

4-Aminopyridine Potentiates Neostigmine and Pyridostigmine in Man

Ronald D. Miller, M.D.,* Leo H. D. J. Booij, M.D.,† Sandor Agoston, M.D., Ph.D.,‡ Jan F. Crul, M.D., Ph.D.§

To elucidate the interaction of 4-aminopyridine with neostigmine and pyridostigmine, the authors studied 57 anesthetized surgical patients using a technique of constant infusion of pancuronium to quantitate antagonist activity. 4-Aminopyridine, 0.15 or 0.35 mg/kg, produced no antagonism, while 0.5 mg/kg produced a mean 24 ± 6 per cent (peak) antagonism. The dose that produced 50 per cent antagonism (ED_{50}) of neostigmine alone was 22 μ g/kg; with 0.35 mg/kg 4-aminopyridine, it was 7 μ g/kg. The ED_{50} of pyridostigmine alone was 110 μ g/kg; with 0.35 mg/kg 4-aminopyridine, it was 27 μ g/kg. 4-Aminopyridine prolonged the onset times of both neostigmine and pyridostigmine, but prolonged the duration of action of neostigmine only. At a given level of antagonism of pancuronium, adding 4-aminopyridine 0.35 mg/kg, to neostigmine and to pyridostigmine decreased the amounts of atropine needed to prevent a change in heart rate by 68 and 70 per cent, respectively. The authors conclude that 4-aminopyridine potentiates antagonism of a pancuronium-induced neuromuscular blockade by neostigmine or pyridostigmine. Also, less atropine is needed to prevent cardiac muscarinic stimulation when 4-aminopyridine is used with either neostigmine or pyridostigmine. (Key words: Antagonists, neuromuscular relaxants: 4-aminopyridine; neostigmine; pyridostigmine. Muscle relaxants: pancuronium. Parasympathetic nervous system: atropine.)

THE ACETYLCHOLINESTERASE INHIBITION produced by neostigmine and pyridostigmine has been used to antagonize nondepolarizing neuromuscular blockades for several years. Two disadvantages of these antagonists are the requirement for concomitant administration of atropine to prevent muscarinic stimulation and their inability to antagonize neuromuscular blockades resulting from the use of many antibiotics, alone and in combination with muscle relaxants.^{1,2} The use of 4-aminopyridine as an antagonist of a nondepolarizing neuromuscular blockade may not be attended by these disadvantages of neostigmine and

pyridostigmine. Preliminary observations in man³ and studies in animals^{4,5} show no muscarinic effect of 4-aminopyridine. In fact, 4-aminopyridine has recently been shown to have sympathetic activity. It potentiated responses of the adrenergically innervated rabbit vas deferens to transmural stimulation by causing norepinephrine release,⁴ and increased both evoked and spontaneous release of norepinephrine from the portal vein.⁶ Also, 4-aminopyridine antagonizes the neuromuscular blockades produced by most antibiotics.⁷ Unfortunately, the doses of 4-aminopyridine necessary for complete antagonism (>1 mg/kg) also stimulate the central nervous system and cause postoperative restlessness and confusion (unpublished data, S. Agoston).

We recently observed synergism between 4-aminopyridine and neostigmine or pyridostigmine in rats.⁸ Such synergism in man could decrease the dose of neostigmine or pyridostigmine necessary for antagonism and thereby attenuate or even eliminate the need for concomitant administration of atropine. In the described herein study we demonstrated that synergism does occur, and that the requirement for atropine is concomitantly decreased.

Methods

Fifty-seven consenting surgical patients, ASA Class I or II, were studied intraoperatively. They were 43 ± 7 (S.D.) years of age and 74 ± 9 kg in weight. They received atropine, 0.25 mg, droperidol, 5 mg, and piritramide (a narcotic analgesic), 11.25 mg, im, approximately an hour prior to anesthesia. Anesthesia was induced with thiopental, 1.5 to 2.0 mg/kg, gamma-hydroxybutyric acid, 50 mg/kg, and fentanyl, 1.5 μ g/kg, iv. Controlled ventilation kept end-tidal carbon dioxide concentration between 4 and 5 per cent.

Through 25-gauge needle electrodes, we delivered supramaximal square-wave bipolar pulses of 0.1-msec duration at 0.1 Hz to the ulnar nerve at the wrist. The resultant force of thumb adduction was quantitated with a force-displacement transducer and recorded on a polygraph.

A technique of constant infusion of pancuronium to

* Professor, Institute of Anesthesiology, Catholic University, Nijmegen, The Netherlands, and Institute of Clinical Experimental Anesthesia, State University of Groningen, Groningen, The Netherlands.

† Staff member, Institute of Anesthesiology, Catholic University, Nijmegen.

‡ Senior Research Associate, Institute of Anesthesiology, Catholic University, Nijmegen, and Institute of Clinical Experimental Anesthesia, State University of Groningen.

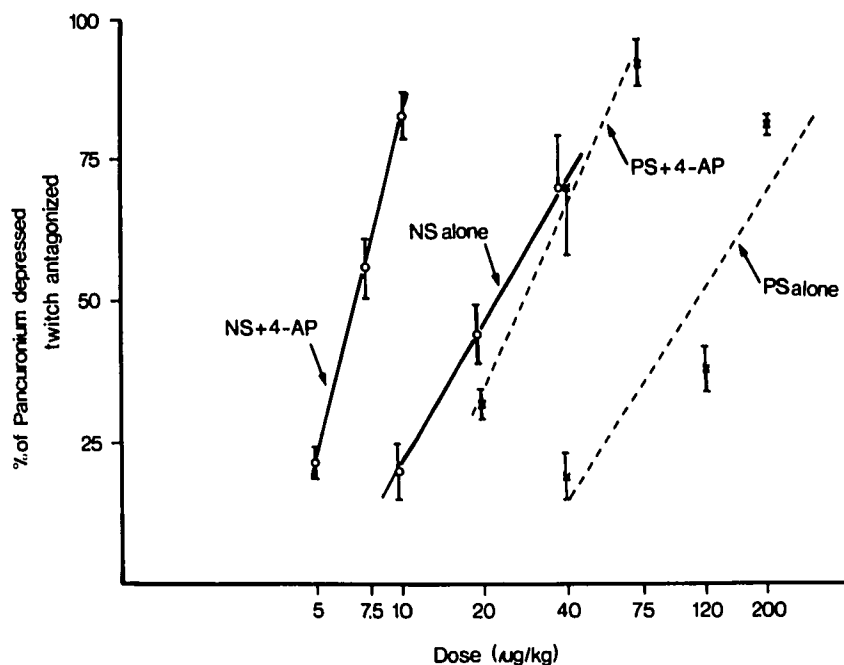
§ Professor and Chairman, Institute of Anesthesiology, Catholic University, Nijmegen.

Work performed while Dr. Miller was a Visiting Professor in The Netherlands. Accepted for publication August 14, 1978.

Address reprint requests to present address: Dr. Miller at his Department of Anesthesia, University of California, San Francisco, California 94143.

¶ Bikhazi GB, Burkett L, Kelly P, et al: The interaction of antibiotics, *d*-tubocurarine and 4-aminopyridine at the neuromuscular junction. Abstracts, Annual Meeting of the American Society of Anesthesiologists, 1976, pp 463-464.

FIG. 1. Correlation between neostigmine (NS) and pyridostigmine (PS) alone and with 4-aminopyridine, 0.35 mg/kg, (4-AP) with percentage of pancuronium-induced depression of twitch tension antagonized. The lines represent analysis of linear regression. Each dot or X represents the mean \pm 1 SE of values for three patients.



quantitate antagonist activity was used for all studies.⁹ After a bolus injection of pancuronium, 20 $\mu\text{g}/\text{kg}$, iv, pancuronium, 200 $\mu\text{g}/\text{ml}$, was infused continuously from a pump to produce a constant 90 per cent depression of twitch tension. The rate needed decreased progressively for the next 15–45 min, after which it remained constant, as determined by at least 15 min of observation. With the pancuronium infusion continued at this rate, the antagonist was given as bolus, iv. The resultant maximum antagonism of twitch depression was recorded and presented as a percentage of the pre-existing 90 per cent depression (*e.g.*, a peak increase to 60 per cent depression of the control twitch tension before pancuronium would be calculated as $(90 - 60) 100/90$, or 33 per cent antagonism). In addition, we measured times from antagonist administration to 50, 70 and 100 per cent of peak effect (onset time) and 70, 50 and 30 per cent return to the pancuronium-induced depressed twitch tension (duration of action). Details of these calculations have been described.⁹

Dose–response curves were determined for neostigmine, pyridostigmine, and 4-aminopyridine alone. Dose–response curves were then determined for neostigmine and pyridostigmine in the presence of 4-aminopyridine, 0.35 mg/kg. Dose–response curves also were determined for 4-aminopyridine in the presence of neostigmine, 7.5 $\mu\text{g}/\text{kg}$, or pyridostigmine, 40 $\mu\text{g}/\text{kg}$. Only one dose of antagonist or antagonist mixture was studied in each patient. The dose of antagonist that produced 50 per cent antagonism (ED_{50}) was derived from curves determined by analysis

of linear regression. The curves were compared for parallelism and shift by analysis of covariance. The onset times and duration of action were compared by *t* test for unpaired data.

Patients who received neostigmine or pyridostigmine alone were also given atropine, 0.5 mg, iv. Those who received 4-aminopyridine alone were not given atropine. Those who received the mixtures of neostigmine or pyridostigmine with 4-aminopyridine were given atropine, 0.25 mg. An additional 0.25 mg atropine was given for every 10 beat/min decrease in heart rate in any patient. The total amount of atropine given was calculated. The amount of atropine needed with each dose of antagonist was plotted against the percentage of antagonism that dose of antagonist produced. The atropine requirements were then compared by analysis of variance.

Results

We did not construct a dose–response curve for 4-aminopyridine alone because neither 0.15 ($n = 3$) nor 0.35 mg/kg ($n = 3$) 4-aminopyridine antagonized the pancuronium-induced depression of twitch tension, but 0.5 mg/kg ($n = 3$) produced 24 ± 6 per cent antagonism. The doses of neostigmine and pyridostigmine that produced 50 per cent antagonism of the pancuronium-induced depression of twitch (ED_{50}) were 22 and 110 $\mu\text{g}/\text{kg}$, respectively (fig. 1). The addition of 4-aminopyridine, 0.35 mg/kg, decreased the ED_{50} s of neostigmine and pyridostigmine to 7 and 27 $\mu\text{g}/\text{kg}$, respectively ($P < 0.01$) (fig. 1). The curves did not deviate from parallelism.

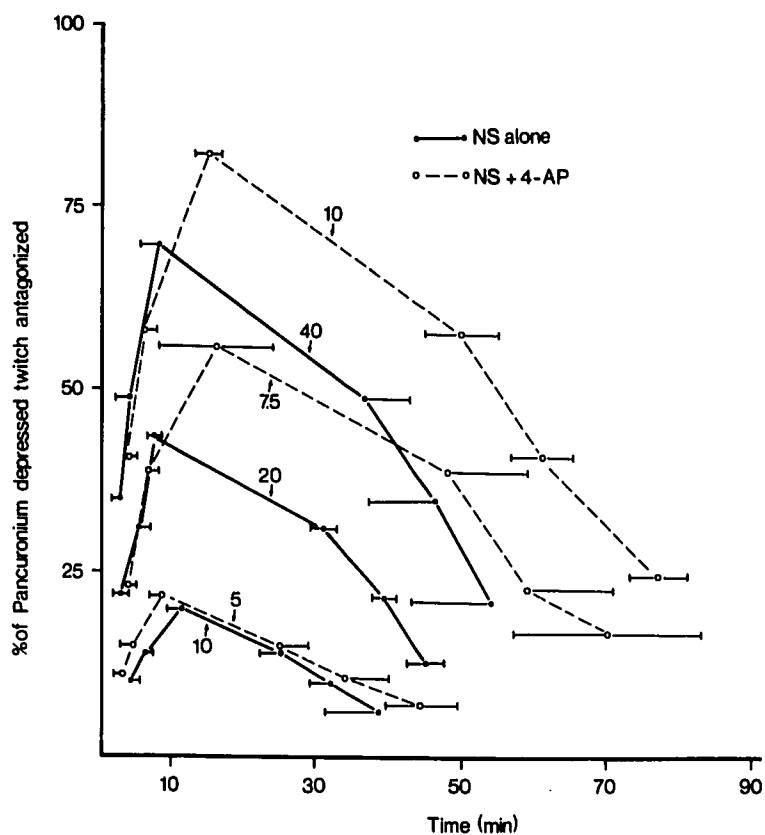


FIG. 2. Plot of time and percentage of pancuronium-induced depression of twitch tension antagonized. The numbers represent doses of neostigmine (NS) in $\mu\text{g}/\text{kg}$. The dose of 4-aminopyridine used was 0.35 mg/kg. The dots and brackets represent means \pm 1 SE. Three patients were studied at each dose.

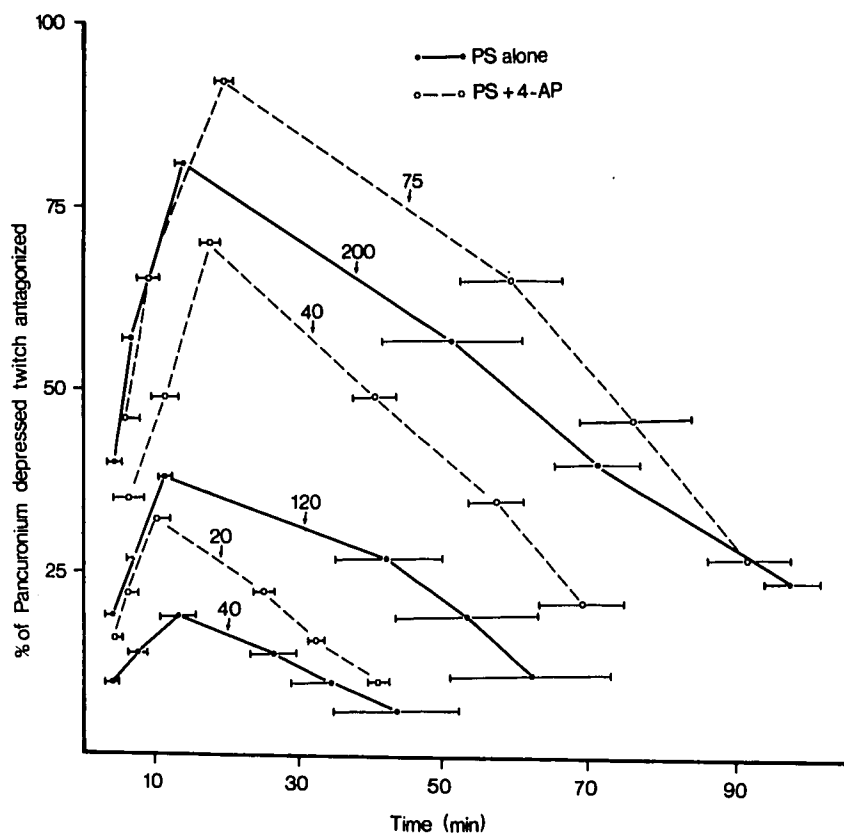
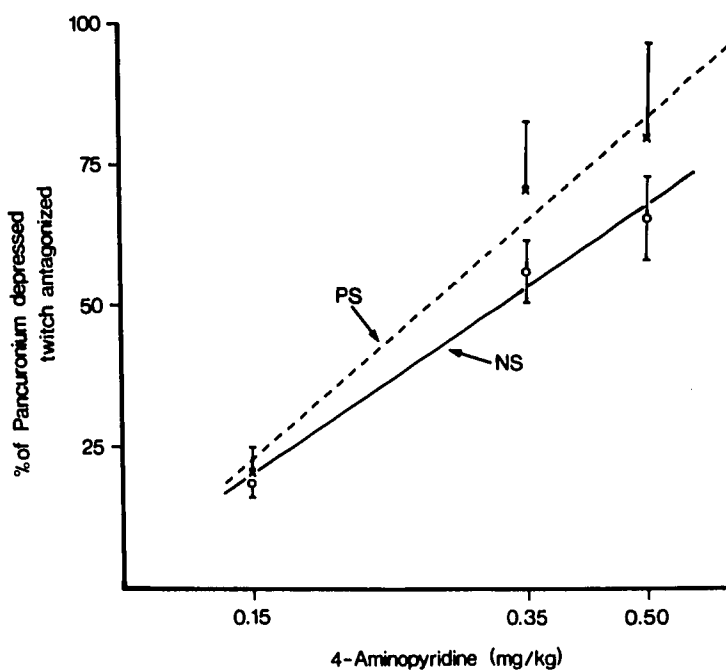


FIG. 3. Plot of time and percentage of pancuronium-induced depression of twitch tension antagonized. The numbers represent doses of pyridostigmine (PS) in $\mu\text{g}/\text{kg}$. The dose of 4-aminopyridine used was 0.35 mg/kg. The dots and brackets represent means \pm 1 SE. Three patients were studied at each dose.

FIG. 4. Correlation between dose of 4-aminopyridine with either neostigmine (NS), 7.5 $\mu\text{g}/\text{kg}$, or pyridostigmine (PS), 40 $\mu\text{g}/\text{kg}$, with percentage of pancuronium-induced depression of twitch tension antagonized. The lines represent analysis of linear regression. Each dot or X represents the mean \pm 1 SE of values for three patients.



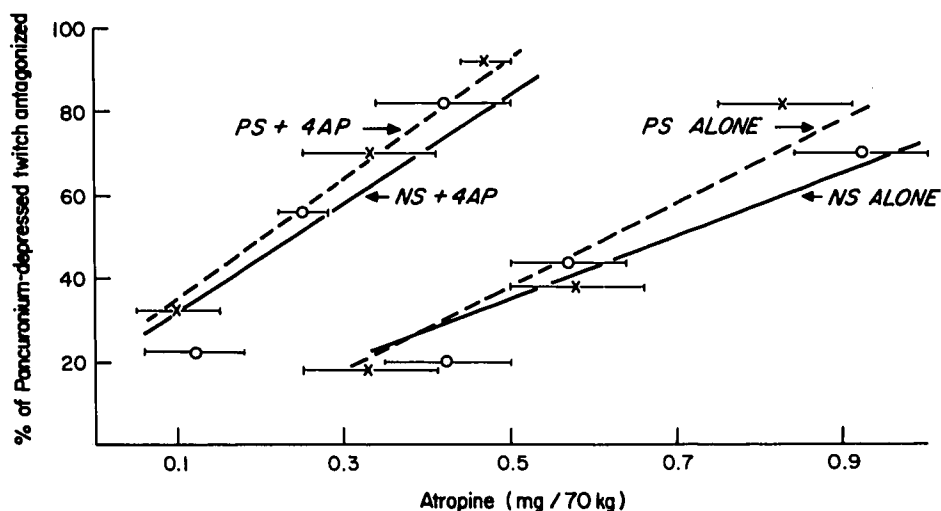
At approximately equipotent doses, 4-aminopyridine increased onset times of neostigmine and pyridostigmine and the duration of neostigmine effect, except in the smallest doses given (fig. 2). There was no difference in times to onset or durations of action of neostigmine, 10 $\mu\text{g}/\text{kg}$, alone, and neostigmine, 5 $\mu\text{g}/\text{kg}$, plus 4-aminopyridine, 0.35 mg/kg (fig. 2).

Comparison of onset times and durations of action of pyridostigmine alone and pyridostigmine plus 4-aminopyridine reveals an inconsistent pattern. Comparison of pyridostigmine, 20 $\mu\text{g}/\text{kg}$, plus 4-aminopyridine, with pyridostigmine, 40–120 $\mu\text{g}/\text{kg}$, alone, shows no difference in onset times. However, pyridostigmine, 40 and 75 $\mu\text{g}/\text{kg}$, with 4-

aminopyridine had longer onset times than pyridostigmine, 200 $\mu\text{g}/\text{kg}$, alone ($P < 0.05$) (fig. 3). Pyridostigmine, 120 $\mu\text{g}/\text{kg}$, alone, had a longer duration of action than did 20 $\mu\text{g}/\text{kg}$ with 4-aminopyridine ($P < 0.05$). These doses produced similar extents of antagonism. Conversely, the durations of action of pyridostigmine, 200 $\mu\text{g}/\text{kg}$, alone, and pyridostigmine 75 $\mu\text{g}/\text{kg}$, with 4-aminopyridine did not differ significantly. 4-Aminopyridine enhanced antagonism by neostigmine and pyridostigmine in a dose-dependent manner (fig. 4).

Less atropine was necessary to prevent bradycardia when 4-aminopyridine was combined with neostigmine or pyridostigmine at a given level of antagonism (fig. 5). When neostigmine and pyridostigmine

FIG. 5. Correlation between dose of atropine necessary to prevent a change in heart rate of more than 10 beats/min from neostigmine (O) or pyridostigmine (X) with and without 4-aminopyridine and percentage of pancuronium-induced depression of twitch tension antagonized.



were given in doses that caused 50 per cent antagonism, atropine, 0.70 mg/70 kg ($r = 0.87$), and 0.62 mg/70 kg ($r = 0.85$), respectively, were needed to prevent a change in heart rate. When 50 per cent antagonism was achieved with 4-aminopyridine, 0.35 mg/kg, and neostigmine or pyridostigmine, the doses of atropine necessary to prevent a change in heart rate decreased to 0.24 mg/70 kg ($r = 0.81$) and 0.20 mg/70 kg ($r = 0.88$), respectively ($P < 0.01$) (fig. 5). The curves did not deviate from parallelism. No blood pressure or heart rate change resulted from administration of 4-aminopyridine alone at any dose used. Although the electroencephalogram was not used, we did not observe any sign of central nervous system stimulation such as spontaneous muscle twitching or seizure activity intraoperatively or in the recovery room.

Discussion

Antagonism of a pancuronium-induced neuromuscular blockade by neostigmine and pyridostigmine is potentiated by 4-aminopyridine. That is, 4-aminopyridine, 0.35 mg/kg, which produced no antagonism by itself, decreased the ED₅₀s of neostigmine and pyridostigmine by 68 and 80 per cent, respectively (fig. 1). This is not surprising since 4-aminopyridine has a different mechanism of action. It antagonizes a pancuronium-induced neuromuscular blockade by increasing both evoked and spontaneous release of acetylcholine from the motor nerve terminal, rather than by inhibition of acetylcholinesterase.⁵

Blood pressure and heart rate were not changed by the doses of 4-aminopyridine administered in this study, which is consistent with previous reports of no muscarinic activity.³⁻⁵ Another advantage of 4-aminopyridine is that it antagonizes neuromuscular blockades produced by antibiotics when neostigmine and pyridostigmine are often ineffective.^{7a} These advantages suggest the use of 4-aminopyridine rather than neostigmine or pyridostigmine. However, doses of 4-aminopyridine greater than those used in our study (*i.e.*, more than 1 mg/kg) cause central nervous system excitation, restlessness and confusion postoperatively (unpublished data, S. Agoston).

The combination of neostigmine or pyridostigmine with 4-aminopyridine may attenuate or eliminate the disadvantages that these drugs have when given individually. We observed no evidence of central nervous system stimulation intra- or postoperatively. The amount of atropine needed to prevent brady-

cardia was significantly decreased by the addition of 4-aminopyridine (fig. 5). Cardiac arrhythmias and hypotension occasionally occur when neostigmine or pyridostigmine is given with atropine for antagonism of neuromuscular blockade.¹⁰⁻¹² We presume that an antagonist that requires less atropine will be associated with less significant cardiovascular problems clinically. This assumption can be documented only by further clinical trials. We believe the advantages of a lesser atropine requirement and possibly a predictable antagonism of an antibiotic-induced neuromuscular blockade are sufficient to warrant further studies.

The authors gratefully acknowledge the editorial advice of Dorothy Urban, William K. Hamilton, M.D., Edmond I. Eger, II, M.D., and Walter L. Way, M.D.

References

1. Fogdall RP, Miller RD: Antagonism of *d*-tubocurarine- and pancuronium-induced neuromuscular blockades by pyridostigmine in man. *ANESTHESIOLOGY* 39:504-509, 1973
2. Miller RD: Antagonism of neuromuscular blockade. *ANESTHESIOLOGY* 44:318-329, 1976
3. Stoyanov E, Vulchev M, Shturbova M, et al: Clinical electromyographic and electromyographic studies in decurarization with pymadine. *Anaesth Resusc Intensive Ther* 4:139-142, 1976
4. Johns A, Golko DS, Lauzon PA, et al: The potentiating effects of 4-aminopyridine on adrenergic transmission in the rabbit vas deferens. *Eur J Pharmacol* 38:71-78, 1976
5. Harvey AL, Marshall IG: The actions of three diaminopyridines on the chick biventer cervicis muscle. *Eur J Pharmacol* 44:303-309, 1977
6. Leander S, Anders A, Johansson B: Effects of 4-aminopyridine on mechanical activity and noradrenaline release in the rat portal vein *in vitro*. *Eur J Pharmacol* 46: 351-361, 1977
7. Booiij LHDJ, Miller RD, Crul JF: Neostigmine and 4-aminopyridine antagonism of lincomycin-pancuronium neuromuscular blockade in man. *Anesth Analg (Cleve)* 57:316-321, 1978
8. Miller RD, Dennissen PAF, van der Pol F, et al: Potentiation of neostigmine and pyridostigmine by 4-aminopyridine in the rat. *J Pharm Pharmacol* 30:699-702, 1978
9. Miller RD, Van Nyhuis LS, Eger EI II: Comparative times to peak effect and durations of action of neostigmine and pyridostigmine. *ANESTHESIOLOGY* 41:27-33, 1974
10. Glisson SN, Fajardo L, El-Etr AA: Amitriptyline therapy increases electrocardiographic changes during reversal of neuromuscular blockade. *Anesth Analg (Cleve)* 57:77-83, 1978
11. Fogdall RP, Miller RD: Antagonism of *d*-tubocurarine- and pancuronium-induced neuromuscular blockades by pyridostigmine in man. *ANESTHESIOLOGY* 39:504-509, 1973
12. Pittinger CB: Antagonists of muscle relaxants, *Muscle Relaxants*. Edited by Foldes FF. Philadelphia, F. A. Davis, 1966, pp 109-110