THE EFFECT OF COLYMYCIN M, GENTAMYCIN AND KANAMYCIN ON DEPRESSION OF NEUROMUSCULAR TRANSMISSION INDUCED BY PANCURONIUM BROMIDE

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INTRODUCTION

THE INTERACTION of antibiotics and neuromuscular blocking agents has been well documented. Neomycin,^{1,2,5} streptomycin,³ kanamycin,³ gentamicin,^{4,5} bacitracin,⁶ polymyxin B⁶ and colymycin⁶ have been shown to potentiate the neuromuscular blockade of d-tubocurarine. The combined action of the muscle relaxant and the antibiotic is often resistant to reversal by both calcium^{12,13,14} and neostigmine.^{12,15}

Administration of d-tubocurarine is often followed by hypotension due to histamine release or ganglionic blockade. Pancuronium bromide is a new non-depolarizing muscle relaxant recently introduced to clinical anaesthesia. It has been found to be free of the adverse effects attributed to d-tubocurarine. However, two clinical reports published recently have shown that kanamycin⁸ and polymyxin B⁷ are capable of prolonging the neuromuscular blockade caused by this new agent. Repeated doses of calcium and neostigmine could not reverse the paralysis.

The purpose of this study was to examine the effect of three members of the mycin group of antibiotics on depression of neuromuscular transmission induced by pancuronium bromide. The agents were administered intravenously to dogs in maximal daily doses recommended for humans. It was the aim of the study to produce a state of partial neuromuscular blockade with pancuronium bromide and then to determine the effect of addition of the test antibiotic on the pre-existing blockade. The antibiotics tested were colymycin (Warner Chilcott Laboratory), gentamycin (Schering) and kanamycin (Bristol).

MATERIALS AND METHODS

The experiments were carried out on 14 dogs of either sex weighing from 11 to 25 kg. Without premedication the animals were anaesthetized with an intravenous injection of nembutal (Abbott) (25 mg/kg). The trachea was intubated without the use of a muscle relaxant. Artificial ventilation with room air was either assisted or controlled using a Bird Mark 8 respirator. Normal blood gases were maintained by adjusting tidal volume according to measured blood gases. Canulae were inserted into a femoral artery and a peripheral vein. The general layout of the experimental set-up is shown in Figure 1.

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FIGURE 1. General layout of the preparation. Shows anaesthetized intubated dog with venous and arterial canulae. The mounting frame holds the experimental limb.

FIGURE 2. Sciatic nerve - tibialis anterior nerve-muscle preparation.

One hind limb was placed in a rigid frame as shown in Figure 2. A pin drilled through the distal end of the femur held the limb firmly in the frame. The sciatic nerve and tibialis anterior muscle were surgically isolated. The nerve was placed on bipolar stimulating electrodes. A silk string attached the tendon of the muscle to an isometric tension transducer (Statham UC3 with UL4-5 cell accessory). The resting tension of the muscle was adjusted to 200 gm using a screw attach-

FIGURE 3. Pharmacological methodology.

ment connected to the transducer. The arterial blood pressure was monitored by a Statham B23A pressure transducer. The experimental responses were monitored on a Beckman dynograph recorder. Additional information may be obtained elsewhere.^{9,10}

Electrical stimuli were delivered to the nerve from a Grass stimulator. The stimuli consisted of square wave pulses of 0.30 mSec duration. The voltage strength ranged from 0.20 to 0.80 volts, with 0.02-volt increments between consecutive stimuli. The minimal stimulus strength was that which elicited a twitch response of 40-80 gm. The three highest stimuli elicited maximal responses. Any preparation requiring a stimulus strength above 1.0 volt to elicit maximal twitch response was rejected. It was assumed that damage to the neuromuscular system had occurred.

The muscle was moistened every 30 minutes with a physiological solution. The animal's body temperature was kept at $37 \pm 0.3^\circ \text{C}$ by a K-thermic unit.

Figure 3 illustrates the pharmacological methodology used in this study. A preliminary study was done with pancuronium, neostigmine and calcium gluconate to establish the base line. Pancuronium bromide in 2.5 to 5 μg dose increments was administered until the control minimal twitch response was depressed by 20 to 60 gm. Thereafter, the muscle responses to the graded stimuli were tested and recorded at 15-minute intervals, for 90 minutes. This was done to study the time course of spontaneous recovery from the paralysis.

In some experiments 15 mg/kg of Calcium gluconate (Glaxo-Allenbury) was administered at the end of 15 minutes. The muscle responses were tested as before 10 and 20 minutes later.

In yet others, the effect of 0.04 mg/kg neostigmine (Roche) was examined by the same methods 10, 15 and 20 minutes from time of drug administration.

With the baseline preliminary study completed, a similar procedure was carried out with pancuronium bromide plus the test antibiotic. The dosage of the antibiotics are shown in Figure 3.

The effect of calcium and neostigmine on the antibiotic plus pancuronium combined blockade was tested as before.

FIGURE 4. Dog (wt. 11 kg) anaesthetized with nembutal. Shows effect of increasing doses of pancuronium bromide on the tibialis anterior muscle twitch responses to graded stimulation of the sciatic nerve.

● = control; doses in mg/kg ○ = 0.16, ▼ = 0.026, △ = 0.037, ■ = after 45 minutes, shows spontaneous recovery. Note some degree of rebound in the lower stimulus range during recovery.

RESULTS

The maximum muscle twitch displacement was 740 ± 40 gm. This was 540 gm over and above the 200 gm resting tension. The mean arterial blood pressure was 150 ± 25 torr and no significant change occurred during the administration of any of the experimental drugs in doses used in this study.

The dose of pancuronium bromide which depressed the minimal muscle twitch by 20 to 60 gm was 0.018 ± 0.004 mg/kg. Increasing the dose of the muscle relaxant caused a greater degree of depression of the muscle twitch response. This was shown by the shift of the dose response curves to the right. Following the administration of pancuronium, the muscle twitch response stabilized at the new level after 10 to 15 minutes. This was maintained from 10 to 30 minutes. Recovery of the twitch response occurred within 90 minutes. Figure 4 documents the rebound which occurred during recovery. This was interpreted as being due to the effect of acetylcholine accumulated at the neuromuscular junction during the period of pancuronium-induced blockade.

FIGURE 5. Dog (wt. 13 kg) anaesthetized with nembutal. Tibialis anterior muscle twitch responses to sciatic nerve stimulation by graded stimuli after administration of colymycin M. 4.5 mg/kg.

● = control, ○ = pancuronium bromide 0.18 mg/kg, ▼ = colymycin M 3.0/kg, ■ = calcium gluconate 15 mg/kg + neostigmine 0.04 mg/kg.

The first antibiotic tested was Colymycin M (Figure 5).

The combined neuromuscular blockade by colymycin and pancuronium bromide was the weakest of the three combinations. It was significantly reversed by both calcium and neostigmine. Generally the reversal by neostigmine was more consistent than that by calcium.

The second antibiotic tested was gentamicin (Figure 6). Gentamicin was shown to potentiate the action of pancuronium bromide to a greater degree than colymycin. The depression of neuromuscular transmission was more progressive. It was partially reversed by neostigmine but not by calcium. This differed from the results obtained with colymycin.

The third antibiotic tested in combination with pancuronium was kanamycin

FIGURE 6. Dog (wt. 15 kg) anaesthetized with nembutal. Tibialis anterior muscle twitch responses to sciatic nerve stimulation by graded stimuli before and after administration of gentamicin 0.85 mg/kg.

● = control, ○ = pancuronium bromide 0.015 mg/kg, ▼ = gentamicin 0.85 mg/kg, Δ = calcium gluconate 15 mg/kg, ■ = neostigmine 0.04 mg/kg.

(Figure 7). Of the three antibiotic-muscle relaxant combinations, this produced the most potent neuromuscular blockade. Sixty minutes after the administration of kanamycin previously supramaximal stimuli elicited less than 50 per cent of the maximal response. Calcium and neostigmine did not reverse the paralysis. These results differ significantly from those obtained with the other two antibiotics.

DISCUSSION

Because of the advantages it has over curare, pancuronium bromide is being used frequently during surgery in severely ill patients. These patients are likely to receive one of the mycin antibiotics by either the parenteral or intraperitoneal route. The problems which are likely to arise due to combining the two agents are simulated in this experimental study. The results obtained indicate that some antibiotic/muscle relaxant combinations produce more severe depression of neuromuscular transmission than others. Gentamicin and kanamycin produce an

FIGURE 7. Dog (wt. 17 kg) anaesthetized with nembutal. Tibialis anterior muscle twitch responses to stimulation of sciatic nerve with graded stimuli before and after the administration of kanamycin 15 mg/kg.

● = control, ○ = pancuronium bromide 0.02 mg/kg, kanamycin 17 mg/kg, ▼ = 45 mins, △ = 75 min, □ = calcium gluconate 15 mg/kg, ■ = neostigmine 0.04 mg/kg.

extremely potent neuromuscular blockade. The resultant paralysis is almost completely refractory to reversal by both calcium and neostigmine. In severely ill patients with pre-existing fluid, electrolyte and acid/base disturbances, a greater degree of blockade would be anticipated.

Several mechanisms have been postulated to explain why the antibiotic/muscle relaxant combination produces such a potent neuromuscular blockade. Vital Brazil and Corrado^{11,16,17} in three separate studies have suggested that the action of antibiotics at the neuromuscular junction was similar to that of excess magnesium ions.¹⁸ Magnesium has been shown to block neuromuscular transmission mainly by preventing the liberation of acetylcholine at the motor nerve terminals. Strong evidence of similar inhibition of acetylcholine release by gentamicin and neomycin has been produced by Vital, Brazil and Prudo-Franceschi,⁴ and by Elmquist.¹ It has also been shown that magnesium ions and antibiotics desensitize the post-synaptic membrane to acetylcholine.^{19,20}

Small doses of d-tubocurarine have been shown to prevent the hypocalcaemic tetany produced in parathyroidectomized animals²¹ as well as *in vitro* prepara-

FIGURE 8. Neuromuscular junction. Suggested mechanism of neuromuscular blockade induced by combination of antibiotic and nondepolarizing muscle relaxant. Left side: normal neuromuscular transmission resulting in muscle action potential (MAP) and contraction. N.A.P., nerve action potential triggers interaction between calcium ions¹ and nerve membrane receptor³ with subsequent release of quanta² acetylcholine from vesicles.

On the right side, the antibiotic, by an action similar to that of magnesium (Mg^{++}) forms an inactive complex with the nerve membrane receptor, displacing calcium and inhibiting the release of acetylcholine. In the presence of decreased calcium ions, the pancuronium bromide (Panc Br.), like curare, would potentiate the antibiotic action.

On the post-synaptic membrane the antibiotic desensitizes⁵ the receptors to acetylcholine, while muscle relaxant⁴ occupies the receptor. Miniature end-plate potentials (MEPP) however continue to occur.

tions in calcium-poor media. The mode of action is believed to be similar to the action of magnesium ions on the presynaptic membrane. The doses of d-tubocurarine able to do this were insufficient to cause paresis in normal animals or in media with adequate amounts of calcium. It is conceivable that pancuronium bromide could have a similar effect.

The mechanism by which nondepolarizing muscle relaxants act on the post-junctional membrane is well documented. Therefore, as shown in Figure 8, it is suggested that the potency of the antibiotic/muscle relaxant combination on neuromuscular junction may be attributed to its multifocal nature.

- (a) The antibiotic inhibits the release of acetylcholine from the presynaptic membrane. It is probable that in the process calcium is dislodged from a specific reception site.²² The antibiotic then forms an inactive complex with the receptor.

- (b) The antibiotic desensitizes the post-junctional membrane receptor to the transmitter.
- (c) With reduced calcium at the presynaptic membrane, a small dose of the muscle relaxant will cause a further inhibition of acetylcholine release.
- (d) Finally, the muscle relaxant blocks the effect of any residual transmitter at the post-junctional membrane in a competitive manner. Thus the antibiotic muscle relaxant induced neuromuscular blockade is both pre- and post-junctional. This accounts for its resistance to reversal by both calcium and neostigmine.

SUMMARY

In summation, the effect of three mycin antibiotics on pancuronium bromide-induced depression of neuromuscular transmission was studied, using a sciatic nerve tibialis anterior muscle preparation of anaesthetized dogs. Muscle twitch responses were recorded before and after administration of non-paralysing doses of the muscle relaxant and the test antibiotic.

It was inferred from the results that colymycin, gentamycin and kanamycin potentiated the neuromuscular blockade induced by pancuronium. Colymycin showed the least potency while kanamycin induced the most effective blockade. Calcium and neostigmine had limited effect in reversing the neuromuscular blockade due to gentamycin and kanamycin. The antibiotics are believed to depress neuromuscular transmission by depleting acetylcholine at the motor end-plate, through inhibition of its release. With decreased calcium levels in the motor nerve terminal membranes, non-depolarizing muscle relaxants exert a similar effect. At the post-synaptic membrane the antibiotic desensitizes the receptors to acetylcholine, while the muscle relaxant acts by occupying the receptor.

The mode of action of the antibiotic and muscle relaxant at the presynaptic membrane is similar to that of magnesium ions. The resistance to reversal of the neuromuscular blockade by both calcium and neostigmine finds explanation in its multifocal nature.

RÉSUMÉ

Nous avons étudié la synergie pharmacologique entre le pancuronium et trois antibiotiques en mycin (colymicine, gentamycine et kanamycine) en mesurant la transmission neuro-musculaire sur une préparation nerf sciatique-jambier antérieur de chien anesthésié. Les réponses du muscle à des stimulations non tétaniques ont été enregistrées avant et après administration de doses sub-limaires (non curarisantes) de pancuronium et de l'antibiotique étudié.

Nous concluons de nos résultats que ces trois antibiotiques potentialisent le blocage neuro-musculaire du pancuronium. Cet effet était minime avec la colymicine mais très puissant avec la kanamycine. Le calcium et la néostigmine ne renversent que médiocrement le blocage neuro-musculaire attribuable à la gentamycine et la kanamycine. On croit que ces antibiotiques exercent leur action bloquante en privant la plaque neuro-musculaire d'acétylcholine par inhibition de sa libération.

Il a déjà été démontré que les relaxants non dépolarisants, eux aussi, produisent un effet présynaptique similaire. Au niveau de la membrane post-synaptique, l'antibiotique réduit l'affinité des récepteurs à l'acétylcholine alors que le myo-résolutif pour sa part occupe d'emblée le site récepteur.

Au niveau pré-synaptique la combinaison antibiotique et relaxant musculaire agit tout comme les ions magnésium, c'est-à-dire, en empêchant la libération d'acétylcholine. Ainsi le bloc neuro-musculaire causé par cette combinaison médicamenteuse est-il résistant à la néostigmine et au calcium en raison de la dualité de son origine, c'est-à-dire, qu'il est à la fois d'origine pré- et post-synaptique.

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