Br. J. Pharmac. (1979), **65**, 53–62

A COMPARISON OF THE FACILITATORY ACTIONS OF 4-AMINOPYRIDINE METHIODIDE AND 4-AMINOPYRIDINE ON NEUROMUSCULAR TRANSMISSION

A.S. HORN*, J.J. LAMBERT & I.G. MARSHALL

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, G1 1XW and *Laboratory for Pharmaceutical and Analytical Chemistry. Rijksuniversiteit, Groningen, The Netherlands

1 4-Aminopyridine methiodide (4-APMI), a quaternary analogue of aminopyridine (4-AP), was tested for neuromuscular facilitatory actions on the chick biventer cervicis and frog sartorius nerve–muscle preparations.

2 In the chick, 4-APMI (10^{-4} to 10^{-2} M) augmented indirectly elicited twitches and reversed tubocurarine-induced neuromuscular block. Reversal of tubocurarine block was observed after treatment of the muscle with an anticholinesterase. 4-APMI did not itself produce contracture but augmented responses to added acetylcholine.

3 4-APMI (10^{-4} M) prolonged the time courses of endplate potentials (e.p.ps) and miniature endplate potentials (m.e.p.ps) in the frog.

4 4-APMI (10^{-4} M) increased e.p.p. quantal content. 4-AP was about 100 times more active than 4-APMI in increasing quantal content. Both compounds prolonged muscle action potentials at similar concentrations.

5 4-APMI (10^{-3} to 3×10^{-3} M) possessed anticholinesterase activity in homogenates of chick biventer cervicis muscle.

6 It is concluded that 4-APMI increases evoked acetylcholine release and also possesses a weak anticholinesterase action. The greater action of 4-AP on quantal content is probably due to an intracellular action, possibly involving the release of calcium ions.

Introduction

In isolated skeletal muscle preparations, aminopyridines increase twitch tension and reverse non-depolarizing neuromuscular blockade (Fastier & MacDowall, 1958; Lemeignan & Lechat, 1967; Sobek, Lemeignan, Streichenberger, Benoist, Goguel & Lechat, 1968; Foldes, Agoston, van der Pol, Amaki, Nagashima & Crul, 1976; Bowman, Harvey & Marshall, 1977; Harvey & Marshall, 1977a,b; Bowman, Khan & Savage, 1977). 4-Aminopyridine has been used clinically as a reversal agent for curare (Stoyanov, Vulchev, Shturbova & Marinova, 1976) and the compound is also effective in relieving neuromuscular block produced by botulinum toxin (Lundh, Leander & Thesleff, 1977) and by some antibiotics (Sobek *et al.*, 1968; Singh, Marshall & Harvey, 1978a,b). Aminopyridines increase muscle contractility, but their main effect is an enhancement of neuromuscular transmission (Lemeignan & Lechat, 1967; Bowman *et al.*, 1977a,b; Harvey & Marshall, 1977a).

The aminopyridines inhibit membrane potassium conductance (Pelhate & Pichon, 1974; Yeh, Oxford, Wu & Narahashi, 1976a,b; Schauf, Colton, Colton & Davis, 1976) and hence they prolong action potentials both in nerve (Schauf *et al.*, 1976; Ulbricht & Wagner, 1976; Yeh *et al.*, 1976a) and in muscle (Molgo, Lemeignan & Lechat, 1975; Gillespie & Hutter, 1975; Lundh *et al.*, 1977). It has been proposed that in nerve-muscle preparations prolongation of the presynaptic spike allows a greater than normal level of calcium influx into the nerve terminal and a subsequent increase of acetylcholine release (Molgo *et al.*, 1975; Harvey & Marshall, 1977a). However, there is evidence that aminopyridines may possess a further mechanism of action causing release of calcium from membrane or intracellular binding sites (Lundh *et al.*, 1977; Harvey & Marshall, 1977c).

We have now synthesized a new quaternary aminopyridine, 4-aminopyridine methiodide (Figure 1) which would be expected to be less lipid soluble than 4-aminopyridine and hence would have mainly extra-

0007-1188/79/010053-10 $01.00

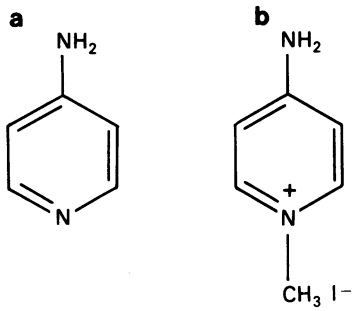

Figure 1 Chemical formulae of (a) 4-aminopyridine (4-AP) and (b) 4-aminopyridine methiodide (4-APMI).

cellular actions. The purpose of this study was to test 4-aminopyridine methiodide (4-APMI) on the same test system as previously used for testing aminopyridines (Bowman *et al.*, 1977a) and diaminopyridines (Harvey & Marshall, 1977a) and to compare the effects of 4-APMI with 4-aminopyridine (4-AP) by the use of intracellular recording techniques.

Methods

Chick biventer cervicis muscle preparation

Biventer cervicis muscles from chicks aged 1 to 6 days were mounted in Krebs-Henseleit (1932) solution at 32°C and bubbled with O_2 containing 5% CO_2 (Ginsborg & Warriner, 1960).

For nerve stimulation the nerve within the tendon was stimulated at a frequency of 0.1 Hz via ring electrodes with rectangular pulses of 0.2 ms duration, and of strength greater than that required to elicit maximal twitches. For direct stimulation, neuromuscular transmission was abolished by the postjunctionally-active irreversible blocking agent, α-bungarotoxin (1 μg/ml). Ring electrodes were placed in contact with the muscle which was stimulated at a frequency of 0.1 Hz with rectangular pulses of 1 ms duration and of strength greater than that required to elicit maximal twitches.

In some experiments nerve stimulation was periodically stopped and acetylcholine (10^{-3} M) or carbachol (2×10^{-5} M) was added to the tissue bath. The acetylcholine and carbachol were allowed to remain in contact with the tissue for 30 s and 90 s respectively before washout by overflow.

Frog sartorius muscle preparation

Frog (*Rana pipiens*) sartorius muscle–sciatic nerve preparations were mounted in a Tris (tris-(hydroxy methyl) aminomethane)-buffered Ringer solution (pH 7.3) at room temperature (18 to 20°C). The composition of the Tris–Ringer was (mM); NaCl 120, KCl 2.5, $CaCl_2$ 1.8 and Tris 1. To record endplate potentials, neuromuscular block was produced by reduction of the $CaCl_2$ concentration of the Tris–Ringer to 0.9 mM and addition of 8 mM $MgCl_2$ (magnesium Tris–Ringer).

Resting membrane potentials, endplate potentials (e.p.ps), miniature endplate potentials (m.e.p.ps) and muscle action potentials were recorded intracellularly with micropipettes filled with 2 M potassium acetate and having resistances in the range 5 to 15 MΩ. Signals were amplified by a WPI M4A preamplifier and displayed simultaneously on Tektronix 5102 and 5103 oscilloscopes. Oscilloscope tracings were recorded either on Polaroid film or on moving 35 mm film by a Grass oscilloscope camera.

Endplate potentials were elicited by stimulation of the sciatic nerve through bipolar platinum electrodes with rectangular pulses of 0.05 ms duration and of sufficient strength to produce a maximal response. Quantal content determinations were made by the ratio of e.p.p. to m.e.p.p. amplitudes (del Castillo & Katz, 1954). One hundred to 150 e.p.ps and 50 to 100 m.e.p.ps were corrected to a standard membrane potential of −80 mV (Katz & Thesleff, 1957) and corrected for non-linear summation (Martin, 1955).

Directly elicited muscle action potentials were produced by stimulation of an individual muscle fibre through a microelectrode inserted in the fibre approximately 1 to 1.5 mm from the recording electrode, with rectangular pulses of 0.2 ms duration and of sufficient strength to elicit an action potential.

Drugs were applied to the solution bathing the entire muscle for action potential measurement. During e.p.p. and m.e.p.p. measurements drugs were microperfused on to the endplate region of individual muscle fibres (Manthey, 1966; Johnson & Parsons, 1972). Microperfusion was achieved by rapidly lowering a micropipette (approx. 100 μm tip diameter) containing the perfusing solution into the bathing medium to a position about 0.1 mm above the muscle fibre surface. The perfusion solution was delivered by hydrostatic pressure from a 5 cm column of solution.

Cholinesterase determination

Cholinesterase activity in the presence of 4-APMI and edrophonium was determined by the colorimetric method of Ellman, Courtney, Andres & Featherstone (1961). The reaction vial contained 15.6 ml phosphate buffer (0.1 M), 1.2 ml chick biventer cervicis muscle homogenate (20 mg/ml), 300 μl 5,5′-dithiobis-(2-nitrobenzoic acid) (0.01 M), 100 μl acetylthiocholine (0.075 M) and was maintained at 32°C. Hydrolysis of acetylthiocholine was shown to be linear with time and

the hydrolysis values quoted were measured after 6 min incubation of substrate and homogenate.

Drugs

The drugs used were acetylcholine chloride, 4-aminopyridine, carbachol chloride, (+)-tubocurarine chloride, acetylthiocholine chloride, 5,5'-dithiobis-(2-nitrobenzoic acid) (all Sigma), edrophonium chloride, neostigmine methylsulphate (both Roche) and α-bungarotoxin (Boehringer).

1-Methyl-4-aminopyridinium iodide (4-APMI) was prepared according to the method of Poziomek (1963). After recrystallization twice from absolute ethanol it had a m.p. of 185 to 186°C; Poziomek reports a value of 179 to 182°C whilst Batts & Spinner (1969) obtained 188 to 188.5°C. The infra red spectrum was consistent with the expected structure and the compound was shown to be pure by differential scanning calorimetry using a Du Pont 910 Differential Scanning Calorimeter.

Statistics

All values quoted in the text, figures and tables represent the mean ± s.e. mean of between 4 and 7 separate observations. When control and drug responses were recorded from the same muscle fibre, differences between means were analysed by paired Students' *t*-test. The unpaired Students' *t*-test was used in the analysis of the cholinesterase determination data.

Results

Chick biventer cervicis muscle preparation

a) *Twitch augmentation and anti-curare action.* 4-AMPI (10^{-4} to 10^{-2} M) produced an augmentation of indirectly elicited twitches whereas its effects on directly elicited twitches were much less in magnitude (Figure 2).

Neuromuscular block produced by (+)-tubocurarine (6×10^{-6} M) was partially reversed (from 25% to 60% control) by 10^{-4} M 4-APMI. 4-APMI (10^{-3} M) produced a complete reversal of the tubocurarine-induced neuromuscular block in 10 to 15 min. Occasionally, the reversal was followed by up to 15% twitch augmentation in the continued presence of tubocurarine.

In preparations treated with neostigmine (3×10^{-6} M) a concentration sufficient to reduce the cholinesterase in the tissue to undetectable levels (Lord, 1975), 4-APMI (10^{-3} M) produced only a partial (75%) reversal of neuromuscular block induced by (+)-tubocurarine (10^{-5} M) (Figure 3). In contrast 4-AP (10^{-4} M) and 3,4-diaminopyridine (10^{-4} M) produced complete reversal followed by twitch augmentation in the continued presence of (+)-tubocurarine (Figure 3). Neostigmine (1.5×10^{-6} M) and edrophonium (10^{-5} M) produced no reversal of the tubocurarine-induced neuromuscular block after pretreatment with neostigmine.

b) *Effects of postjunctional sensitivity.* At concentrations up to 10^{-2} M, 4-APMI produced no contracture of either indirectly or directly stimulated preparations.

The effects of 10^{-3} M 4-APMI, a concentration that produced an approximately 120% increase in twitch tension, produced a large increase (181 ± 19%) in the response to added acetylcholine whereas the response to carbachol was slightly increased in one experiment (20%) and slightly reduced (mean = 16%) in four other experiments.

4-APMI (5×10^{-4} M) produced a parallel 1.2 log unit shift to the left of the dose-response curve to acetylcholine (Figure 4). The augmentation produced by 4-APMI on responses to acetylcholine was rapidly reversed by one 15 s washout of the drug from the tissue bath.

α-Bungarotoxin (1 μg/ml) produced neuromuscular block after pretreatment of tissues with, and in the continued presence of 4-APMI (10^{-3} M). The bungarotoxin block was not reversed by washing the tissue at 5 min intervals for up to 1 hour.

Cholinesterase activity

The effects of 4-APMI were compared with those of the anticholinesterase edrophonium on the cholinesterase activity of chick biventer cervicis muscle homogenates. 4-APMI was approximately one thousand times less active than edrophonium in inhibiting the cholinesterase (Figure 5). 4-APMI (3×10^{-3} M) and edrophonium (3×10^{-6} M) both produced approximately 45% inhibition of the enzyme activity.

Frog sartorius muscle preparation

Neither 4-APMI (10^{-5} to 10^{-3} M) nor 4-AP (10^{-6} to 10^{-4} M) produced depolarization when microperfused directly onto the endplate regions of individual muscle fibres.

In magnesium Tris–Ringer solutions, both 4-APMI (10^{-4} M) (Figure 6 and Table 1) and 4-AP (10^{-6} M) (Figure 7 and Table 1), produced an increase in e.p.p. amplitude. 4-APMI caused a simultaneous increase in m.e.p.p. amplitude, but 4-AP had no effect on m.e.p.p. amplitude. The rise time and duration of e.p.ps was unaffected by 4-AP (Table 1), but 4-APMI increased the time course of both e.p.ps and m.e.p.ps (Tables 1 and 2), suggesting an inhibitory action of 4-APMI on acetylcholinesterase. In addition to this action, both 4-APMI (10^{-4} M) and 4-AP (10^{-6} M)

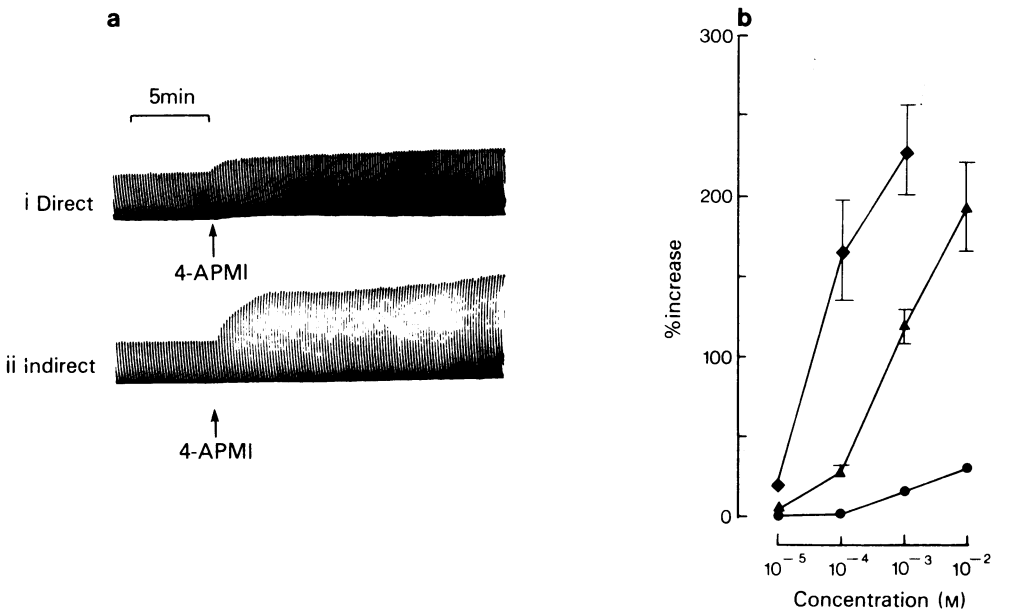

Figure 2 Chick biventer cervicis nerve-muscle preparations. Effects of 4-aminopyridine methiodide (4-APMI) on directly and indirectly elicited twitches. (a) Shows the effects of 4-APMI (10^{-2} M) on two preparations, (i) stimulated directly in a solution containing α-bungarotoxin (1 μg/ml) and (ii) stimulated indirectly. (b) Shows concentration-effect curves for 4-APMI in augmenting directly (●) and indirectly (▲) stimulated preparations. The effects of 4-aminopyridine on indirectly elicited twitches (from Bowman, Harvey & Marshall, 1977) are also shown for comparison (◆). All points represent the mean; vertical bars show s.e. mean except where smaller than the symbols.

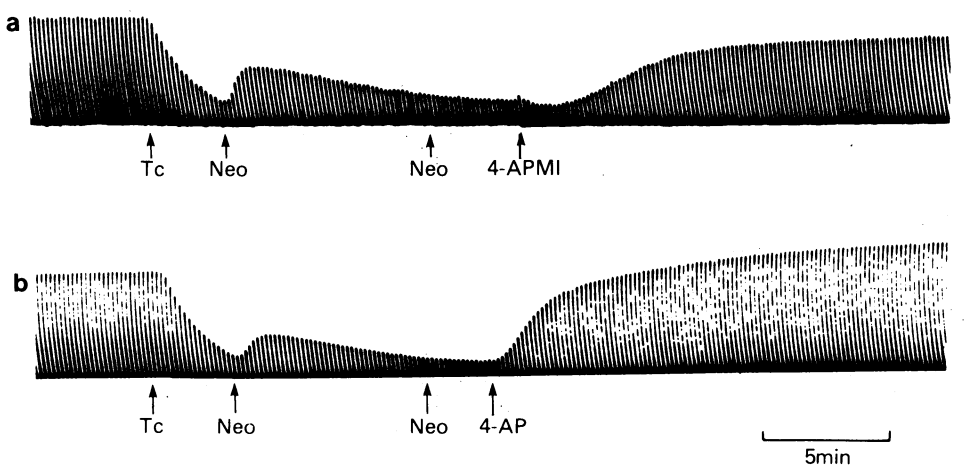

Figure 3 Chick biventer cervicis muscle preparations. Both preparations were treated with a large concentration (10^{-5} M) of tubocurarine (Tc) followed by neostigmine (3×10^{-6} M) at Neo. A subsequent challenge with neostigmine produced no further relief of the neuromuscular block. In (a) 4-aminopyridine methiodide (4-APMI, 10^{-3} M) produced a partial recovery of twitch height whereas in (b) 4-aminopyridine (4-AP, 10^{-4} M) produced complete recovery.

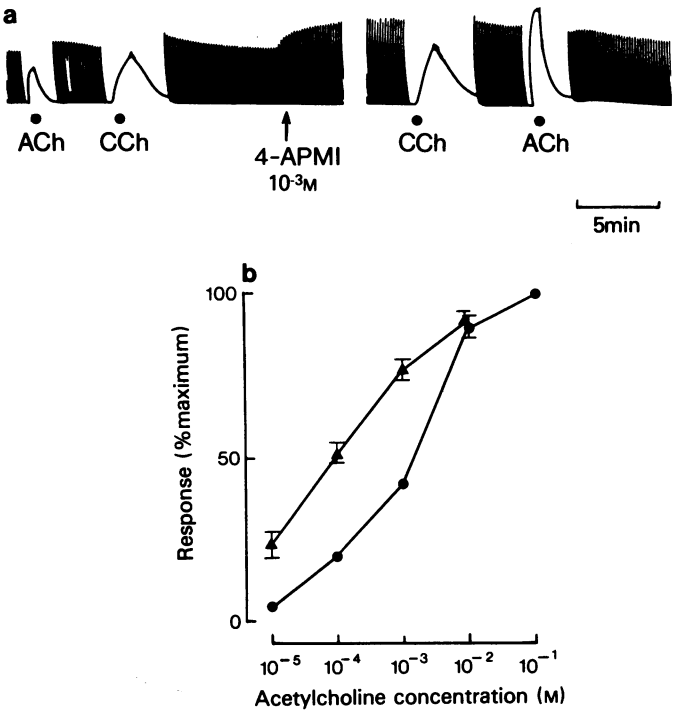

Figure 4 Chick biventer cervicis preparations. In (a) the preparation was stimulated indirectly and acetylcholine (ACh, 10^{-3} M) and carbachol (CCh, 2×10^{-5} M) were added to the tissue bath. In the continued presence of 4-aminopyridine methiodide (4-APMI) responses to carbachol are little changed but those to acetylcholine are greatly augmented. (b) Shows concentration-effect curves for acetylcholine in the absence (●) and presence (▲) of 4-APMI (5×10^{-4} M). Vertical bars show s.e. means except where these are smaller than the symbols.

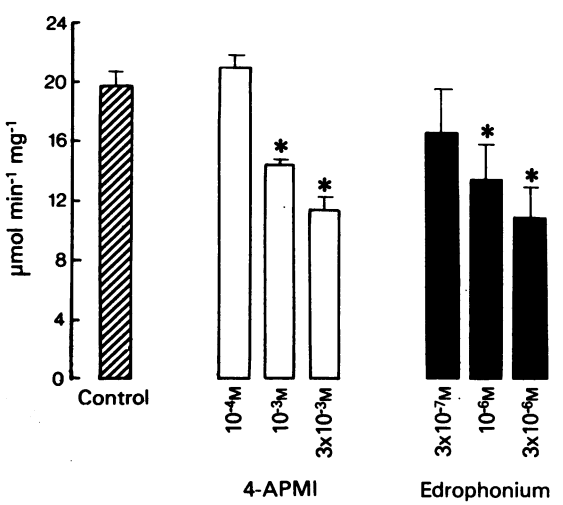

Figure 5 A comparison of the effects of 4-aminopyridine methiodide (4-APMI, 10^{-4} to 3×10^{-3} M) and edrophonium (10^{-7} to 3×10^{-6} M) on cholinesterase activity of chick biventer cervicis muscle homogenates. * Significantly different ($P < 0.05$) from control.

caused an increase in e.p.p. quantal content. This was assessed by the direct method of e.p.p./m.e.p.p. ratio (Table 1) and confirmed by a reduction in the number of failures.

Both 4-AP (10^{-6} M) and 4-APMI (10^{-4} M) produced a slight increase in m.e.p.p. frequency (Table 1) in magnesium Tris–Ringer. In normal Tris–Ringer 4-APMI (10^{-4} M) had no marked effects on m.e.p.p. frequency (Table 2) but occasionally giant m.e.p.ps were observed.

Repetitive e.p.ps in response to single shock nerve stimulation were occasionally observed in the presence of either 4-APMI (10^{-4} M) or 4-AP (10^{-5} M).

Both 4-APMI (10^{-4} to 10^{-3} M) and 4-AP (10^{-4} to 10^{-3} M) prolonged the duration of directly stimulated muscle action potentials (Figure 8). Prolongation of overshoot, as measured by the duration of the action potential at the zero potential was usually increased, but the effect of the drugs was more marked when the action potential duration was measured at −40 mV (Table 3).

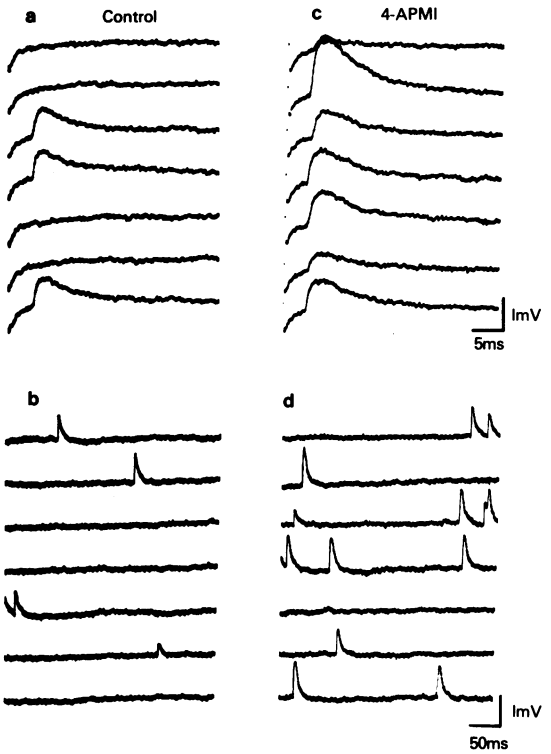

Figure 6 Frog sartorius muscle–sciatic nerve preparation. Effects of 4-aminopyridine methiodide (4-APMI) on endplate potentials (e.p.ps) and miniature endplate potentials (m.e.p.ps). (a) Shows e.p.ps and (b) m.e.p.ps before treatment with 4-APMI; (c) shows e.p.ps and (d) m.e.p.ps after 20 min exposure to 4-APMI (10^{-4} M).

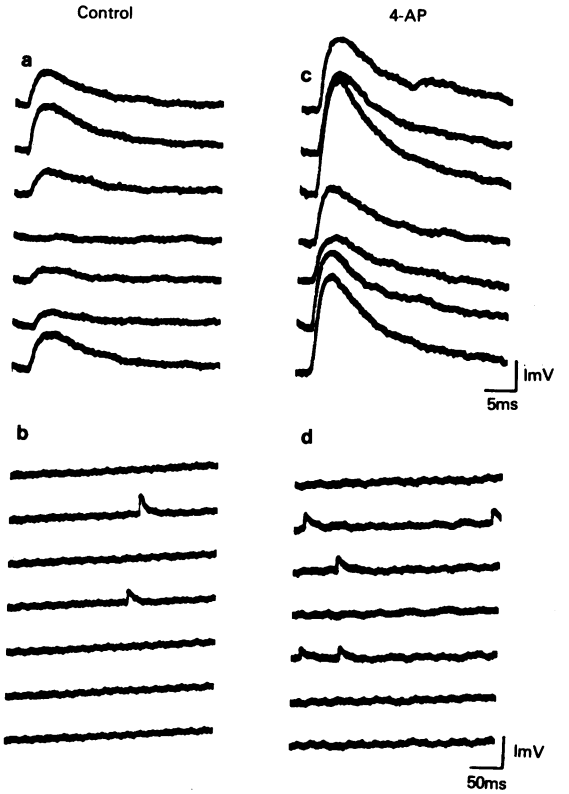

Figure 7 Frog sartorius muscle–sciatic nerve preparation. Effects of 4-aminopyridine (4-AP, 10^{-6} M) on e.p.ps and m.e.p.ps. Legend as for Figure 6.

Table 1 Effects of 4-aminopyridine methiodide (4-APMI) and 4-aminopyridine (4-AP) on endplate potentials (e.p.ps), miniature endplate potentials (m.e.p.ps) and mean e.p.p. quantal content in magnesium–Tris solution

	4-AP (10^{-6} M)			*4-APMI (10^{-4} M)*		
	Control	*Drug*	*% increase*	*Control*	*Drug*	*% increase*
m.e.p.p. frequency/s	1.03 ± 0.17	2.71 ± 1.09	163	1.58 ± 0.34	3.16 ± 0.65*	100
m.e.p.p. amplitude (mV)	0.56 ± 0.06	0.54 ± 0.07	−4	0.49 ± 0.06	0.67 ± 0.11*	37
e.p.p. amplitude (mV)	1.13 ± 0.08	3.43 ± 0.36*	204	0.89 ± 0.53	2.18 ± 0.93*	145
mean quantal content	2.11 ± 0.29	6.52 ± 0.9*	209	2.18 ± 1.42	3.61 ± 1.46*	65
e.p.p. rise time (ms)	1.42 ± 0.29	1.43 ± 0.32	1	1.51 ± 0.19	2.63 ± 0.26*	74
e.p.p. ½ decay time (ms)	4.1 ± 0.59	4.13 ± 0.63	1	5.17 ± 0.25	6.81 ± 0.37*	32

* Significantly different ($P < 0.05$) from control.

Discussion

The results show that like the aminopyridines and diaminopyridines, 4-APMI possesses twitch facilitatory and anti-curare activity. The lack of appreciable twitch augmenting action of 4-APMI in directly stimulated chick biventer cervicis muscles shows that the compound acts mainly on neuromuscular transmission and that its direct effect on muscle contractility is weak. Comparison of the activity of 4-APMI with those of 4-AP and 3,4-diaminopyridine, previously shown to be the most active aminopyridines on neuromuscular transmission (Bowman *et al.*, 1977a; Harvey & Marshall, 1977a) shows 4-APMI to be approximately ten times less active than the other two compounds in facilitating neuromuscular transmission.

As 4-APMI is a quaternary compound, it might be expected that it would possess postjunctional agonist or antagonist activity at the neuromuscular junction. However, concentrations as high as 10^{-2} M produced no contracture of the chick biventer cervicis muscle and no depolarization was seen in the frog sartorius muscle, indicating a lack of agonist action. The compound did not reduce carbachol contractions in the chick and did not reduce m.e.p.p. amplitude in the frog, indicating a lack of postjunctional blocking activity. Additional evidence for 4-APMI not acting directly on acetylcholine receptors was that the compound did not protect the receptors against α-bungarotoxin binding in the chick.

4-APMI greatly increased responses to acetylcholine in the chick, indicating a possible anticholinesterase action. Such an action is not seen with aminopyridines or diaminopyridines (Bowman *et al.*, 1977a; Harvey & Marshall, 1977a). The anticholinesterase action was rapid in onset and easily removed by washing, indicating that the compound, like edrophonium, associates with and dissociates from the acetylcholinesterase receptor molecule very quickly. Like that of edrophonium, the structure of 4-APMI would allow binding to the receptor at the anionic site only. Carbamates such as physostigmine and neostigmine bind at both anionic and esteratic sites and the carbamylated esteratic site reactivates only very slowly (Wilson & Bergmann, 1950; Wilson, 1951). When tested on the cholinesterase activity of chick muscle homogenates, 4-APMI exhibited an anticholinesterase activity 1,000 times weaker than that of edrophonium.

The anticholinesterase action of 4-APMI also explains the increase in m.e.p.p. amplitude and in the rise times and times to half decay of both m.e.p.ps and e.p.ps seen in the frog. Similar effects are observed with other anticholinesterase agents (Eccles & MacFarlane, 1949; Fatt & Katz, 1951).

Thus the anticholinesterase activity of 4-APMI could explain the twitch augmenting and anti-curare action of the compound. However, we have reason to believe that the compound possesses a second mechanism of action as 4-APMI can still partially reverse the action of (+)-tubocurarine in preparations in which the acetylcholinesterase was inhibited. In these preparations neostigmine and edrophonium were completely inactive indicating that the cholinesterase was completely inhibited. Studies of acetylcholine release in the frog indicate that 4-APMI produces a small increase in quantal content and we believe that this increase in acetylcholine release explains the anti-curare action of 4-APMI seen in the absence of functional cholinesterase. 4-APMI was much less active than 4-AP in increasing quantal content and this difference was reflected by the greater anti-curare activity of 4-AP in the absence of cholinesterase. 4-AP did not alter the time course of e.p.ps, confirming its lack of anticholinesterase action.

On occasions, repetitive e.p.ps were observed in the presence of either 4-APMI or 4-AP, presumably as a result of repetitive nerve terminal activity in response to single shock stimulation. Repetitive firing has also been observed in the squid axon (Yeh *et al.*, 1976b) in the presence of aminopyridines and it has been suggested that the compounds may increase membrane excitability (Hue, Pelhate, Callec & Chanelet, 1976; Leander, Arner & Johansson, 1977). Our results suggest that prejunctional repetitive firing may contribute to the action of the aminopyridines at the neuromuscular junction. 4-AP and 4-APMI

Table 2 Effects of 4-aminopyridine methiodide (4-APMI) on miniature endplate potentials (m.e.p.ps) recorded in normal Tris–Ringer solution

		4-APMI (10^{-4} M)	
	Control	*Drug*	*% increase*
Amplitude (mV)	0.76 ± 0.08	0.98 ± 0.14*	29
Frequency/s	1.79 ± 0.52	1.52 ± 0.25	−15
Rise time (ms)	1.15 ± 0.03	1.84 ± 0.14*	60
½ decay time (ms)	4.44 ± 0.47	6.4 ± 0.52*	44

* Significantly different ($P < 0.05$) from control.

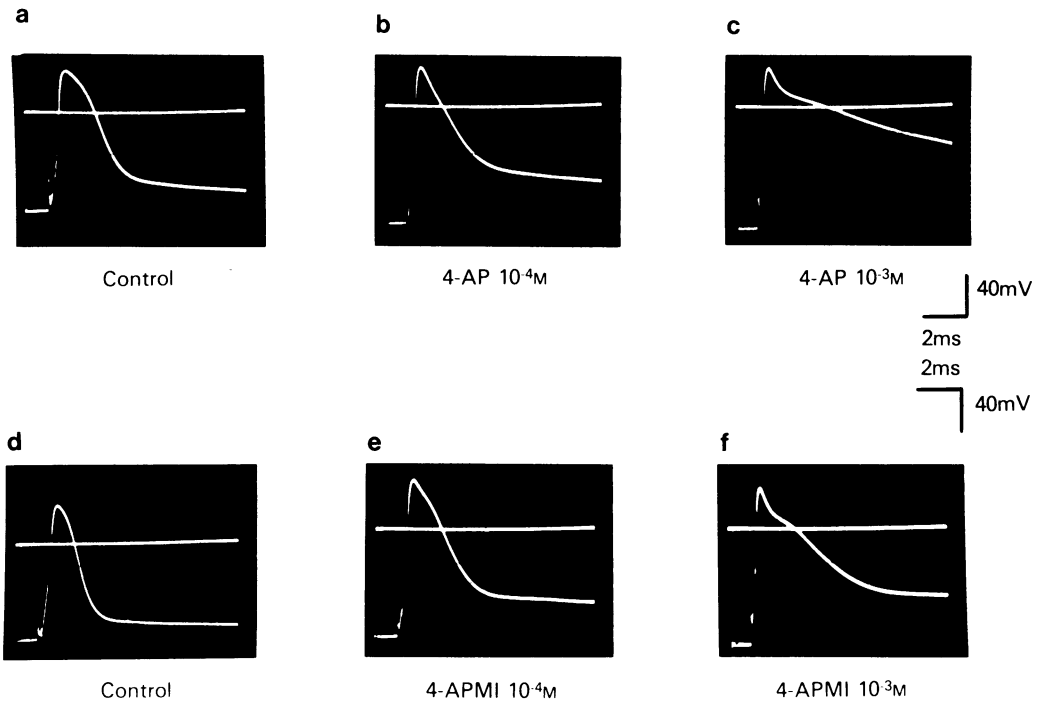

Figure 8 Frog sartorius muscle preparation. (a, b and c) Effects of 4-aminopyridine (4-AP, 10^{-4} and 10^{-3} M) and (d, e and f) 4-aminopyridine methiodide (4-APMI, 10^{-4} and 10^{-3} M) on intracellularly recorded directly elicited muscle action potentials. In each case the upper horizontal line corresponds to the zero potential of the fibre.

prolonged muscle action potentials in the same concentration ranges, probably by an inhibitory action on potassium conductance. Assuming a similar effect occurs at nerve terminals, a prolongation of nerve terminal action potentials and the consequent increased influx of calcium probably accounts for the whole of the effect of 4-APMI on quantal content. However, 4-AP has a much more potent action on quantal content and a release of calcium from membrane or intracellular stores may also be involved. It would be expected that 4-APMI, a poorly lipid soluble quaternary compound, would have poor access to such stores of calcium, whereas the lipid-soluble 4-AP would have easy access. Thus we conclude that the difference in chemical structure determines the access to sites of action of the compounds and that the observed differences in activity are due to differences in membrane penetrating properties.

J.L. is supported by a Ministry of Defence grant. We are grateful to Dr S. Agoston for assistance in organizing the project.

Table 3 Effects of 4-aminopyridine methiodide (4-APMI) and 4-aminopyridine (4-AP) on muscle action potential duration measured at 0 mV and −40 mV

	Action potential duration (ms)				
	0 mV	*% increase*	*−40 mV*	*% increase*	n
Control	1.06 ± 0.07		1.70 ± 0.07		7
10^{-4} M 4-APMI	1.60 ± 0.03	51	2.58 ± 0.07	52	6
10^{-3} M 4-APMI	1.52 ± 0.15	43	3.03 ± 0.20	78	6
Control	1.50 ± 0.04		2.73 ± 0.18		4
10^{-4} M 4-AP	1.65 ± 0.03	10	3.18 ± 0.21	16	6
10^{-3} M 4-AP	2.74 ± 0.38	83	>10	>266	5

References

BATTS, B.D. & SPINNER, E. (1969). Vibration-spectral and structural comparison of the 4-aminopyridine cation with the 4-hydroxypyridinium ion and 4-pyridone. The protio parent ions, N- and C-deuterated, and N-methylated ions. Relevant N.M.R. spectral studies. *Aust. J. Chem.*, **22**, 2595–2610.

BOWMAN, W.C., HARVEY, A.L. & MARSHALL, I.G. (1977a). The actions of aminopyridines on avian muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **297**, 99–103.

BOWMAN, W.C., KHAN, H. & SAVAGE A. (1977b). Some antagonists of dantrolene sodium on the isolated diaphragm muscle of the rat. *J. Pharm. Pharmac.*, **29**, 616–625.

DEL CASTILLO, J. & KATZ, B. (1954). Quantal components of the end-plate potential. *J. Physiol.*, **124**, 560–573.

ECCLES, J.C. & MACFARLANE, W.V. (1949). Actions of anticholinesterases on end-plate potential of frog muscle. *J. Neurophysiol.*, **12**, 59–80.

ELLMAN, G.L., COURTNEY, K.D., ANDRES, V. JR. & FEATHERSTONE, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmac.*, **7**, 88–95.

FASTIER, F.N. & MACDOWELL, M.A. (1958). A comparison of the pharmacological properties of the three isometric aminopyridines. *Aust. J. exp. Biol. med. Sci.*, **36**, 365–372.

FATT, P. & KATZ B. (1951). An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol.*, **115**, 320–370.

FOLDES, F.F., AGOSTON, S., VAN DER POL, F., AMAKI, Y., NAGASHIMA, H. & CRUL, J. (1976). The *in vitro* neuromuscular effects of 4-aminopyridine and its interaction with neuromuscular blocking agents. *Abstracts American Society of Anesthetists' Meeting*, October 1976, 179–180.

GILLESPIE, J.I. & HUTTER, O.F. (1975). The actions of 4-aminopyridine on the delayed potassium current in skeletal muscle fibres. *J. Physiol.*, **252**, 70P.

GINSBORG, B.L. & WARRINER, J. (1960). The isolated chick biventer cervicis nerve-muscle preparation. *Br. J. Pharmac. Chemother.*, **15**, 410–411.

HARVEY, A.L. & MARSHALL, I.G. (1977a). The actions of three diaminopyridines on the chick biventer cervicis muscle. *Eur. J. Pharmac.*, **44**, 303–309.

HARVEY, A.L. & MARSHALL, I.G. (1977b). A comparison of the effects of aminopyridines on isolated chicken and rat skeletal muscle preparations. *Comp. Biochem. Physiol.*, **58C**, 161–165.

HARVEY, A.L. & MARSHALL, I.G. (1977c). The facilitatory actions of aminopyridines and tetraethylammonium on neuromuscular transmission and muscle contractility in avian muscle. *Naunyn-Schmiedebergs Arch. Pharmac.*, **299**, 53–60.

HUE, B., PELHATE, M., CALLEC, J.J. & CHANELET, J. (1976). Synaptic transmission in the sixth ganglion of the cockroach: action of 4-aminopyridine. *J. exp. Biol.*, **65**, 517–527.

JOHNSON, E.W. & PARSONS, R.L. (1972). Characteristics of postjunctional carbamylcholine receptor activation and inhibition. *Am. J. Physiol.*, **222**, 793–799.

KATZ, B. & THESLEFF, S. (1957). On the factors which determine the amplitude of the 'miniature end-plate potential'. *J. Physiol.*, **137**, 267–278.

KREBS, H.A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seylers Z. Physiol. Chem.*, **210**, 33–66.

LEANDER, S., ARNER, A. & JOHANNSSON, B. (1977). Effects of 4-aminopyridine on mechanical activity and noradrenaline release in the rat portal vein *in vitro*. *Eur. J. Pharmac.*, **46**, 351–362.

LEMEIGNAN, M. & LECHAT, P. (1967). Sur l'action anticurare des aminopyridines. *C.R. hebd. Seanc. Acad. Sci., Paris*, (D) **264**, 169–172.

LORD, J.A.H. (1975). Some aspects of the effects of anticholinesterases in the skeletal muscle of the chick. *Ph.D. Thesis. University of Strathclyde.*

LUNDH, H., LEANDER, S. & THESLEFF, S. (1977). Antagonism of the paralysis produced by botulinum toxin in the rat. The effects of tetraethylammonium, guanidine and 4-aminopyridine. *J. Neurol. Sci.*, **32**, 29–42.

MANTHEY, A.A. (1966). The effects of calcium on the desensitization of membrane receptors at the neuromuscular junction. *J. gen. Physiol.*, **49**, 963–976.

MARTIN, A.R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol.*, **130**, 114–122.

MOLGO, J., LEMEIGNAN, M. & LECHAT, P. (1975). Modifications de la libération du transmetteur à la jonction neuromusculaire de Grenouille sous l'action de l'amino-4-pyridine. *C.R. hebd. Séanc. Acad. Sci., Paris* (D) **281**, 1637–1639.

PELHATE, M. & PICHON, Y. (1974). Selective inhibition of potassium current in the giant axon of the cockroach. *J. Physiol.*, **242**, 90–91P.

POZIOMEK, E.J. (1963). Experiments in the synthesis of pyridinium amidines and imino esters. *J. org. Chem.*, **28**, 590–591.

SCHAUF, C.L., COLTON, C.A., COLTON, J.S. & DAVIS, F.A. (1976). Aminopyridines and sparteine as inhibitors of membrane potassium conductance. Effects on Myxicola giant axons and the lobster neuromuscular junction. *J. Pharmac. exp. Ther.*, **197**, 414–425.

SINGH, Y.N., MARSHALL, I.G. & HARVEY, A.L. (1978a). Some effects of the aminoglycoside antibiotic amikacin on neuromuscular and autonomic transmission. *Br. J. Anaesth.*, **50**, 109–117.

SINGH, Y.N., MARSHALL, I.G. & HARVEY, A.L. (1978b). Reversal of antibiotic-induced muscle paralysis by 3,4-diaminopyridine. *J. Pharm. Pharmac.*, **30**, 249–250.

SOBEK, V., LEMEIGNAN, M., STREICHENBERGER, G., BENOIST, J.M., GOGUEL, A. & LECHAT, P. (1968). Etude sur le diaphragme isole de rat de l'antagonisme entre substances curarisantes et aminopyridines. *Archs int. Pharmacodyn. Ther.*, **171**, 356–368.

STOYANOV, E., VULCHEV, P., SHTURBOVA, M. & MARINOVA, M. (1976). Clinical electromyomechanographic and electromyographic studies with pymadine. *Anaesth. Resus. Intern. Therap.*, **4**, 139–142.

ULBRICHT, W. & WAGNER, H.H. (1976). Block of potassium channels of the nodal membrane by 4-aminopyridine and its partial removal on depolarization. *Pflügers Arch.*, **367**, 77–78.

WILSON, I.B. (1951). Acetylcholinesterase. XI Reversibility of tetraethylpyrophosphate inhibition. *J. biol. Chem.*, **190,** 111–125.

WILSON, I.B. & BERGMANN, F. (1950). Studies on cholinesterase. VII The active surface of acetylcholinesterase derived from effects of pH on inhibitors. *J. biol. Chem.*, **185,** 479–489.

YEH, J.Z., OXFORD, G.S., WU, C.H. & NARAHASHI, T. (1976a). Dynamics of aminopyridine block of potassium channels in squid axon membrane. *J. gen. Physiol.*, **68,** 519–535.

YEH, J.Z., OXFORD, G.S., WU, CH. & NARAHASHI, T. (1976b). Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys. J.*, **16,** 77–81.

(*Received April 10, 1978.*
Revised July 10, 1978.)