J. Pharm. Pharmacol. 1986, 38: 156–158
Communicated July 26, 1985

Antagonism of non-depolarising neuromuscular blockade by aminopyridines in cats

MIKLOS RIESZ*, EGON KÁPÁTI, LÁSZLÓ SZPORNY, *Pharmacological Research Centre, Chemical Works of Gedeon Richter Ltd., H-1475 Budapest 10, P.O. Box 27, Hungary*

The antagonism of pipecuronium- and vecuronium-induced neuromuscular blockade by 4-aminopyridine (4AP), 3,4-diaminopyridine (3,4AP) and 3-[(dimethylamino)-carbonyl] amino-4-aminopyridine (LF14) were studied in anaesthetized cats during constant infusion of the relaxants. Aminopyridines were administered cumulatively at steady state 90% block level. The ED50 values of 4AP, 3,4AP and LF14 were 243, 106 and 254 μg kg^{-1} in pipecuronium, and 232, 195 and 235 μg kg^{-1} in vercuronium blockade. It has been assumed that in cats the anticurare effect of aminopyridines is mainly a result of K^+ channel blockade on motor nerve terminals which enhances the evoked release of acetylcholine.

Aminopyridines are thought to enhance synaptic transmission by blocking the outward K^+ current in nerve terminals and thereby prolonging the duration of action potentials (Pelhate & Pichon 1974; Yeh et al 1976). This effect results in an increase of the amount of transmitter released by nerve impulses (for reviews see Bowman 1982, Paskov et al 1985). At skeletal muscle myoneural junctions aminopyridines effectively antagonize non-depolarizing (but not depolarizing) neuromuscular blockade by elevation of acetylcholine output from motor nerve terminals (Vohra & Pradhan 1964; Lemeignan & Lechat 1967; Bowman et al 1976). Cats are particularly useful models for the evaluation of the myoparalysant action of curare-like substances, considering that the potency ratios of these drugs obtained on cat nerve-muscle preparations are similar to those registered in man (Bowman 1964). The present study was designed to evaluate the antagonistic action of three aminopyridine derivatives, 4-aminopyridine (4AP), 3,4-diaminopyridine (3,4AP) and 3-[(dimethylamino)-carbonyl] amino-4-aminopyridine (LF14) in cats, under the conditions of deep steady-state curarization. Neuromuscular block was maintained by pipecuronium or vecuronium (two clinically used steroidal muscle relaxants, see Fig. 1) in order to compare the antagonist potencies of aminopyridine derivatives against different curare-like compounds.

Methods and materials

Male cats, 2·0–4·6 kg, were used in experimental conditions similar to those previously described by Miller et al (1975), with minor modifications. Briefly, the animals (n = 30) were anaesthetized with chloralose (80–100 mg kg^{-1} i.p.), and after tracheotomy they were artificially ventilated. Both external jugular veins were cannulated for drug administration. Arterial blood pressure and heart rate were monitored by means of a cannula inserted in the carotid artery. The tendon of the anterior tibialis muscle was freed and cut near its point of attachment to the bone and was connected to a Grass FT 10 force-displacement transducer. The sciatic nerve was sectioned centrally and the peripheral end of the isolated peroneal nerve was stimulated supramaximally with rectangular pulses (0·2 ms duration, 0·1 Hz) through shielded bipolar platinum electrodes. The resulting twitch tension of the muscle and circulatory parameters were recorded on a polygraph (Hellige). The rectal temperature of the animals was kept at 37 ± 0·5 °C by IR lamp connected to an appropriate thermoregulator. Following an i.v. loading dose of pipecuronium (10–17·5 μg kg^{-1}) or vecuronium (40 μg kg^{-1}), an infusion of 5 μg ml^{-1} pipecuronium or 20 μg ml^{-1} vecuronium was started from a precision infusion pump (AHS) at a rate that depressed contraction force by a constant of 90%. When the infusion rate required to maintain this pre-determined block level remained unchanged at least for 15 min, i.v. boluses of

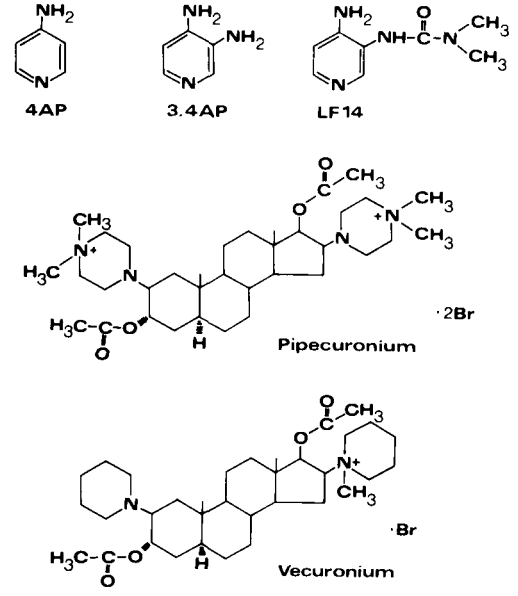

FIG. 1. Structures of aminopyridines and relaxants used.

* Correspondence.

an antagonist were given cumulatively during the continued infusion of the relaxant. Within an experiment only one relaxant + anticurare combination was examined.

The degree of antagonism caused by a given dose of aminopyridines was expressed as a percentage of the pre-existing 90% depression of contraction force:

$$\text{Antagonism (\%)} = \frac{\text{Pre-existing depression (\%)} - \text{Depression after antagonist (\%)} \times 100}{\text{Pre-existing depression (\%)}}$$

ED50 values (the dose of antagonist producing 50% antagonism) were determined by linear regression analysis. The median effective doses of aminopyridines were compared by the unpaired Student's test, values of $P < 0.05$ being regarded as significant.

Drugs used were 4AP, 3,4AP (Janssen), LF14 (kindly supplied by Dr F. Foldes, Montefiore Hospital, Albert Einstein University, New York), pipecuronium (Richter), vecuronium (Organon Teknika), each dissolved in physiological saline just before administration.

Results and discussion

In preliminary experiments, carried out under the same conditions, single boluses of aminopyridines produced a long-lasting increase of twitch tension with a sufficiently prolonged plateau phase, allowing the cumulative administration of these drugs without the overestimation of median effective doses. The infusion rates maintaining 90% neuromuscular block were 0·223 ± 0·029 $\mu g\,kg^{-1}\,min^{-1}$ for pipecuronium, and 2·86 ± 0·360 $\mu g\,kg^{-1}\,min^{-1}$ for vecuronium (mean ± s.e.m., n = 15 for each). Although considerably greater relaxant requirement was observed when vecuronium was used as a curare-like compound, compared with pipecuronium, no significant difference between the effectiveness of aminopyridines against the two drugs could be detected (Table 1). 3,4AP seemed to be more active against pipecuronium paralysis than against vecuronium paralysis, although the difference did not attain significance. Booij et al (1980) demonstrated the nearly equal ED50 values of 4AP in vecuronium and pancuronium neuromuscular blockade in the rat sciatic nerve-anterior tibialis preparation. Similarly, 4AP and LF14 had almost equal potencies against both vecuronium and pipecuronium (this study). The possible difference in 3,4AP effect needs further experimental support.

In cats, 3,4AP possessed more marked anticurare action than 4AP and LF14, the deviation being significant in pipecuronium but not in vecuronium paralysis. According to Molgo et al (1980), 3,4AP is 6–7 times more potent than 4AP in facilitating the stimulus-evoked release of acetylcholine in rat and mouse neuromuscular synapses. Similarly, more powerful enhancement of transmission by 3,4AP than by the monoamino derivative was demonstrated on avian (Harvey & Marshall 1977) and rat (Foldes, personal communication) isolated nerve-muscle preparations. Interestingly, in cats the anticurare activity of LF14 remained at the level of 4AP. Previously, the comparative experiments of Biessels et al (1984) revealed more pronounced cholinergic stimulation by LF14 than by 4AP, when in-vitro and in-vivo curare-antagonist actions in rats and spasmogenic properties on guinea-pig isolated ileum were investigated. The discrepancy between our observations, and the earlier findings of Biessels et al (1984) can presumably be explained by the peculiar pharmacological profile of LF14. This compound structurally resembles pyridostigmine and in fact it has a considerable anticholinesterase activity (IC50 = 8×10^{-7} M, Duncalf et al 1984), while 4AP and 3,4AP are much weaker acetylcholinesterase inhibitors (although this effect of 4AP and 3,4AP is pharmacologically detectable, Marshall et al 1979). Therefore, it is reasonable to assume that LF14 has a dual effect on cholinergic transmission; prolongation of nerve action potential (Den Hertog et al 1983) and cholinesterase inhibition (Duncalf et al 1984), each leading to the accumulation of the transmitter acetylcholine in the synaptic cleft and thereby a more pronounced postjunctional response. It is possible that this dual action is reflected in the higher potency of LF14 on rodent preparations (Biessels et al 1984), than in cats, because in cats the acetylcholinesterase activity in the anterior tibialis muscle is rather low (Silver 1974). It can be hypothesized that anticurare effect of aminopyridines in cats is mainly a result of K^+ channel blockade on motor nerve terminals. Direct action of these substances on the contractility of cat anterior tibialis muscle can probably be excluded, considering that effective anticurare doses of 4AP are lower than those producing twitch augmentation on chronically denervated muscle (Agoston et al 1982), and it seems probable that the same holds for other derivatives.

Table 1. Anticurare effects of the three aminopyridines in cats.

Muscle relaxant	Antagonist	Cumulative doses of antagonists ($\mu g\,kg^{-1}$ i.v.) 50	100	200	300	400	ED50 of antagonists ± s.e.m. ($\mu g\,kg^{-1}$ i.v.)
Pipecuronium	4AP		14 ± 3·6§	37 ± 7·2	63 ± 7·3	78 ± 7·9	243 ± 45·7
	3,4AP	29 ± 5·8	52 ± 5·8	80 ± 11	93 ± 7·9		106 ± 28·9
	LF14		14 ± 1·6	40 ± 9·0	66 ± 13	84 ± 12	254 ± 39·0
Vecuronium	4AP		25 ± 7·4	47 ± 11	61 ± 10	76 ± 7·0	232 ± 41·8
	3,4AP		29 ± 6·2	54 ± 7·6	72 ± 9·1	83 ± 8·4	195 ± 35·3
	LF14		18 ± 3·6	35 ± 6·3	60 ± 4·4	80 ± 4·9	235 ± 21·1

§ Numbers below the doses refer to % antagonism, mean ± s.e.m. of 5 experiments.

In conclusion, cat preparations may be useful in the evaluation of drugs enhancing acetylcholine release (or in the determination of this quality of effect of a drug which similarly to LF14 produces a mixed type of cholinergic stimulation).

The authors are greatly indebted to Professors Sandor Agoston (Department of Pharmacy, University of Groningen, The Netherlands) and Francis Foldes (Montefiore Hospital, Albert Einstein University, New York, USA) for helpful discussion and criticism.

REFERENCES

Agoston, S., Bowman, W. C., Houwertjes, M. C., Rodger, I. W., Savage, A. O. (1982) Clin. Exp. Pharmacol. Physiol. 9: 21–34

Biessels, P. T. M., Agoston, S., Horn, A. S. (1984) Eur. J. Pharmacol. 106: 319–325

Booij, L. H. D. J., van der Pol, F., Crul, J. F., Miller, R. D. (1980) Anaesth. Analg. 59: 31–34

Bowman, W. C. (1964) in: Laurence, D. R., Bacharach, A. L. (eds) Evaluation of drug activities: Pharmacometrics. Academic Press, London, pp 325–351

Bowman, W. C. (1982) Trends in Pharmacol. Sci. 3: 183–185

Bowman, W. C., Harvey, A. L., Marshall, I. G. (1976) J. Pharm. Pharmacol. 28: Suppl. 79P

Den Hertog, A., Pielkenrood, J., Biessels, P., Agoston, S. (1983) Eur. J. Pharmacol. 94: 353–355

Duncalf, D., Lalezari, I., Nagashima, H., Foldes, F. F. (1984) in: Vizi, E. S., Magyar, K. (eds) Regulation of transmitter function: basic and clinical aspects. Akadémiai Kiadó, Budapest, p 307

Harvey, A. L., Marshall, I. G. (1977) Eur. J. Pharmacol. 44: 303–309

Lemeignan, M., Lechat, P. (1967) C.R. Acad. Sci. (Paris) Ser. D. 264: 169–172

Marshall, I. G., Lambert, J. J., Durant, N. N. (1979) Eur. J. Pharmacol. 54: 9–14

Miller, R. D., Van Nyhuis, L. S., Eger, E. I. (1975) J. Pharmacol. Exp. Ther. 195: 237–241

Molgo, J., Lundh, H., Thesleff, S. (1980) Eur. J. Pharmacol. 61: 25–34

Paskov, D. S., Agoston, S., Bowman, W. C. (1985) in: Kharkevich, D. A. (ed.) New curare-like agents. Handbook of experimental pharmacology, Springer-Verlag, Berlin (in press)

Pelhate, M., Pichon, Y. (1974) J. Physiol. (Lond.) 242: 90–91P

Silver, A. (1974) in: Neuberger, A., Tatum, E. L. (eds) Frontiers of biology. Vol. 36. North-Holland, Amsterdam, pp 177–302.

Vohra, M. M., Pradhan, N. N. (1964) Arch. Int. Pharmacodyn. 150: 413–424

Yeh, J. Z., Oxford, G. S., Wu, C. H., Narahashi, T. (1976) J. Gen. Physiol. 68: 519–535

J. Pharm. Pharmacol. 1986, 38: 158–160
Communicated June 5, 1985

Lack of effect of omeprazole, a potent inhibitor of gastric (H^+ + K^+) ATPase, on hepatic lysosomal integrity and enzyme activity

SUSAN GRINPUKEL, RICHARD SEWELL*, NEVILLE YEOMANS, GEORGE MIHALY, RICHARD SMALLWOOD, *Gastroenterology Unit, Department of Medicine, Austin Hospital, Heidelberg 3084, Victoria, Australia*

The substituted benzimidazole, omeprazole, is a potent inhibitor of the ATP-dependent proton pump of the parietal cell. Since there is accumulating evidence that hepatic lysosomes also possess an ATP-dependent proton pump system to maintain internal acidification, and since antibodies to the putative lysosomal proton pump protein are immunologically similar to the parietal cell (H^+ + K^+) ATPase, we studied the effects in rats of six days of omeprazole treatment on hepatic lysosomal function. Omeprazole, 5 mg kg^{-1}, a dose five times the ED50 for gastric acid secretion inhibition in rats, did not alter the activity of three representative lysosomal enzymes in liver (acid phosphatase, β-galactosidase and *N*-acetyl-β-glucosaminidase) nor did it alter lysosomal enzyme latency, a measure of the integrity of the lysosomal membrane. Furthermore, bile flow and the secretion of lysosomal enzymes into bile were also unaffected by omeprazole. These data indicate that in rats short-term treatment with omeprazole, in doses that markedly inhibit gastric acid secretion, has no major biological effect on liver lysosomal integrity and lysosomal enzyme activity.

Omeprazole, a substituted benzimidazole, is a potent new inhibitor of gastric acid secretion, which acts on the (H^+ + K^+) ATPase at the secretory canaliculus of the parietal cell (Fellenius et al 1981; Wallmark et al 1983). This ATP-dependent proton pump is responsible for the final step of gastric acid secretion (Rabon et al 1983).

The lysosome is an intracellular organelle which is responsible for the metabolism of a variety of endogenous and exogenous molecules, and which requires an acidic internal milieu for its degradative function (de Duve & Wattiaux 1966). There is strong evidence to indicate that an ATP-dependent proton pump is also present on the membrane of the lysosome (Dell 'Antone 1984; Ohkuma et al 1982; Schneider 1981), and furthermore, antibodies directed at the putative hepatic lysosomal proton pump protein, cross react specifically with the (H^+ + K^+) ATPase of the parietal cell, but not with other ATPase proteins (Reggio et al 1984). It is presently not known whether omeprazole interacts with the hepatocyte lysosomal proton pump, or whether it alters lysosomal function. Any impairment of lysosomal integrity by such a drug might constitute a serious side effect. For example, chloroquine, an antimalarial drug used in the treatment of rheumatoid arthritis is known

* Correspondence.