J. Pharm. Pharmac., 1978, **30**, 699–702 *Received March* 13, 1978

Potentiation of neostigmine and pyridostigmine by 4-aminopyridine in the rat

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The interaction between 4-aminopyridine and neostigmine or pyridostigmine was studied *in vivo* in the rat sciatic nerve-anterior tibialis preparation using the constant infusion of pancuronium technique. The ED50 (dose of drug which produced a 50% antagonism) of neostigmine, pyridostigmine and 4-aminopyridine were 18, 49 and 440 μg kg^{-1} respectively. The addition of 100 μg kg^{-1} of 4-aminopyridine, which produced no antagonism by itself, decreased the neostigmine ED50 to 7·4 μg kg^{-1}. The addition of 200 μg kg^{-1} of 4-aminopyridine, which produced a 30% antagonism by itself, decreased the ED50 of pyridostigmine to 11 μg kg^{-1}. We conclude that both neostigmine and pyridostigmine interact with 4-aminopyrine synergistically.

Neostigmine and pyridostigmine are used clinically to antagonize a neuromuscular blockade produced by pancuronium or (+)-tubocurarine (Miller, 1976). Neostigmine and pyridostigmine act primarily by inhibition of acetylcholinesterase. Recently, 4-aminopyridine has been used clinically as an antagonist of (+)-tubocurarine (Stoyanov, Vulchev & others, 1976). However, the mechanism of this antagonism differs from that of neostigmine or pyridostigmine. 4-Aminopyridine does not inhibit acetylcholinesterase activity (Molgo, Lemeignan & Lechat, 1977), but increases the amount of acetylcholine released by the motor nerve terminal in response to a nerve impulse (Bowman, Harvey & Marshall, 1977, Molgo & others, 1977). Since neostigmine or pyridostigmine antagonize a pancuronium-induced neuromuscular blockade by a different mechanism from that of 4-aminopyridine, we propose that these agents may not act in an additive manner. We tested this possibility in the following study.

METHODS

Eighty-five rats, 260 to 430 g were anaesthetized with pentobarbitone, 40 mg kg^{-1} and urethane, 500 mg kg^{-1}, intraperitoneally. Both jugular veins were cannulated and all drugs were administered through these cannulae. A cannula in the carotid artery permitted transduction of arterial blood pressure. A tracheostomy was performed and ventilation was controlled with a Braun air pump. The tendon of the left tibialis anterior muscle was freed, sectioned and connected to a Grass F103 force displacement transducer. The resting tension was adjusted to 20 g. Stimuli of 0·2 ms duration and of strength sufficient to produce a maximal twitch were applied to the sciatic nerve through a bipolar electrode at a frequency of 0·1 Hz. Twitch tension was recorded on a polygraph. Rectal and muscle temperatures were maintained between 37 and 38°.

Pancuronium was administered intravenously by continuous infusion to achieve and maintain 90% depression of twitch tension. When the infusion rate required to maintain 90% depression of twitch tension was constant for at least 15 min, neostigmine, pyridostigmine or 4-aminopyridine or a combination of these drugs was given as an intravenous bolus. Except when 4-aminopyridine was given alone, atropine 17 μg kg^{-1} was given intravenously before administration of drugs (see Miller, Van Nyhuis & others, 1974 for technique). Time from antagonist administration to peak effect (onset), magnitude of antagonism, and time from antagonist administration to 50% return to the pancuronium-depressed twitch tension (duration) were calculated. Only one dose of antagonist or combination of antagonists was studied with each animal.

Initially, dose-response curves were determined for neostigmine, pyridostigmine and 4-aminopyridine alone. Then neostigmine or pyridostigmine was combined with various doses of 4-aminopyridine to determine whether these drugs interact with each other in an additive or synergistic manner. Specifically, dose response curves were determined for neostigmine with 4-aminopyridine 100 μg kg^{-1} and for

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*Work performed while Doctor Miller was a visiting professor at the above institutions.

pyridostigmine with 4-aminopyridine 200 μg kg^{-1}. Dose response curves were determined for 4-aminopyridine with neostigmine 3·5 and 7·5 μg kg^{-1} and with pyridostigmine 25 μg kg^{-1}. The dose response curves were compared by analysis of variance.

RESULTS

The ED50 (dose of antagonist which produced a 50% antagonism of the pancuronium-depressed twitch tension) of neostigmine, pyidostigmine and 4-aminopyridine were 18, 49 and 440 μg kg^{-1} (Fig. 1).

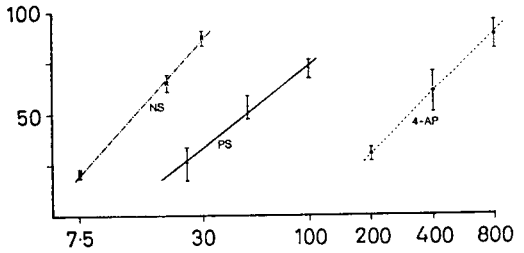

FIG. 1. Correlation between dose (μg kg^{-1}) (abscissa) of neostigmine (NS), pyridostigmine (PS) and 4-aminopyridine (4-AP) on % antagonism (ordinate) of the pancuronium-depressed twitch. The lines represent analysis of linear regression. The vertical lines represent the mean ± s.e. in four rats.

The dose response curves did not significantly deviate from parallelism. The 1200 μg kg^{-1} dose of 4-aminopyridine caused generalized twitching of all muscles which resembled convulsive activity in spite of the animal being anaesthetized with pentobarbitone. This dose was not included in the calculations because it caused no more antagonism than did the 800 μg kg^{-1} dose. Addition of 100 μg kg^{-1} of 4-aminopyridine decreased the ED50 of neostigmine from 18 to 7·4 μg kg^{-1} (Fig. 2). Addition of 200 μg kg^{-1} of 4-aminopyridine decreased the ED50 of pyridostigmine from 49 to 11 μg kg^{-1} (Fig. 3). Addition of 3·5 and 7·5 μg kg^{-1} of neostigmine decreased the ED50 of 4-aminopyridine from 440 to

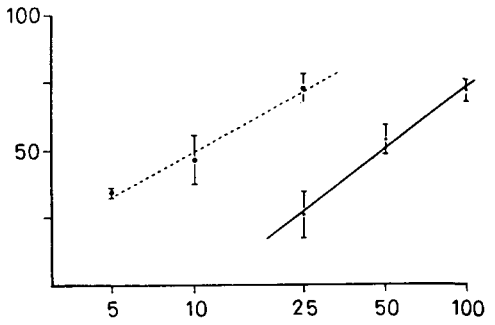

FIG. 3. The effect of pyridostigmine alone (—) and given with 4-aminopyridine (200 μg kg^{-1}, - - -) on % antagonism (ordinate) of the pancuronium-depressed twitch. The lines represent analysis of linear regression. The vertical lines represent the mean ± s.e. of four rats. Abscissa: Pyridostigmine (μg kg^{-1}).

220 μg kg^{-1} and to 82 μg kg^{-1} respectively (Fig. 4). The ED50 of 4-aminopyridine also was reduced from 440 μg kg^{-1} to 76 g kg^{-1} by pyridostigmine, 25 μg kg^{-1} (Fig. 5).

The ED50 values of the various combinations of 4-aminopyridine plus neostigmine or pyridostigmine were plotted against an isobologram constructed from the ED50 values of the drugs alone. Either combination produced an effect greater than simple addition (Fig. 6).

When equivalent levels of antagonism are compared, 4-aminopyridine increased the onset times but not the duration of action of neostigmine (P <0·05)

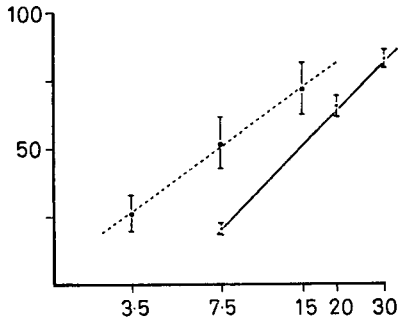

FIG. 2. The effect of neostigmine alone (———) and given with 4-aminopyridine (100 μg kg^{-1}, - - - - -) on % antagonism (ordinate) of the pancuronium-depressed twitch. The lines represent analysis of linear regression. The vertical lines represent the mean ± s.e. of four rats. Abscissa: Neostigmine (μg kg^{-1}).

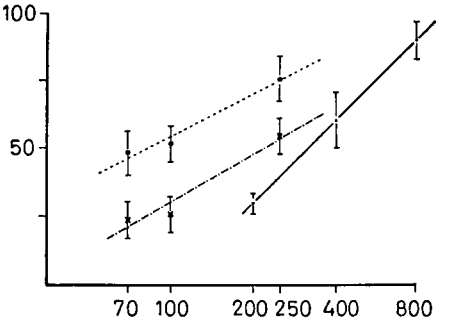

FIG. 4. The effect of 4-aminopyridine alone (—) and given with neostigmine (—·—·—, 3·5 μg kg^{-1}; - - - - 7·5 μg kg^{-1}) on % antagonism (ordinate) of the pancuronium-depressed twitch. The lines represent analysis of linear regression. The vertical lines represent the mean ± s.e. of four cats. Abscissa: 4-aminopyridine (μg kg^{-1}).

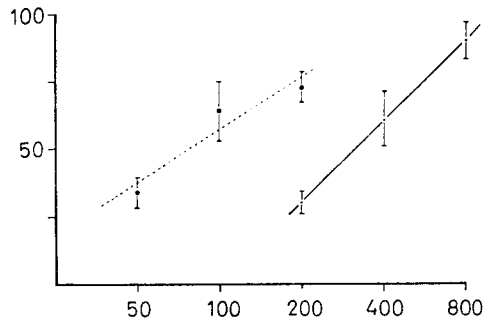

FIG. 5. The effect of 4-aminopyridine alone (—) and given with pyridostigmine (25 μg kg^{-1}, - - - -) on % anatagonism (ordinate) of the pancuronium-depressed twitch. The lines represent analysis of linear regression. The vertical lines represent the mean ± s.e. for four rats. Abscissa: 4-Aminopyridine (μg kg^{-1}).

(Fig. 7A). In contrast, 4-aminopyridine had no effect on either the onset times or duration of pyridostigmine action with one exception (Fig. 7B). The pyridostigmine 10 μg kg^{-1}, plus 4-aminopyridine 200 μg kg^{-1} combination had a longer onset time than pyridostigmine alone ($P < 0{\cdot}05$) (Fig. 7B).

Blood pressure was not changed by the above drugs alone or combinations.

DISCUSSION

4-Aminopyridine potentiated the effect of neostigmine or pyridostigmine (Fig. 6). That is, the effects of the neostigmine or pyridostigmine and 4-aminopyridine combinations were greater than would have been predicted from the effects of the drugs given alone. An effect other than simple addition might have been predicted from the fact that 4-aminopyridine has a different mechanism of action from neostigmine and pyridostigmine. 4-Aminopyridine antagonizes a neuromuscular blockade by increasing both evoked and spontaneous release of acetylcholine from the motor nerve terminal rather than by inhibition of acetylcholinesterase (Bowman & others, 1977).

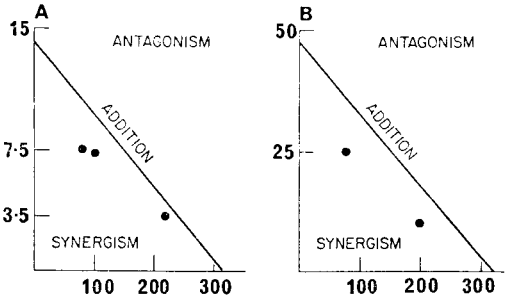

FIG. 6. Correlation of the ED50 (dose which produced a 50% antagonism of the pancuronium depressed twitch) of 4-aminopyridine, neostigmine and pyridostigmine alone from which the line of identity was formed. The dots represent the ED50 of various combinations of the above drugs. Ordinate: μg kg^{-1}. Abscissa: 4-Aminopyridine (μg kg^{-1}).

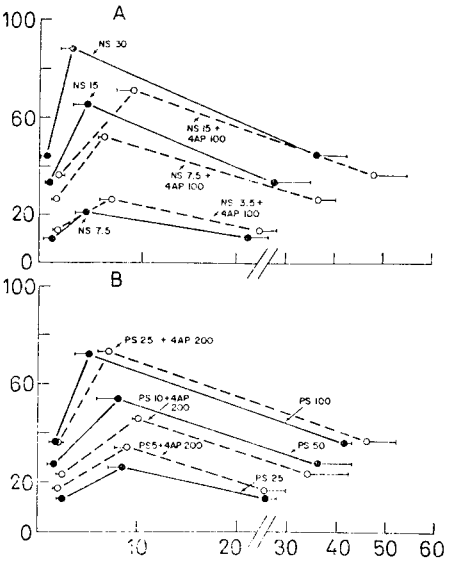

FIG. 7. Correlation between time (min) (abscissa) and % antagonism (ordinate) of the pancuronium-depressed twitch with neostigmine (NS) (A) or pyridostigmine (PS) (B) alone and with 4-aminopyridine (4-AP). The numbers beside the drug abbreviations are the doses in μg kg^{-1}.

Neostigmine and pyridostigmine are drugs usually used to antagonize a non-depolarizing neuromuscular blockade (Miller, 1976). However, 4-aminopyridine may offer some advantages. The apparent absence of muscarinic effects eliminates the need for concomitant administration of atropine as is required with neostigmine and pyridostigmine (Fogdall & Miller, 1973). In fact, recently, 4-aminopyridine has been shown to have some sympathetic activity. It potentiated responses of the adrenergically innervated rabbit vas deferens to transmural stimulation by causing increased noradrenaline release (Johns, Golko & others, 1976). Also, 4-aminopyridine increased both evoked and spontaneous release of adrenaline from the portal vein (Leander, Arner & Johansson, 1977). Despite this evidence for sympathetic stimulation, the blood pressure in this study was not altered by administration of 4-aminopyridine.

Another advantage of 4-aminopyridine is that it will antagonize an antibiotic neuromuscular blockade

where neostigmine and pyridostigmine are often ineffective (Bikhazi, Burkett & others, 1977). With these advantages, perhaps 4-aminopyridine should be used alone rather than in combination with neostigmine or pyridostigmine. However, 4-aminopyridine readily crosses the blood brain barrier. Doses of 4-aminopyridine which completely antagonize a non-depolarizing neuromuscular blockade cause central nervous system stimulation which we observed with the 1200 $\mu g\ kg^{-1}$ dose; the precise relation between dose and central nervous system stimulation needs to be defined. Perhaps the combination of neostigmine or pyridostigmine plus 4-aminopyridine will attenuate or eliminate the disadvantages of these drugs individually. That is, the dose of 4-aminopyridine will be low enough not to cause central nervous system stimulation, yet may reduce the doses of neostigmine or pyridostigmine sufficiently to attenuate or eliminate the need for atropine to prevent muscarinic stimulation. Also, perhaps the combination of neostigmine or pyridostigmine plus 4-aminopyridine can be used to antagonize the antibiotic-induced neuromuscular blockades where neostigmine or pyridostigmine alone often fail (Miller, 1976).

The above advantages of the drug combination are speculative and need further study. That 4-aminopyridine markedly enhances the ability of neostigmine or pyridostigmine to antagonize a pancuronium neuromuscular blockade needs confirmation in other species including man.

Acknowledgements

The authors gratefully acknowledge the editorial advice of Dr Edmond I. Eger, II and Dorothy Urban.

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