

Reversal of Dantrolene Sodium-induced Depression of Skeletal Muscle in the Cat

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Dantrolene sodium, a muscle relaxant, does not have a clinically useful antagonist. The present study was undertaken to test the efficacies of germine monoacetate, 4-aminopyridine, neostigmine, and calcium chloride as possible agents for the reversal of the direct skeletal muscle depression produced by dantrolene sodium in the cat anesthetized with α -chloralose. Depression of the indirectly elicited twitch responses (0.1 Hz) of the tibialis anterior muscle by 25, 50, 75 and 84 per cent was produced by dantrolene, 0.16, 0.36, 0.88 and 1.13 mg/kg respectively; spontaneous recovery of twitch tension during the subsequent 30 min was negligible. After the 30-min observation period had elapsed, one of the reversal agents under study was given (iv) in divided doses at intervals of 10 min (five cats for each agent). Germine monoacetate (2×0.5 mg/kg) immediately reversed the dantrolene-induced twitch depression, with an overshoot that persisted for an hour. 4-Aminopyridine (4×0.5 mg/kg) caused a steady but incomplete reversal to 17 per cent depression, 30 min after the last dose. Neostigmine (4×0.04 mg/kg) caused an immediate, but transient, reversal of the twitch depression, with overshoot. Calcium chloride (4×10 mg/kg) was without effect. It is concluded that germine monoacetate is the drug of choice for reversal of the muscle depression produced by dantrolene sodium in the cat. (Key words: Antagonists, neuromuscular relaxants: 4-aminopyridine; calcium; germine monoacetate; neostigmine. Neuromuscular relaxants: dantrolene.)

DANTROLENE SODIUM (DS), a hydantoin compound, is used in the treatment of chronic skeletal muscle spasticity occurring in conditions such as cerebral palsy.¹ It has recently been advocated and marketed for the prophylaxis and treatment of malignant hyperthermia and depresses the contraction of the muscle directly without blocking neuromuscular transmission.² The depression of muscle power may be undesirable, and overdosage may result in or predispose to unintentional paralysis, yet counteracting drugs are not available clinically. Bowman *et al.*³ have studied the effects of potential antagonists of DS, including uranyl ions, thiocyanate ions, epinephrine, caffeine, quazodine, quinine, calcium ionophore A23187, and 4-aminopyridine (4-AP), on neurally evoked twitches of isolated hemidiaphragm of the rat. All of these compounds facilitate excitation-contraction coupling in one way or another.

The authors found that 4-aminopyridine and quinine were the most potent of these on a molecular basis; however, with the exception of 4-AP, none of these compounds possesses clinical potential. Therefore, we tested the reversal of DS-induced muscle depression by several drugs that have already been either used or tested clinically for the reversal of various types of muscle paralysis. These included the experimental drugs germine monoacetate (GMA) and 4-AP, and large doses of neostigmine and calcium.

Methods and Materials

Twenty cats of either sex weighing 2.5–5.5 kg were anesthetized with a mixture of α -chloralose, 60 mg/kg, and pentobarbital sodium, 10 mg/kg, injected intraperitoneally. The lungs were artificially ventilated with 18 ml/kg air at a rate of 24 breaths/min. Esophageal temperatures were maintained at 36–38° C throughout the experiments. Arterial blood pressure of each cat was recorded via a polyethylene cannula placed in a carotid artery and connected to a Statham® P23Db pressure transducer. All drugs were administered intravenously.

The sciatic nerve in the popliteal space was stimulated by rectangular pulses of 0.2-msec duration at a frequency of 0.1 Hz. The stimulus strength was adjusted to evoke maximal twitches of the tibialis anterior muscle, which were recorded by a Grass® FT 10 C displacement transducer. The corresponding electromyographic (EMG) response was amplified by a method previously described.⁴ All recordings were made on a Beckman® R-612 dynograph.

Muscle depression was produced by cumulative injections of DS until the twitch tension finally was decreased by approximately 84 per cent. The nerve-muscle preparation was observed to ensure that no further depression would ensue. When the twitch response had recovered very slightly to 20 per cent of control, the rate of further spontaneous recovery, if any, was measured during the following 30 min. Groups of five cats each then received as many as four boluses of one of the following drugs, injected at 10-min intervals; GMA, 0.5 mg/kg; 4-AP, 0.5 mg/kg; neostigmine methylsulfate, 0.04 mg/kg; calcium chloride, 10 mg/kg. In the case of GMA, only two doses were used, because this was sufficient to produce complete reversal of DS-induced

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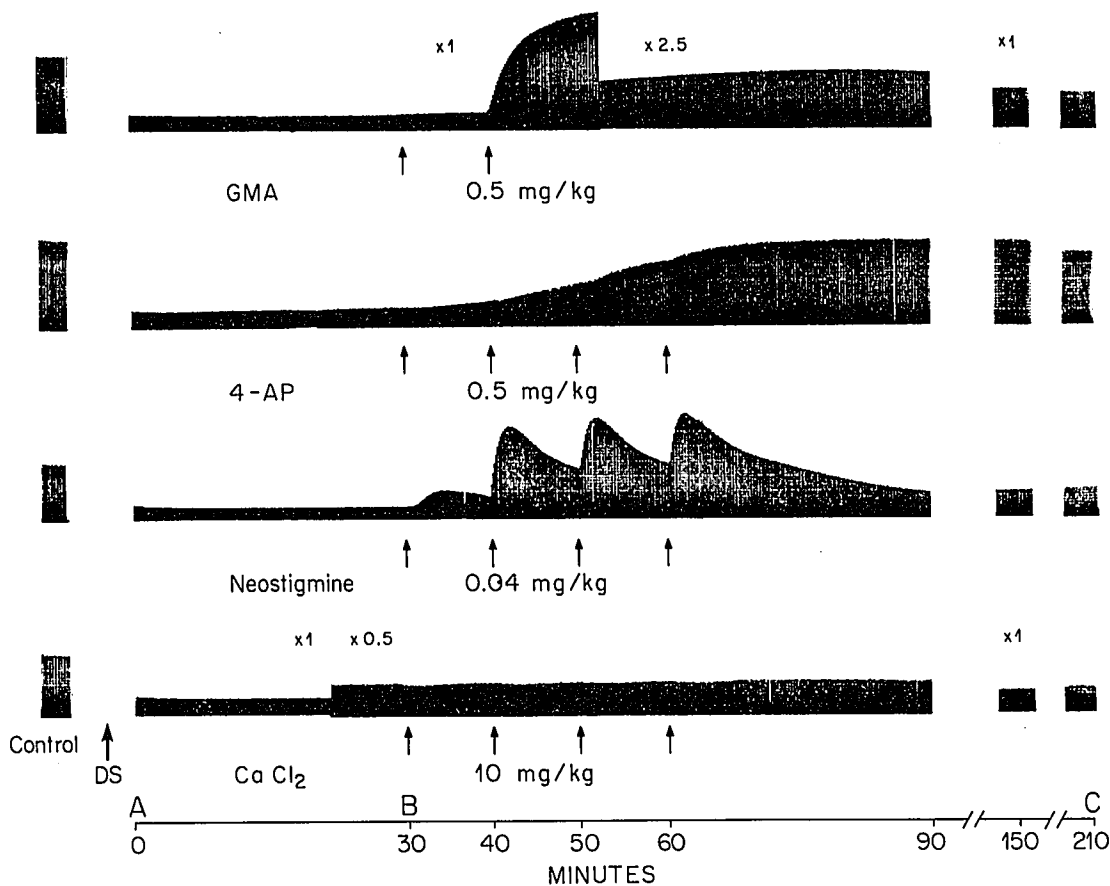


FIG. 1. Typical responses of the neurally evoked twitches of the cat tibialis anterior muscle to dantrolene sodium (DS) and counteracting drugs (GMA = germine monoacetate; 4-AP = 4-aminopyridine). Twitches were elicited at 0.1 Hz. Each arrow signifies a single administration of the drug in the dose indicated below. A, 80 per cent twitch depression by DS; A to B: slight spontaneous recovery during 30 minutes; B to C: drug-induced reversal.

muscle depression, with marked overshoot. All data are reported as means \pm SEM.

Dantrolene sodium \ddagger was obtained in pure form, and dissolved in polyethylene glycol 400 in a concentration of 3 mg/ml (the maximum solubility of DS in polyethylene glycol 400 is 80 mg/ml). Polyethylene glycol itself, in the volume injected (less than 0.1 ml/kg in each bolus), had previously been verified to lack neuromuscular effects. Germine monoacetate ** was supplied in pure form and dissolved in 0.9 per cent saline solution to a concentration of 1 mg/ml; 4-AP $\dagger\dagger$ was also dissolved in 0.9 per cent saline solution to a concentration of 1 mg/ml. Calcium chloride and neostigmine methylsulfate were diluted to make the total volume of each injection just less than 1 ml.

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Results

While DS did not appreciably depress the amplitude of the EMG response to neural stimulation, it depressed the twitch response by 25 per cent at the cumulative dose of 0.16 ± 0.01 mg/kg, by 50 per cent at 0.36 ± 0.03 mg/kg, by 75 per cent at 0.88 ± 0.09 mg/kg, and by 84 per cent at 1.13 ± 0.09 mg/kg ($n = 20$). Following each injection of DS, the onset of twitch depression began immediately, and 95 per cent of the final twitch depression in response to each bolus was achieved in 5.5 ± 0.3 min ($n = 20$). The duration of DS-induced twitch depression was long, and the exact time when spontaneous recovery began could not accurately be determined. During a subsequent period of 30 min of observation the recovery of the twitch tension was minimal (about one per cent) though discernible. The DS itself caused no significant change in blood pressure or pulse rate.

Figure 1 illustrates the typical responses of the twitch tension to the drugs tested as reversal agents,

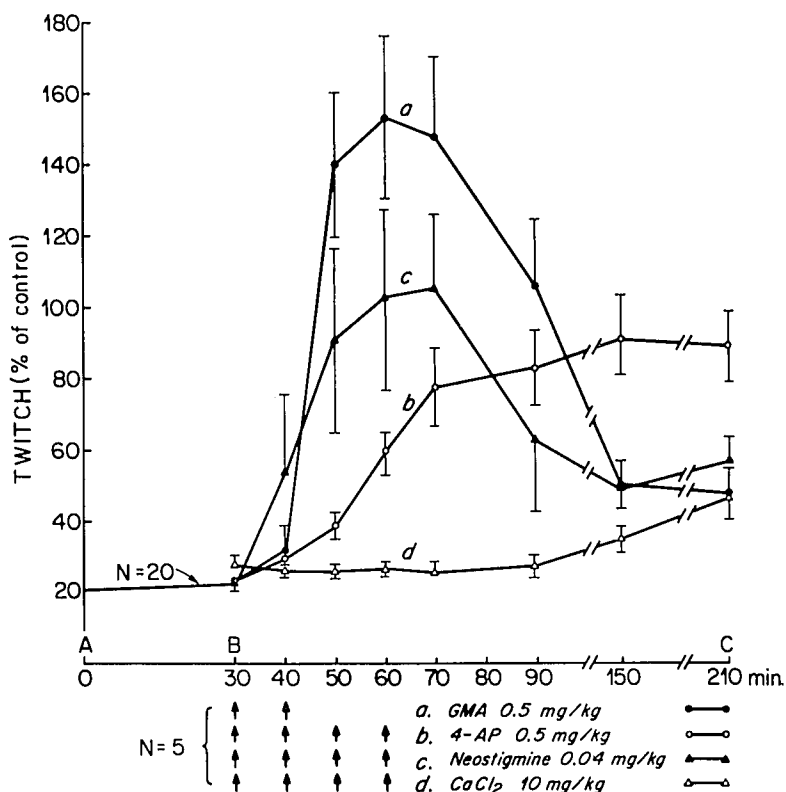
and figure 2 presents the group data and the time courses. Germine monoacetate caused an increase of the DS-depressed twitch response, with an overshoot of muscle twitch that lasted approximately an hour following the second dose of 0.5 mg/kg. 4-Aminopyridine also increased twitch tension, but the effect was gradual and the reversal was incomplete even after the fourth dose of 0.5 mg/kg, at which point all cats began to convulse. Neostigmine caused an immediate partial reversal of the DS-depressed muscle twitch and an overshoot following the second dose of 0.04 mg/kg; however, both the reversal and the overshoot were transient. Peak effect occurred within 2 min of injection of each dose of neostigmine, and maximum twitch tensions after individual administrations were 63 ± 24 , 168 ± 52 , 190 ± 48 , and 207 ± 54 per cent of control. Up to the fourth dose of 10 mg/kg, calcium chloride was without discernible effect on DS-induced depression of muscle twitch.

Discussion

Germine monoacetate, 4-AP, neostigmine, and calcium all reverse nondepolarizing neuromuscular block. However, they differ both chemically and

pharmacologically. Germine monoacetate is a semi-synthetic veratrum alkaloid derivative that reverses both depolarizing and nondepolarizing neuromuscular block.⁵ It acts primarily by causing the axon and the muscle fiber to respond to a single stimulus with repetitive firing.⁶ Clinically, it has had some application in the treatment of myasthenia gravis.⁷ 4-Aminopyridine is currently being used clinically in parts of Europe⁸ and experimentally in the United States, and acts by prolonging the potassium current during depolarization of the nerve terminal membrane, thus delaying rectification and prolonging the nerve terminal action potential.⁹ At the neuromuscular junction, the primary resultant action of 4-AP is an increase of transmitter release due to the prolonged depolarization of the motor nerve terminal and at high doses, it also causes repetitive firing.¹⁰ Neostigmine, at high doses, and particularly in the absence of neuromuscular block, may cause repetitive firing of the motor nerve terminal and consequently, the muscle fiber. Calcium is essential for transmitter release and is used as an adjunct for the restoration of neuromuscular transmission, especially when the neuromuscular block is the result of the aminoglycoside antibiotics or magnesium, both of which act mainly prejunctionally.

FIG. 2. Time courses and dose schedules of spontaneous and drug-induced reversal of dantrolene sodium (DS)-induced twitch depression of skeletal muscle in the anesthetized cat. Arrows indicate single intravenous administrations of drug in the doses shown below. All measurements of drug-induced reversal were made 10 min after the respective administrations; however, in the case of neostigmine the maximum effect had passed before this period (see fig. 1). A, 80 per cent twitch depression by DS; A to B: slight spontaneous recovery during 30 min; B to C: drug-induced reversal. Bars indicate SEM; GMA = germine monoacetate; 4-AP = 4-aminopyridine.



Dantrolene sodium does not block neuromuscular transmission.¹ Its mechanism of action of muscle depression is uncoupling of excitation-contraction by reduction of calcium release from the sarcoplasmic reticulum.² During DS-induced muscle depression, the chain of events beginning from activation of the nerve, through transmission of impulse across the neuromuscular junction, and ending with the generation of the muscle action potential, is uninterrupted. This accounts for the absence of diminution of the amplitude of the neurally evoked compound EMG of the muscle *in vivo*.¹¹ Among the drugs we tested, GMA is the most effective in the reversal of the action of DS. This we believe is because GMA causes repetitive firing of the muscle fiber, which in turn causes repetitive release of calcium from the sarcoplasmic reticulum, to result in a prolonged activation of the contractile mechanism. In essence, repetitive firing of the muscle is equivalent to a brief tetanus, and Nott and Bowman² have shown that compared with twitch, tetanus is spared during DS-induced muscle depression. By comparison, 4-AP is less effective; the reason is probably that at the dosage tested 4-AP acts prejunctionally, and unless marked repetitive firing of the muscle fibers occurs, the effect of 4-AP will be diminished by the direct effect of DS on the muscle. Neostigmine is probably effective against DS due to repetitive firing of the nerve terminal and thus the muscle fiber; this action of neostigmine is probably transient, because the concentration necessary is reached only just after administration. Elevation of the extracellular calcium concentration was ineffective against DS, since calcium influx into the muscle fiber would be insufficient and too slow to have an effect.

The doses of GMA (0.5 ~ 1.0 mg/kg), 4-AP (0.5 ~ 1.0 mg/kg), neostigmine (0.04 ~ 0.08 mg/kg), and calcium chloride (10 mg/kg) are approximately equipotent, and usually sufficient for complete reversal of an 80 per cent neuromuscular block produced by curariform drugs in the cat (personal observation). Thus, if a single injection sufficed, the depression would be regarded as readily reversible; on the other hand, if four doses were needed the depression would be regarded as very difficult to reverse or almost irreversible. In either case, four doses of the counteracting drugs cannot be injected intravenously without producing signs of overdose in the cat. Therefore, for reversal of DS only GMA can be considered effective; neostigmine is too short-acting, 4-AP lacks efficacy, and calcium is ineffective.

From the viewpoint of potential clinical usefulness,

we believe that the order of preference for the drugs tested is GMA, 4-AP, neostigmine, then calcium. Besides effectiveness, GMA has a rapid onset and a sufficiently long duration of action. It is also recognized to provide long-lasting improvement of the muscular power in myasthenic patients,⁷ to reverse α -bungarotoxin-induced neuromuscular block (personal observation), and to reverse not only non-depolarizing but also depolarizing neuromuscular block.⁵ 4-Aminopyridine is also long-lasting, a quality necessary for the treatment of DS-induced paralysis,¹ and although its initial effect appears inferior to that of neostigmine, its delayed effect is superior. However, 4-AP has a slow onset of action, does not have sufficient efficacy, and has a narrow therapeutic ratio. In a separate study, we found that 4-AP causes hyperreflexia in the cat at 1 mg/kg, iv, may cause a long-lasting convulsion at 2 mg/kg, and will cause death at 5 mg/kg (in spite of respiratory support). However, the narrow margin of safety does not necessarily preclude eventual clinical use because, as in the case of neostigmine, antidotes to the non-neuromuscular effects of 4-AP may be developed. Neostigmine has a rapid onset on action against DS, but its duration of action is too short. Conceivably, a combined use of neostigmine and 4-AP may improve the result, since 4-AP does potentiate the ability of neostigmine to reverse the neuromuscular block produced by pancuronium in the rat.¹² Not only is calcium ineffective against DS, but it is often regarded as contraindicated in the treatment of malignant hyperthermia for which DS is currently advocated. Finally, it should be understood that until our observations are confirmed clinically, mechanical ventilatory support, rather than drug reversal, remains the treatment of choice for DS-depressed patients.

In summary, results of the present study suggest that GMA, 4-AP, neostigmine, and a combination of the latter two warrant further investigation for their possible clinical usefulness in the reversal of DS-induced muscle depression. Germine monoacetate is the single drug of choice in the cat, and its clinical potential warrants consideration.

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