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4-Aminopyridine Reversal of Sympathetic Ganglionic Blockade in the Anesthetized Cat

N. N. Durant, Ph.D.,* C. Lee, M.D.,† R. L. Katz, M.D.‡

The effects of 4-aminopyridine on the blockades of transmission of the superior cervical ganglion produced by hexamethonium, *d*-tubocurarine, and polymyxin B were investigated in 15 anesthetized cats. Isometric contractions of the nictitating membrane resulting from pre- and postganglionic stimulation were quantified. A 75–85 per cent ganglionic blockade produced by hexamethonium, 3 mg/kg, or by *d*-tubocurarine, 1.0 to 1.2 mg/kg, was completely reversed by 4-aminopyridine, 1 mg/kg. After injection of 4-aminopyridine, the times required for the contractions of the nictitating membrane to increase from 25 to 75 per cent of control with preganglionic stimulation were 6 ± 3 (mean ± SEM) and 7 ± 2 min for hexamethonium and *d*-tubocurarine, respectively. Both values are significantly ($P < 0.05$) shorter than their respective control spontaneous recovery times of 26 ± 8 and 25 ± 7 min. Polymyxin B, 20–35 mg/kg, depressed the contractions of the nictitating membrane resulting from pre- and postganglionic stimulation by 84 ± 2 and 66 ± 10 per cent, respectively. 4-Aminopyridine, 1 mg/kg, lessened the polymyxin B-induced depression of the contractions of the nictitating membrane resulting from preganglionic stimulation to 50 ± 13 per cent, but had less effect, 61 ± 13 per cent, on the contractions resulting from postganglionic stimulation. It is concluded that 4-aminopyridine rapidly and completely reverses the sympathetic ganglion blockade produced by hexamethonium or *d*-tubocurarine, and is partially effective in the reversal of the autonomic effects of polymyxin B. (Key words: Antagonists, neuromuscular relaxants: 4-aminopyridine. Antibiotics: polymyxin B. Neuromuscular relaxants: *d*-tubocurarine. Sympathetic nervous system: ganglionic blocking agents, hexamethonium.)

THE FACILITATORY ACTION of 4-aminopyridine on neuromuscular transmission is well documented[1] and is postulated to be due to an increase of the evoked release of acetylcholine.[2] As a result, 4-aminopyridine reverses the neuromuscular blockade produced by *d*-tubocurarine.[1,3] Clinically, 4-aminopyridine has had limited use as an agent for the reversal of nondepolarizing neuromuscular blockades[4] and in the treatment of the Eaton-Lambert syndrome.[5] While the effects of 4-aminopyridine on nondepolarizing neuromuscular blockade are well known, its ability to reverse blockade of cholinergic transmission at a site other than the neuromuscular junction has not been described. The aim of the present study was to investigate the ability of 4-aminopyridine to reverse ganglion blockades produced by hexamethonium, *d*-tubocurarine, and polymyxin B.

Materials and Methods

Fifteen cats of either sex weighing between 2.4 and 5.2 kg were anesthetized with a mixture of α-chloralose, 80 mg/kg, and pentobarbital sodium, 5 mg/kg, injected intraperitoneally. The lungs were artificially ventilated with 18 ml/kg air at a rate of 24 breaths/min. Esophageal temperature was maintained at 36–38 C throughout the experiments. Arterial blood pressure was recorded via a polyethylene cannula placed in a femoral artery and connected to a Statham P23Db® pressure transducer. A Gould® tachograph was used to measure heart rate.

The left cervical sympathetic nerve was stimulated preganglionically, distal to a ligation, with trains of supramaximal square pulses of 0.5 msec duration, 10 Hz for 10 sec, repeated once every 100 sec. The right cervical sympathetic nerve was stimulated postganglionically, distal to a ligation, with an

* Staff Research Associate.
† Associate Professor.
‡ Professor and Chairman.

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Address reprint requests to Dr. Durant.

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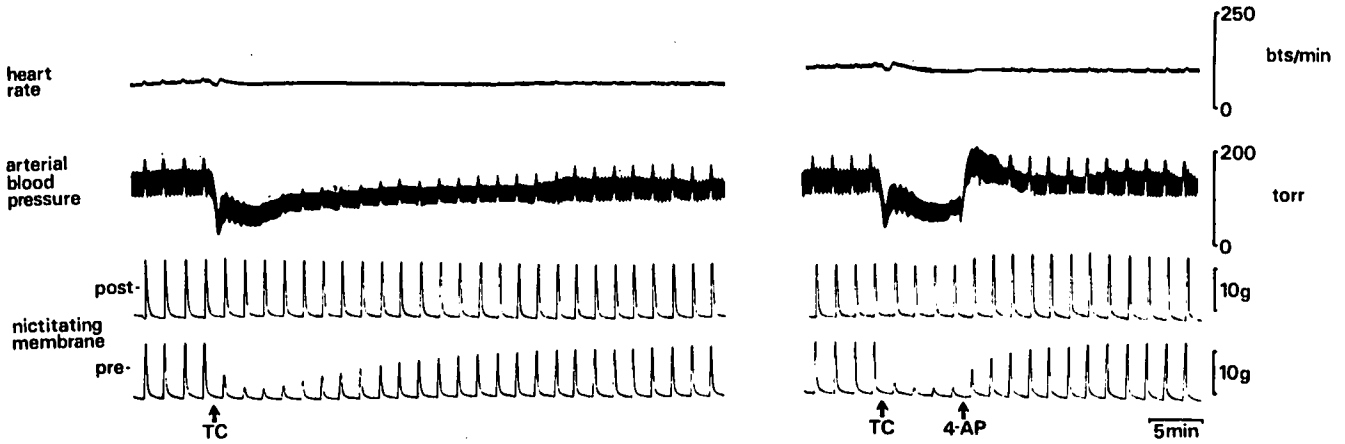

FIG. 1. The actions of 4-aminopyridine, 1 mg/kg (4-AP), on the autonomic effects of *d*-tubocurarine, 1 mg/kg (TC), in the anesthetized cat. *Left*, control time course of blockade without 4-aminopyridine; *right*, the action of 4-aminopyridine on the blockade. Pre- and post- indicate contractions of the nictitating membranes from pre- and postganglionic stimulation.

independent stimulation. The contractile response of each nictitating membrane was separately transduced by a Grass FTO3® transducer. All recordings were made on a Gould Brush 440® recorder.

Drugs used were 4-aminopyridine (Aldrich), hexamethonium bromide (Sigma), pentobarbital sodium solution (Abbott), polymyxin B (Pfizer) and *d*-tubocurarine (Squibb). α-Chloralose was dissolved in polyethylene glycol; all other drugs were dissolved in saline solution, 0.9 per cent, and were administered intravenously via a cannula placed in a femoral vein. Each cat received only one ganglionic blocking drug.

Following stabilization of all variables measured, either hexamethonium, 3 mg/kg, or *d*-tubocurarine, 1.0–1.2 mg/kg, was administered to each cat, to produce an approximately 80 per cent inhibition of contractions of the nictitating membrane resulting from preganglionic stimulation. The blockade was produced twice in each cat, the second time an hour after complete recovery from the first. The time course of the first blockade served as the control. After the maximal effect of the second blockade had been verified, 4-aminopyridine, 1 mg/kg, was injected, and its effect was quantified by comparison of the time course of the second blockade with that of the control. Compared with hexamethonium and *d*-tubocurarine, the doses of polymyxin B required to produce 80 per cent inhibition of the contractions of the nictitating membrane resulting from preganglionic stimulation were more variable, and blockade was more protracted. Therefore, the antibiotic was administered cumulatively in one 10-mg/kg increment and subsequent 5-mg/kg increments to a total dose of 20–35 mg/kg of the base, as needed. Spontaneous recovery, if any, was observed for 45 min, after which 4-aminopyridine, 1 mg/kg, was injected in an attempt to reverse residual blockade. All results are presented as the mean ± the standard error of the mean of five observations. Differences between means were tested with Wilcoxon's test for paired values, $P < 0.05$ being regarded as significant.

Results

Hexamethonium, 3 mg/kg, and *d*-tubocurarine, 1.0–1.2 mg/kg, produced intensities of ganglionic blockade that are not significantly different (table 1). Neither of these drugs produced any inhibition

TABLE 1. Actions of 4-Aminopyridine (1 mg/kg) on Time Courses of Ganglionic Blockades Produced by Hexamethonium and *d*-Tubocurarine*

	Per Cent Maximum Block	Duration of Block to 90 Per Cent Recovery (Min)	Recovery Time 25–75 Per Cent (Min)
Hexamethonium, 1 mg/kg			
Spontaneous recovery	78 ± 4	42 ± 10	26 ± 8
4-Aminopyridine reversal	78 ± 6	9 ± 1†	6 ± 3†
d-Tubocurarine, 1.0–1.2 mg/kg			
Spontaneous recovery	80 ± 6	43 ± 8	25 ± 6
4-Aminopyridine reversal	82 ± 7	18 ± 2†	7 ± 2†

* 4-Aminopyridine was injected when the blockade of the contractions of the nictitating membrane from preganglionic stimulation was maximal (n = 5).

† Significant difference, $P < 0.05$, spontaneous recovery *vs.* 4-aminopyridine reversal.

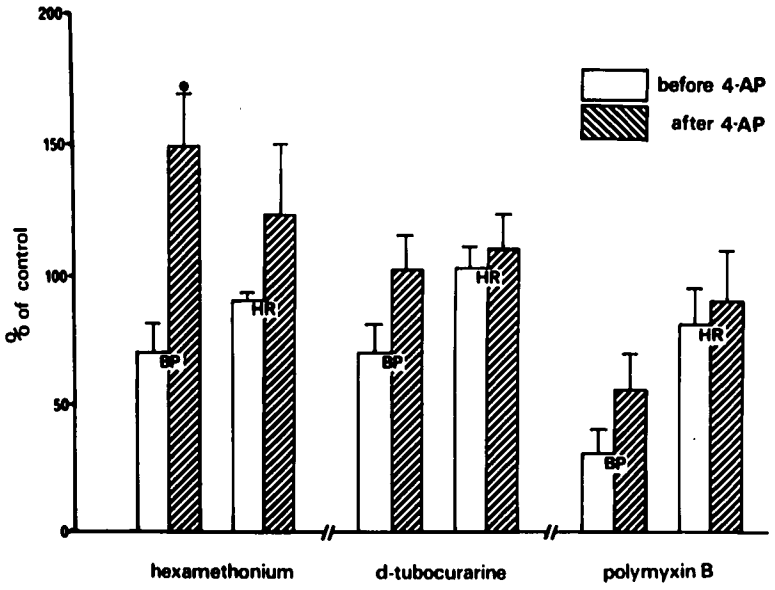

FIG. 2. The actions of 4-aminopyridine, 1 mg/kg (4-AP), on the hypotension and bradycardia produced by hexamethonium (3 mg/kg), *d*-tubocurarine (1.0 to 1.2 mg/kg) and polymyxin B (20 to 35 mg/kg). BP indicates mean arterial blood pressure and HR indicates heart rate. The vertical bars represent SEM (* indicates statistically significant difference at $P < 0.05$, n = 5).

of the contractions of the nictitating membrane resulting from postganglionic stimulation. The ganglionic blockade produced by either hexamethonium or *d*-tubocurarine (fig. 1) was completely reversed by 4-aminopyridine, 1 mg/kg, in a time which is significantly shorter than the time required for spontaneous recovery (table 1). 4-Aminopyridine, 1 mg/kg, facilitated the undepressed contractions of the nictitating membrane resulting from postganglionic stimulation by 10 ± 2 and 18 ± 7 per cent in the hexamethonium- and *d*-tubocurarine-treated cats, respectively. The hypotension and bradycardia produced by both ganglion-blocking agents were fully reversed by 4-aminopyridine (fig. 2).

Polymyxin B, 20 to 35 mg/kg, inhibited the contractions of the nictitating membrane produced by preganglionic stimulation by 84 ± 2 per cent. It also inhibited, although slightly less markedly, the contractions of the nictitating membrane resulting from postganglionic stimulation, by 66 ± 10 per cent, indicative of postganglionic inhibition in addition to ganglionic blockade (fig. 3).

During the 45 min after administration of polymyxin B, the maximum rate of spontaneous recovery of the contractions of the nictitating membrane resulting from preganglionic stimulation was 0.6 ± 0.1 per cent/min, and in all cats this spontaneous recovery was transient. Immediately before 4-aminopyridine, 1 mg/kg, was injected, the polymyxin B-induced depression of the contraction was 77 ± 7 per cent, and after 4-aminopyridine, depression decreased to 50 ± 13 per cent, with a maximum rate of recovery of 8 ± 5 per cent/min ($P < 0.05$). Also, during the 45 min after administration of polymyxin B, the maximum rate of spontaneous recovery of the contractions of the nictitating membrane resulting from postganglionic stimulation was 0.7 ± 0.2 per cent/min. 4-Aminopyridine did not produce any significant change in the polymyxin B-induced depression of the contraction or in the maximum rate of recovery. Thus, the action of 4-

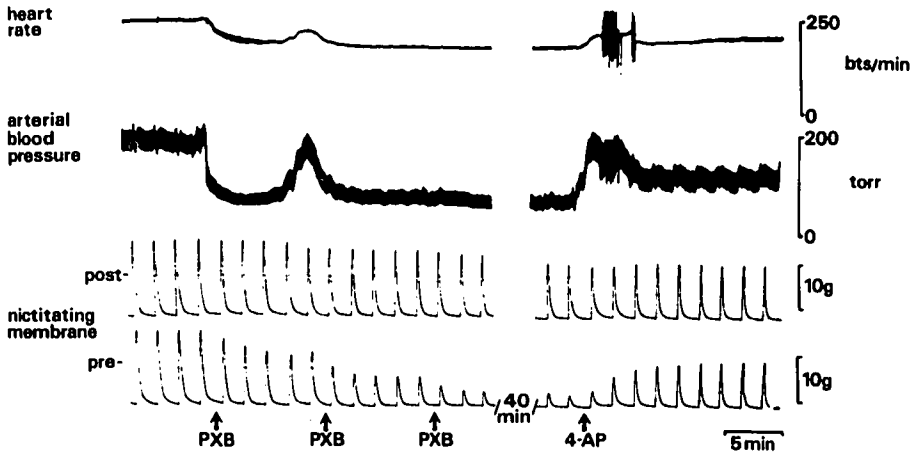

FIG. 3. The actions of 4-aminopyridine, 1 mg/kg (4-AP) on the autonomic effects of polymyxin B, 20 mg/kg (PXB, total dose = 10 + 5 + 5 mg/kg) in the anesthetized cat. Pre- and post- indicate the contractions of the nictitating membranes from pre- and postganglionic stimulation. A transient cardiac arrhythmia following injection of 4-aminopyridine is manifest as a rapid fluctuation in heart rate.

aminopyridine was confined mainly to incomplete reversal of the ganglionic effects of polymyxin B, with minimal reversal of postganglionic depression. The partial reversal of the autonomic effects of polymyxin B by 4-aminopyridine was manifested as a partial reversal of the hypotension and bradycardia (fig. 2), and in three of the five cats this was accompanied by a transient cardiac arrhythmia (fig. 3).

Discussion

The results with hexamethonium and *d*-tubocurarine clearly demonstrate that 4-aminopyridine reverses ganglionic blockade. The ganglionic site of action of these two blocking drugs is confirmed by their selective depression of the contractions of the nictitating membrane resulting from preganglionic but not postganglionic stimulation. In contrast, while polymyxin B did block the ganglia, its action was not confined to this site, since the contractions of the nictitating membrane resulting from postganglionic stimulation were also depressed. During the reversal of the effects of polymyxin B with 4-aminopyridine, contractions of the nictitating membrane resulting from postganglionic stimulation were little affected, nor was the resting tension of the nictitating membrane increased. Thus, in all these circumstances, the action of 4-aminopyridine would seem to be located primarily at the ganglion.

In terms of mechanism of action, 4-aminopyridine is known to block potassium conductance,[6] prolong the action potential in nerves,[7] create a regenerative calcium current,[2] and increase the evoked release of acetylcholine.[2] The resultant increase of released acetylcholine may result in stimulus-bound repetitive firing at the neuromuscular junction.[2,8] In addition to its action on the neuromuscular junction, 4-aminopyridine has been shown to cause repetitive firing in the spinal cord of the cat[9] and at the sixth ganglion of the cockroach.[10] Thus, it is likely that 4-aminopyridine can increase the evoked release of acetylcholine not only at the neuromuscular junction but also at other cholinergic synapses. Such an action may have been responsible for the reversal of the ganglionic blockades produced by hexamethonium and *d*-tubocurarine that we observed in the present study.

Polymyxin B has been shown to depress sensitivity to acetylcholine and to possess a local anesthetic-like action at the neuromuscular junction.[11] Although there is no direct evidence to suggest that it has the same action at the ganglia as at the neuromuscular junction, the same mechanisms of action might explain its effects on the superior cervical ganglion that we observed in the present study. These noncompetitive actions of polymyxin B at the ganglion might not be fully reversible by 4-aminopyridine despite an increased release of acetylcholine.

The action of 4-aminopyridine at mammalian ganglia is of clinical interest because some neuromuscular blocking agents block sympathetic in addition to neuromuscular transmission and because the clinical use of 4-aminopyridine to reverse neuromuscular blockade is contemplated. In view of the fact that the pharmacologic characteristics of different cholinergic synapses vary greatly, it is also of interest to establish that 4-aminopyridine will reverse competitive blockade of cholinergic transmission at synapses other than the neuromuscular junction.

In summary, 4-aminopyridine totally and rapidly reverses the sympathetic ganglionic block produced by hexamethonium or by *d*-tubocurarine and partially reverses the autonomic effects of polymyxin B. We postulate that the mechanism of action of 4-aminopyridine at sympathetic ganglia is similar to its mechanism of action at the neuromuscular junction, that is, an increase of the evoked release of acetylcholine.

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