Antagonism of Polymyxin B-induced Neuromuscular and Cardiovascular Depression by 4-Aminopyridine in the Anesthetized Cat

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In an attempt to find a better antidote to polymyxin B-induced neuromuscular blockade, the authors tested 4-aminopyridine, 0.4–1 mg/kg, in ten anesthetized cats. The sciatic–tibialis anterior nerve–muscle preparation was used. The neuromuscular blockade was successfully reversed in all cats. Rapidity of reversal depended on the dose of 4-aminopyridine administered. At 0.6–1 mg/kg, reversal of the twitch response from 20 per cent of control to 80 per cent of control required 2.4 (SE, 0.5) min; to 100 per cent, 14.4 (SE, 3.7) min. An overshoot of the recovery of neuromuscular blockade of 6–10 hours’ duration followed the reversal. The mechanical twitch response reached a peak of approximately 140 per cent of control at 2 hours. At a lower dosage, 0.4 mg/kg, two of three cats took more than an hour for complete recovery of the twitch response, and the overshoot was approximately 10 per cent of control. The hypotensive effect of polymyxin B was partially reversed, but the bradycardia was not. It is concluded that 4-aminopyridine is an effective antidote to polymyxin B-induced neuromuscular blockade in the cat. (Key words: Antibiotics; polymyxin B. Antagonists, neuromuscular relaxants; 4-aminopyridine.)

Neuromuscular blockade produced by polymyxin B is notoriously difficult to reverse. Drugs used to reverse neuromuscular blockades produced by other muscle relaxants and other antibiotics may not antagonize polymyxin B.1,2 The reversing effect of calcium is incomplete and unreliable. The effects of cholinesterase inhibitors have been inconsistent, sometimes ineffective, sometimes worsening the situation.3,4 Lee et al. described enhancement of the blockade by edrophonium, neostigmine, and physostigmine in the cat.5 They also observed a profound and protracted cardiovascular depression with this antibiotic.

4-Aminopyridine was originally known for its effect as a convulsant in many birds and mammals.6 It facilitates neuromuscular transmission and antagonizes various nondepolarizing neuromuscular blocking agents.6,7 It has been used clinically for reversal of neuromuscular blockade, and has been claimed to have few undesirable side effects and to necessitate no atropine administration.8 Since its action is not specific to the cholinergic system, it may reverse neuromuscular blockade that is irreversibly by cholinesterase inhibitors, such as that produced by polymyxin B. It may also reverse the cardiovascular depression that can be associated with polymyxin B administration. This study was intended to confirm and to quantify these effects.

Methods

Ten cats, weighing 2–3 kg, were anesthetized by intraperitoneal injection of alpha-chloralose, 70 mg/kg, and pentobarbital, 10 mg/kg, without overnight fasting. The trachea, a common carotid artery, and a peripheral vein were cannulated for purposes of ventilation, monitoring of the arterial blood pressure and pulse rate, and injection of drugs. Ventilation was controlled with a Palmer pump delivering room air at 300 ml/kg/min. At this setting, blood-gas values were within normal limits for cats. Arterial blood pressure was continuously recorded on a Brush oscillographic recorder by use of a Statham transducer and appropriate preamplifier. The pulse rate was counted from the arterial pressure tracing, or with a stop watch during the experiment. The sciatic nerve was exposed in the sciatic notch, crushed and severed. The bundle of the sciatic nerve innervating the tibialis anterior muscle was separated and stimulated distally. Stimulus was a supramaximal square pulse of 0.1 msec duration derived from a Grass S88 peripheral nerve stimulator via an SIUS Stimulus Isolation Unit. Stimulation was repeated every 12 sec except where otherwise specified. The tibialis anterior muscle was detached from its insertion with a small piece of bone, freed from the surrounding tendinous sheath, and attached to a Grass FT 10C force transducer with prestretched heavy silk. The proximal head of the tibia was immobilized on a heavy metal frame by use of pins. The ankle was immobilized by clamps. The force of contraction of the muscle was thereby transduced and recorded. In addition, the compound electromyographic response of the muscle was analyzed and recorded simultaneously.9 The core temperature of the cat was maintained at 36–37.5°C.
by a circulating-warm-water mattress, additional heat being applied by a heating lamp when needed. The baseline stretch of the muscle necessary for the maximal tension output on stimulation of the nerve was determined and applied. Several tetanic contractions were then elicited to set the preparation. If the baseline tension was lost, it was restored. When the muscle had no longer lost its baseline tension, the twitch response was allowed to stabilize.

Each run of tests consisted of a continuously repeated twitch evoked every 12 sec, a train of several twitches at 2 Hz (as in a train-of-four), a tetanic contraction elicited by a train of stimuli at 50 Hz lasting 6 sec, and posttetanic twitches evoked 10 sec after the end of the tetanus. The run of tests was repeated to ensure stability of response. Intravenous injection of 3 ml of 0.89 per cent saline solution was made twice during the control period to assure its lack of cardiovascular effect. Then control values were obtained and polymyxin B injected intravenously to block 80–95 per cent of the mechanical twitch response. Doses ranged from 6 to 12 mg/kg, injected in two or three increments over a period of 10 min. The first increment was 6 mg/kg. When neuromuscular transmission had recovered to 20 per cent of control (80 per cent blocked) responses to train-of-four, tetanus, and posttetanic stimuli were evoked. After these observations, appropriate doses of polymyxin B were added to produce once again an 80 per cent block of the twitch response. At that point, 4-aminopyridine 0.4–1.0 mg/kg was injected intravenously as a bolus. Besides the possible reversal of neuromuscular blockade, effects of 4-aminopyridine on arterial blood pressure, heart rate, and salivary secretion, as well as hyperreflexia, fasciculation, convolution, defecation, or urination, if any, were also recorded. All cats were observed for at least eight hours, three for 24 hours.

Polymyxin B and 4-aminopyridine were prepared fresh from powder in 0.89 per cent saline solution. The volume of each injection was limited to 2.5 ml. All cats were hydrated with dextrose, 5 per cent in saline solution, 0.45 per cent, at a rate of approximately 25 ml/kg body weight over each period of eight hours.

Results

4-Aminopyridine consistently reversed the neuromuscular blockade produced by polymyxin B (fig. 1). The reversal began immediately, and was complete and long-lasting, with no recurrence of blockade during subsequent observation periods. The times required for the depressed twitch to be reversed from 20 per cent of control to 80 and to 100 per cent of control depended on the dosage of 4-aminopyridine used (fig. 2). The values were 2.4 ± 0.5 (SE) and 14.4 ± 5.7 min, respectively, in cats receiving 4-aminopyridine, 0.6–1 mg/kg. Cats receiving 4-aminopyridine, 0.4 mg/kg, had variable responses, and an hour or longer might elapse before the twitch response reached the control value (fig. 2). The reversal of
polymyxin B-induced neuromuscular blockade by 4-aminopyridine was evident in all variables examined, including the mechanical force, the amplitude of the neurally evoked compound electromyogram, and twitch responses to the train-of-four, tetanic, and posttetanic stimulation.

An overshoot of 6–10 hours’ duration of the twitch response and the corresponding electromyogram followed the reversal. After 4-aminopyridine, 0.6–1 mg/kg, the peak twitch response was 142 ± 19 (SD) per cent of its control, reached in 125 ± 38 min except in one cat. This exceptional cat received 4-aminopyridine, 0.6 mg/kg. A slow, steady increase in twitch response occurred, but did not reach peak effect in five hours, at which time it was 115 per cent of control. After 4-aminopyridine, 0.4 mg/kg, the peak twitch response was approximately 110 per cent of control.

The decrease of arterial blood pressure produced by polymyxin B was also reversed, but the bradycardia was not. Mean arterial blood pressure averaged 112 ± 10 (SE) torr prior to and 75 ± 12 torr 4 min after injection of the first incremental dose of polymyxin B, and 62 ± 8 torr at the lowest point. The hypotensive effect occurred before neuromuscular blockade (fig. 1). Blood pressure increased immediately upon injection of 4-aminopyridine and was 95 ± 12 torr approximately 2.5 min later, at the time when the twitch response was restored to 80 per cent of control. The corresponding values for heart rate were 192 ± 12, 131 ± 8, 128 ± 7, and 131 ± 7 min, respectively. Levels of statistical significance using Student’s t test were P < 0.05 for paired values for polymyxin B-induced hypotension and bradycardia, as well as for 4-aminopyridine reversal of hypotension. 4-Aminopyridine caused a slight hyperreflexia of one to two hours’ duration in one cat, which after receiving 0.8 mg/kg of the drug responded to the sound of clapping hands with a slight jerk. Salivation, urination, defecation, cardiac arrhythmias, fasciculations, convulsions, and arousal were not observed.

**Discussion**

In view of the difficulty in the reversal of paralysis produced by polymyxin B,[10,11] availability of a new antidote is encouraging. However, 4-aminopyridine itself may have serious side effects. In our preliminary observations of unparalyzed cats, we noticed that 4-aminopyridine in doses in excess of 5 mg/kg will cause hyperreflexia, profuse salivation, and prolonged clonic convulsions. In the dose range of 0.4–1 mg/kg, no such effect was observed except for slight hypertension and hyperreflexia, which invariably followed administration of the drug. This may be the result of the use of alpha-chloralose as the primary anesthetic agent in our studies. In the presence of neuromuscular blockade, 4-aminopyridine 0.4–1 mg/kg caused only an occasional slight hyperreflexia in the cat.

Neither polymyxin B nor 4-aminopyridine is specifically active at the neuromuscular junction. Polymyxin B is a cationic detergent. It changes the permeability of the cell membrane.[10] 4-Aminopyridine is a convulsant. It inhibits potassium conductance and decreases the potassium current of cell membranes.[11,12] Reversal of polymyxin B-induced neuromuscular blockade by 4-aminopyridine apparently results from a complex mechanism of interaction.[12–14] We do not know whether doses of 4-aminopyridine necessary for reversal of the neuromuscular effect may significantly decrease the antibacterial effect of polymyxin B.

Ability of 4-aminopyridine to reverse polymyxin B-induced hypotension is a definite advantage. The intrinsic hypertensive effect of 4-aminopyridine, however, may be disadvantageous when it is used to reverse neuromuscular blockade in the absence of pre-existing hypotension.

The duration of action of 4-aminopyridine is very long. This, again, may be advantageous or not, depending on the circumstances. Neuromuscular blockade produced by antibiotics may be protracted.[1–3] A sustained antagonism is what is necessary for its
continuous reversal. However, we do not know whether difficulties may arise when it becomes necessary to provide muscle relaxation again shortly after use of 4-aminopyridine.

The optimal dose of 4-aminopyridine for reversal of polymyxin B-induced neuromuscular blockade in the cat appears to be 0.6–0.8 mg/kg. Smaller doses are inadequate. Except for the initial improvement of the twitch, 4-aminopyridine, 0.4 mg/kg, did not satisfactorily alter the overall time course of recovery. Larger doses are unnecessary, and may incur undesirable side effects. The dose requirement of 4-aminopyridine, the rapidity and long duration of reversal, and the virtual absence of complications from the reversal agree with the findings of Folds et al. and Stoyanov et al., who used 4-aminopyridine to antagonize a variety of nondepolarizing neuromuscular blocking agents in rats and in man.

In conclusion, 4-aminopyridine, 0.4–1.0 mg/kg, completely reversed the neuromuscular blocking and partially reversed the hypotensive effects of polymyxin B in the cat. An overshoot in the twitch response of as much as 60 per cent of control which lasted as long as 6–10 hours, followed the reversal. The optimal dosage appears to be 0.6–0.8 mg/kg. Smaller doses did not reverse with satisfactory rapidity. The reversal was not complicated by cardiac arrhythmias, profuse salivation, incontinence, fasciculation, bronchospasm, or convulsions. Slight hyperreflexia, seen in one cat, may be dependent on the use of alpha-chloralose as the primary anesthetic. 4-Aminopyridine may be a valuable antidote to antibiotic-induced neuromuscular blockade.

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References