his hypothesis with a reference to a publication by Hava, Sobek & Mikulaskova (61) which purported a reduction of ionized calcium by neomycin. He related the consequence of the lowering of calcium ions to a reduction of the potential of the motor end plate as reported by Brink (62). This postulation of Corrado stressed a post-junctional action of the antibiotics. A pre-junctional effect was neither explicitly stated nor excluded.

The chelation hypothesis was not only consistent with calcium antago-

nism of antibiotic-induced blockade (18, 25–27, 30, 63, 64) but also with decreased toxicity of neomycin and streptomycin administered with calcium salts (24, 65), decreased streptomycin toxicity when administered as the calcium chloride complex, and synergism of the neuromuscular blocking action of neomycin by the chelating agent, sodium citrate (24).

Previous observations of others were consistent with Corrado's concept: the chelating potentiality of certain antibiotics including streptomycin (66, 67), the inverse relationship between streptomycin toxicity and residual calcium content of impure samples of the drug (49), its anticoagulant action (49), and the effect of the antibiotic in reducing ionized calcium in Ringer's solution as measured by the bioassay technique of McLean & Hastings (68). More recently, Sobek & coworkers (69, 70) inferred the prolongation of magnesium-induced depression by neomycin and the antibiotic's enhancement of ammonium oxalate toxicity to calcium binding. Sakurai & associates (71) suggested the same mechanism responsible for the diminished antibacterial activity of kanamycin in the presence of added calcium chloride.

In 1968 the current concept of neuromuscular and ganglionic blockades due to antibiotics associated with anesthesia was appraised by Adriani (72). His summary reiterated essentially the chelation hypothesis of Corrado (60) in attributing the neuromuscular blocking actions of neomycin, streptomycin, dihydrostreptomycin, bacitracin, polymyxin B, and kanamycin to their binding proclivity with calcium, the consequent reduction of the calcium level, and disturbance of the integrity of the myoneural junction with augmentation of the effect of magnesium. The basis for including bacitracin is not evident. Its neuromuscular blocking action is not documented in the appraisal. Contrariwise, Adamson et al (10) and Timmerman and coworkers (14) reported no such activity. Other than the fact that polymyxin B possesses neuromuscular blocking activity, the rationale for its inclusion among the streptomycin group of antibiotics is likewise obscure.

In 1969, Vital Brazil & Prado-Franceschi (15) proposed the competitive hypothesis concerning the neuromuscular blocking action of the streptomycin-neomycin group of antibiotics. In this group they included neomycin, gentamicin, paromomycin, streptomycin, kanamycin, and dihydrostreptomycin, whose respective potencies as ascertained with the rat phrenic nerve-diaphragm preparations were 1.00, 0.54, 0.44, 0.16, 0.12, and 0.06 (16).

The competitive hypothesis.—This concept was postulated by Vital Brazil & Prado-Franceschi (15) in a report of the nature of neuromuscular block produced by neomycin and gentamicin. This hypothesis also related the antibiotic effects to those of magnesium ions. It recognizes the decreased sensitivity of the motor end plate to the depolarizing action of acetylcholine but stresses the inhibition of its pre-junctional release. The competitive aspect of the hypothesis relates to the latter effect. The proponents postulated that combination of calcium ions with receptors on the nerve terminal triggers release of acetylcholine; however, the complexing of magne-

sium ions with the same sites is not effective in this respect. They suggest that the streptomycin-neomycin group of antibiotics, like magnesium, also combine with the same receptors to form complexes incapable of releasing acetylcholine. Thus these antibiotics are considered to compete with calcium ions for receptor sites on the nerve terminal.

The hypothesis evolved from considerations of the actions of the antibiotics in relation to the effects of magnesium ions at the neuromuscular junction as reported by Castillo & associates (58, 59), who had observed that: (*a*) an excess of magnesium ions blocked neuromuscular transmission as a result of reduction in amplitude of end plate potential; (*b*) the ion decreased the sensitivity of the end plate to the depolarizing effect of applied acetylcholine and also depressed the direct excitability of muscle fibers; (*c*) quantitative comparisons of these actions indicated that the main effect of magnesium was to decrease the amount of acetylcholine liberated by a motor nerve impulse; (*d*) calcium increased the liberation of acetylcholine and antagonized the effect of magnesium on the motor nerve endings, thereby antagonizing the neuromuscular blockade; (*e*) high magnesium concentrations, like calcium deficiency, produced quantal fluctuations of end plate potential recorded at single junctions with intracellular electrodes. These relationships suggested that the amount of acetylcholine release by a nerve stimulus was a function of the relative concentrations of calcium and magnesium ions.

In 1960, Vital Brazil (73) observed that large doses of streptomycin produced neuromuscular blockade and a simultaneous depression of muscular responses evoked by intra-arterial injections of acetylcholine. This suggestion of a direct effect of streptomycin upon muscle was pursued by studying its action on denervated tibial muscle in dogs. Streptomycin depressed contractions caused by direct electrical stimulation and in some instances produced a slight elongation of the denervated muscle. Calcium ions synergized this action but antagonized the hypotensive and respiratory depressant effects of the antibiotic. Contractions of the denervated muscle produced by intra-arterial injections of acetylcholine or potassium chloride were abolished by streptomycin. Intra-arterial injections of gallamine did not affect the contractures produced by potassium ions.

The above effects of streptomycin were consistent with observations previously reported concerning magnesium. Hof & Schneider (74) had demonstrated its antagonism of contractures of the frog rectus abdominus muscle provoked by acetylcholine and potassium. Maaske & Gibson (75) showed the depressant effect of magnesium on directly stimulated, denervated muscle. These similarities, and the fact that gallamine did not depress contractions of denervated muscle, led to Vital Brazil's postulation that the antibiotics stabilized muscle membranes rather than combined with acetylcholine receptors. He speculated that streptomycin blocked the effect of acetylcholine by a dual mechanism: the desensitization of the end plate to the depolarizing action of the transmitter and diminution of membrane excitability

in regions close to the end plate. The fact that calcium chloride antagonized the neuromuscular blockade produced by streptomycin (30) but enhanced its depressant effect on denervated muscle (73) led to rejection of chelate formation as the basis for calcium-streptomycin antagonism.

In 1962, Elmqvist & Josefsson (25) studied neomycin in relation to its magnesium-like actions (58, 59). Their particular interest related to the pre-junctional release of acetylcholine and the sensitivity of the post-junctional membrane to it (58, 76). In a study of the action of acetylcholine in frog sartorius muscle, neomycin at concentrations of 0.3 mg/ml reduced depolarization by 55%. The antibiotic also caused a 59% reduction of the acetylcholine-induced contracture of denervated rat diaphragm immersed in a bath containing a 10^{-6} concentration of the transmitter. The latter depressant effect was not influenced by increasing the external concentration of calcium ions (25).

The mean resting potential of rat diaphragm in normal bathing solution was 81 ± 1.3 mv for 30 fibers. In a solution containing 0.3 mg/ml of neomycin the resting potential was 81.5 ± 1.3 mv, indicating no change (25).

Measurements of miniature end plate potentials of the rat diaphragm (25) indicated that a blocking concentration of neomycin (0.3 mg/ml) did not change spontaneous pre-junctional activity. In normal bathing solution the resting discharge frequency was 1.02 ± 0.15 per second; with neomycin the frequency was 1.04 ± 0.15 per second. Amplitude was somewhat lower in the presence of neomycin, as would be expected on the basis of a decreased sensitivity at the post-junctional membrane. Increase in the potassium concentration of normal bathing solution from 5 to 20 millimolar resulted in a marked increase in the frequency of miniature end plate potentials. Such an increase did not occur in the presence of neomycin even when potassium concentrations were as high as 30 millimolar. However, when external calcium ion concentration was increased from 2 to 8 millimolar, response to high potassium in the presence of neomycin was normalized. These events were interpreted as pre-junctional depression by the antibiotic. Measurements of calcium ion activity using the murexide technique of Schwarzenbach & Gysling (77) indicated no evidence of calcium binding by neomycin (25).

The amplitude of successive end plate potentials fluctuated at random in the rat phrenic nerve-diaphragm preparation during neomycin blockade. There was no decline in the amplitude with high frequency stimulation at a rate of 50 per second as occurred during repetitive nerve stimulation during d-tubocurarine blockade (25).

On the basis of their findings Elmqvist & Josefsson (25) postulated that since calcium ions were not bound by neomycin, the pre-junctional action of the antibiotic, like that of magnesium, was competition with calcium ions at some step in the pre-junctional process, thus interfering with the release of acetylcholine. This pre-junctional effect would be antagonized by an excess of calcium ions. Like magnesium ions, neomycin also decreased the sensitiv-

ity of the post-junctional end plate to the depolarizing action of acetylcholine. Whereas the post-junctional actions were directly observed, the suggested pre-junctional effect, i.e., inhibition of acetylcholine release, was deduced from the fact that neomycin prevented increase in the frequency of miniature end plate potentials normally resulting from increased potassium concentrations.

The indirect evidence (25) of a pre-junctional effect of neomycin stimulated Vital Brazil & Prado-Franceschi (15) to investigate quantitatively the release of acetylcholine from nerve endings. Using the rat phrenic nerve-diaphragm in an organ bath, determinations of spontaneously released acetylcholine were alternated with measurements of its release during a control period of nerve stimulation, during indirect stimulation in the presence of 310 μg/ml of neomycin or 608 μg/ml of gentamicin and finally in the presence of these amounts of antibiotics with calcium chloride added to a concentration of 0.16 mg/ml. Organ bath fluids were analyzed for acetylcholine by the blood pressure bioassay procedure in cats. The mean release of acetylcholine during the first period of spontaneous output was 8.07 ng. During subsequent periods of spontaneous release the quantities were similar. A mean of 60.5 ng were released during a 23 minute control period of indirect stimulation. During a similar period of stimulation in the presence of neomycin the output was reduced to a mean of 8.07 ng, the equivalent of the spontaneous release. With neomycin plus added calcium ions, mean release of acetylcholine increased to 52.4 ng. In studies of the effects of gentamicin, mean spontaneous release was 8.75 ng and mean control release during nerve stimulus was 68.6 ng. During stimulation in the presence of gentamicin mean release was 8.74 ng and with gentamicin plus calcium ions, 68.6 ng. These observations convincingly demonstrated the pre-junctional depression of acetylcholine release by neomycin and gentamicin, and confirmed the indirect evidence of Elmqvist & Josefsson (25) regarding the former antibiotic.

Vital Brazil & Prado-Franceschi (15) also investigated the effects of the same antibiotics upon acetylcholine-induced contractions of the isolated and chronically denervated hemidiaphragm of the rat. Dose-response curves shifted toward the right with both drugs and increased with successively higher doses. This observation confirmed the post-junctional desensitization reported by Elmqvist & Josefsson (25) for neomycin.

The depression of acetylcholine release during indirect stimulation was interpreted as evidence that the main cause of the blockade was this pre-junctional effect.

These definitive studies by Vital Brazil (73), Vital Brazil & Prado-Franceschi (15) and Elmqvist & Josefsson (25) established the pre- and post-junctional roles of these antibiotics in producing neuromuscular blockade. The observations of these investigators, consistent with reports concerning the actions of magnesium ions at the neuromuscular junction

(58, 59, 62, 76) provided a substantial basis for the emergence of the "competitive hypothesis."

Comparison of the Chelation and Competitive Hypotheses

The chelation and competitive hypotheses concurred in the magnesium-like neuromuscular blocking action of the streptomycin group of antibiotics. The chelation hypothesis of Corrado (60) attributed their major effect to the formation of complexes with calcium ions and consequent reduction in end plate potential. This stressing of the post-junctional action did not negate the possibility of a magnesium-like pre-junctional effect.

Contrariwise, the competitive hypothesis of Vital Brazil & Prado-Franceschi (15) rejected the concept of diminished calcium ionization and postulated that the antibiotics, like magnesium, were in competition with calcium ions for receptor sites on the nerve terminal. While emphasizing this pre-junctional effect, evidence of depression of end plate potential was also presented.

The credibility of either hypothesis concerned the issue of calcium binding. Corrado (60) referred to the abstract by Hava & associates (61) as evidence that ionized calcium was lowered by neomycin, whereas total serum calcium was unchanged. Unfortunately, that abstract provided no insight into the nature of the technique employed for measuring ionized calcium. However, since a subsequent report (70) by the investigators indicated an increased sleeping time from magnesium salts in mice treated with neomycin, and enhanced toxicity to ammonium oxalate, these indirect suggestions were presumably the basis for the concept of calcium binding.

Several early indirect evidences opposed the concept of chelation of calcium ions by streptomycin and related antibiotics. In 1956, Swain & associates (78) reported an ingenious experiment involving the heart-lung preparation of dogs. The intravenous administration of disodium EDTA as a chelating agent led to cardiac failure evidenced by increased venous pressure and cardiac enlargement. Induced failure was repeatedly amenable to treatment with infusions of calcium chloride for as many as 8 times. Tetracycline-induced cardiac failure could be reversed only twice by calcium administration. An attempt to rectify the third induced failure was invariably unsuccessful due to massive plugging of the pulmonary vasculature with a product presumed to be a complex of the antibiotic with calcium. With streptomycin-induced failure and attempted restoration of normal function with calcium, fibrillation occurred on the second attempt. This was interpreted as indicating a lack of chelation by streptomycin and the consequent accumulation of a toxic concentration of calcium ions sufficient to induce fibrillation. The investigators concluded that streptomycin must cause failure by some mechanism other than binding of calcium ions.

In 1960, Vital Brazil (73) demonstrated that streptomycin depressed the response of denervated, striated muscle to direct stimulation and the aug-

mentation of this effect by calcium. If streptomycin chelated calcium ions of the muscle, infusion of calcium salts would have antagonized, rather than augmented, the depressant action. Chelation of calcium by streptomycin was therefore excluded as a plausible explanation.

In 1962, Elmqvist & Josefsson (25) utilized the murexide analysis of Schwarzenback & Gysling (77) to study the in vitro effect of neomycin on calcium ionization. Calcium ion activity in solutions containing sodium, potassium, and calcium salts in concentrations similar to those in the bathing fluid of their rat phrenic nerve-diaphragm experiments was not altered by neomycin. Until the recent advent of the calcium-selective electrode, the murexide procedure was considered the technique of choice for research studies of ionized calcium (79).

Additional indirect evidence opposing chelation was the progressive, flaccid paralysis observed without tetany in unanesthetized animals treated parenterally with streptomycin and neomycin (26, 47, 64). In addition, tetany has not been reported as an accompaniment of muscle weakness associated with neomycin, streptomycin, and dihydrostreptomycin administrations to unanesthetized persons (6).

The recent advent of the calcium-selective electrode provided the first practical, direct technique for measuring ionic calcium levels. Such an instrument was employed by Pittinger (47) in 1970 to study the effects of pH and streptomycin upon ionic calcium in serum. In analyses of control samples of pooled human serum, mean ionic calcium was 1.17 ± 0.20 mM/L. In aliquots of these sera to which 3.3 mg/ml of streptomycin sulfate had been added and whose pH was maintained to within 0.04 unit of their respective controls, mean ionic calcium was 1.15 ± 0.15 mM/L. In a series of rabbits the mean ionic calcium level was 1.60 ± 0.43 mM/L; following paralysis from infused streptomycin sulfate mean ionic calcium was 1.64 ± 0.38 mM/l. Therefore, streptomycin sulfate in paralyzing concentrations had no significant effect upon the level of ionized calcium in either of the above experiments.

The discrepancy between the electrode data of Pittinger (47) and those derived by Keller and associates (50) with the isolated frog heart is explainable. McLean & Hastings (68) recognized that their bioassay procedure yielded erroneously low indications of ionic calcium in artificial perfusates at pH 7.35 or lower. Since aqueous solutions of streptomycin sulfate are acidic (80), the relatively unbuffered, lactated Ringer's solutions employed by Keller and co-workers (50) should have had pH values considerably lower than the critical level of 7.35. The *apparently* low levels of ionic calcium would have suggested chelation.

Thus, the chelation hypothesis (60) seems no longer tenable whereas the competitive hypothesis (15) seems well substantiated and credible, on the basis of current evidences, with regard to streptomycin, neomycin, and related antibiotics. It is consistent with the recent evidences of Katz & Miledi

(81, 82) and Blaustein (83) concerning the significant role of calcium ions in the functioning of presynaptic nerve elements and their relationship with magnesium ions.

Stanley and associates (84) called attention to the high concentration of calcium salts required for effective antagonisms of neomycin-induced paralysis in both laboratory and clinical situations. They reported the antagonistic action of sodium bicarbonate and suggested its use as an adjunct to calcium salts. Sodium bicarbonate rather than its separate ionic constituents was essential for the antagonism. The unexplained action was not considered related to the correction of acidosis. Elevations of pH through the use of sodium bicarbonate would reduce, rather than increase, calcium ionization (47). An in vitro study of molar relationships between concentrations of magnesium ions and the various antibiotics required for the same degree of depression of acetylcholine-induced muscle contractions and the amounts of calcium ions or sodium bicarbonate required for antagonism of the depressant effects would serve to support the concept of competitive antagonism between calcium and the antibiotics, or conversely, indicate noncompetitive antagonism.

The capacity of the tetracyclines to bind divalent metals (43, 85, 86), their tendency toward accumulation in bone and areas of newly formed calcium complexes (42, 85, 86), and the intravascular deposit from calcium treatment of tetracycline-induced cardiac failure in the dog heart-lung preparation (78) suggest a likelihood of calcium binding by this group of antibiotics. However, it is not known that calcium binding is involved in neuromuscular blockade. Considerations of these evidences of calcium binding and dissimilarities in chemical structure (Table 1) suggest the probability of a mechanism of action for the tetracyclines different from that for the streptomycin-neomycin type of antibiotics.

The polymyxins are polypeptides and differ structurally from the streptomycin-neomycin and tetracycline groups of antibiotics. Timmerman et al (14), Adamson et al (10), and Naiman & Martin (44) have reported that neuromuscular blockades from polymyxins are not antagonized by either calcium salts or anticholinesterases. Their production of a flaccid paralysis in the chicken indicates that these antibiotics are not depolarizing agents. However, their mechanisms of neuromuscular blockade have not been elucidated.

The mechanisms of action of lincomycin, an antibiotic which does not conform to the three groups previously described, is also unknown.

Conclusions

The streptomycin-neomycin group of antibiotics, like magnesium ions, primarily inhibit the prejunctional release of acetylcholine and also depress post-junctional sensitivity to the humoral agent. Their relationship with calcium ions is suggestively competitive at a common receptor site on the mem-

brane of the nerve ending. The prior concept that this group of antibiotics suppresses serum ionized calcium through chelation is no longer tenable.

The mechanisms of action of the tetracycline and polymyxin groups, and of lincomycin, are not known. Evidences of calcium binding by tetracyclines suggest such as a possible contributory mechanism in their neuromuscular blocking activity.

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