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Effect of Dantrolene on Neuromuscular Block by d-*Tubocurarine and Subsequent Antagonism by Neostigmine in the Rabbit*

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The effects of dantrolene on neuromuscular blockade produced by *d*-tubocurarine (*d*Tc) and subsequent reversal with neostigmine were studied *in vivo* in a rabbit model. Two groups of rabbits were given a constant rate of infusion of *d*Tc at a concentration of 0.1 or 0.2 mg/ml to produce approximately 95 per cent twitch depression, as measured by plantar flexion of the hind foot in response to sciatic-nerve stimulation. An additional two groups of rabbits first received dantrolene, 1.5 mg/kg, producing approximately 60 per cent depression of twitch amplitude, and then received the *d*Tc infusion to attain a combined 95 per cent twitch depression. The paralytic effects of initial dantrolene and subsequent *d*Tc were additive. Prior dantrolene therapy produced greater twitch depression with initial doses of *d*Tc, but as greater *d*Tc-induced paralysis was achieved, the dantrolene effect became insignificant. It appears that *d*Tc and dantrolene act on proximal and distal sites, respectively, in the process of muscle excitation, and that these sites are in tandem. Thus, there was no difference between the amounts of *d*Tc (.2 mg/kg) necessary to produce 95 per cent twitch depression with and without prior administration of dantrolene. When neostigmine, .02 mg/kg, with atropine was administered, reversal was complete in rabbits receiving only *d*Tc, but in those receiving prior dantrolene, neostigmine appeared to antagonize only the *d*Tc effect, without influencing the dantrolene-induced block. (Key words: Antagonists, neuromuscular relaxants: neostigmine. Neuromuscular relaxants: dantrolene; *d*-tubocurarine.)

DANTROLENE, a skeletal muscle relaxant, is used for the treatment of spasticity associated with various diseases, and is unique in its mechanism of action. Unlike *d*-tubocurarine (*d*Tc), dantrolene does not appear to interfere with neuromuscular transmission, alter membrane excitability, or affect the central nervous system.[1–3] Dantrolene appears to block the excitation–contraction coupling mechanism of skeletal muscle.[4–6] In rat diaphragm–phrenic nerve preparations, dantrolene did not alter the dose–response curve of *d*Tc *in vitro*.[3] Physostigmine has produced a transient reversal of dantrolene-induced twitch depression in rats.[4]

Patients taking dantrolene orally may need general anesthesia, but the interactions of dantrolene and other muscle relaxants, such as *d*Tc, have not been clearly established. The present experiment was designed to investigate the effect of dantrolene on the dose response to *d*Tc. The antagonistic effect of neostigmine on combined dantrolene–*d*Tc-induced block was also evaluated.

Methods

Twelve male albino rabbits weighing 2.8–3.8 kg were anesthetized by intravenous administration of pentobarbital sodium, 6 mg/ml. A cumulative dose of 140 mg/kg was necessary for orotracheal intubation. Ventilation was assisted to maintain a peak expired CO_2 of 3.0 ± 0.2 per cent by continuous monitoring with a Godart-Statham capnograph. This CO_2 level resulted in an arterial blood CO_2 tension of approximately 42 torr. Incremental doses of pentobarbital, 12 mg, were used to maintain anesthesia (cumulative dose, 58 ± 10 mg/kg), and physiologic saline solution was infused at 60 ml/hr during the procedure. Rectal temperatures ranged from 38.5 to 39.3 C among animals.

Plantar flexion of the foot in response to sciatic-nerve stimulation was quantitated as previously described.[7] Briefly, each animal was placed in a supine position and restrained. The right hind leg was secured in a clamp in a manner which allowed unimpeded plantar flexion of the foot. Two 25-gauge metal needles were placed just posterior to the knee joint and connected to a Grass Model SD5 Stimulator.® A square-wave pulse of 60–80 volts, 0.04 msec duration, and a frequency of 0.2 pulse/sec was used. Resting tension of the foot was adjusted to produce maximum twitch tension. Twitch amplitude and peak expired CO_2 were recorded with a Grass recorder. A control period of 30 min was allowed for stabilization before treatments were initiated.

The 12 rabbits were randomly assigned to four treatment groups with three rabbits per group. Group I received a constant infusion of *d*Tc, 0.1 mg/ml, until 95 ± 2 per cent depression of twitch amplitude occurred. Group II was treated similarly, except that the *d*Tc concentration was 0.2 mg/ml. Groups III and IV first received dantrolene, 1.5 mg/kg, intravenously over 60 sec. When twitch depression by dantrolene

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TABLE 1. Treatment Group Means and Standard Deviations

	Dantrolene, 0.0 mg/kg		Dantrolene, 1.5 mg/kg	
	*d*Tc, 0.1 mg/ml Group I	*d*Tc, 0.2 mg/ml Group II	*d*Tc, 0.1 mg/ml Group III	*d*Tc, 0.2 mg/ml Group IV
Rabbit weight	3.2 ± 0.3 kg	3.2 ± 0.5 kg	3.4 ± 0.2 kg	3.4 ± 0.2 kg
d-Tubocurarine	0.199 ± .041 mg/kg	0.247 ± .045 mg/kg	0.203 ± .011 mg/kg	0.220 ± .023 mg/kg
*d*Tc infusion time	303 ± 107 sec†	237 ± 35 sec	410 ± 40 sec†	223 ± 35 sec
*d*Tc lag time*	183 ± 35 sec†	120 ± 17 sec	217 ± 6 sec†	130 ± 30 sec
Dantrolene depression	—	—	61 ± 6 per cent	58 ± 7 per cent
Per cent of control before neostigmine	63 ± 39 per cent	69 ± 6 per cent	80 ± 13 per cent	80 ± 22 per cent
Per cent of control after neostigmine	97 ± 7 per cent	108 ± 15 per cent	52 ± 7 per cent	53 ± 16 per cent

* Lag time is duration of *d*Tc infusion prior to onset of twitch depression.

† $P < 0.01$ for Group I *vs.* Group II or Group III *vs.* Group IV.

reached a steady state of approximately 60 per cent, *d*Tc infusions were initiated. Group III was given *d*Tc, 0.1 mg/ml, and Group IV received *d*Tc, 0.2 mg/ml, until a cumulative twitch depression of approximately 95 per cent was achieved. All *d*Tc infusions were administered with an infusion pump at a constant rate of 1 ml/min. The dose of *d*Tc (mg/kg) administered at any infusion time can be calculated using subject weight and concentration infused (table 1). Ten minutes after *d*Tc infusion was terminated, each animal was given neostigmine, 0.02 mg/kg, as a bolus injection equal to 10 per cent of the previously administered *d*Tc dose. Atropine, 0.01 mg/kg, was administered concurrently with the neostigmine.

Dantrolene for intravenous administration was prepared by dissolving 7.5 mg powdered dantrolene, 1.72 mg sodium hydroxide, and 66 mg mannitol in 15 ml distilled water. The final concentration of dantrolene was 0.5 mg/ml.

From the four possible combinations of two concentrations of *d*Tc, with or without dantrolene, cumulative data for analytical variables were analyzed as a 2 × 2 factorial arrangement via analysis-of-variance procedures. Dose responses, measured across time, were analyzed and studied using regression techniques. $P < 0.01$ was regarded as significant.

Results

The concentration of *d*Tc administered affected the infusion time necessary to produce 95 per cent block. Groups I and III needed significantly longer infusion times to produce 95 per cent block than did Groups II and IV, while the presence or absence of dantrolene had no appreciable effect on total infusion time for any test dose of *d*Tc (table 1). Similar results were observed for lag times between start of *d*Tc infusion and perceptible twitch depression; *i.e.*, lag time was significantly shorter for the 0.2 mg/ml *d*Tc concentration, and dantrolene had no effect on lag times for any dose of *d*Tc (table 1).

The concentration of *d*Tc did not significantly affect the cumulative dose of *d*Tc necessary to produce 95 per cent twitch depression. Also, the presence of dantrolene did not significantly alter the cumulative dose of *d*Tc. That is, the total amount of *d*Tc needed to achieve 95 per cent twitch depression was essentially the same regardless of the presence or absence of dantrolene.

For each concentration of *d*Tc, changes in twitch tension across the measurement period had an asymptotic relationship (fig. 1). Thus, early in the infusion period the dantrolene-treated groups experienced a greater degree of twitch block for any given amount of *d*Tc. However, as total paralysis was approached, the effect of dantrolene on the response to *d*Tc became insignificant.

Dose responses were also studied by considering percentage change in twitch tension from control twitch as a function of lapsed time from beginning of *d*Tc infusion (fig. 2). The control twitch tension was that tension that existed just prior to initiation of *d*Tc infusion regardless of prior dantrolene administration. No significant dantrolene effect on the response to *d*Tc (in terms of *percentage block* from control) was observed with either concentration of *d*Tc at any time period.

During the time interval after maximal depression with *d*Tc and prior to neostigmine administration, all groups showed partial spontaneous recovery of twitch. Animals changed from 95 per cent block of pretreatment control level to an average of only 63 per cent block in Group I and 80 per cent block in Group III (table 1). A similar pattern was evident for Groups II and IV, with average residual blocks of 69 per

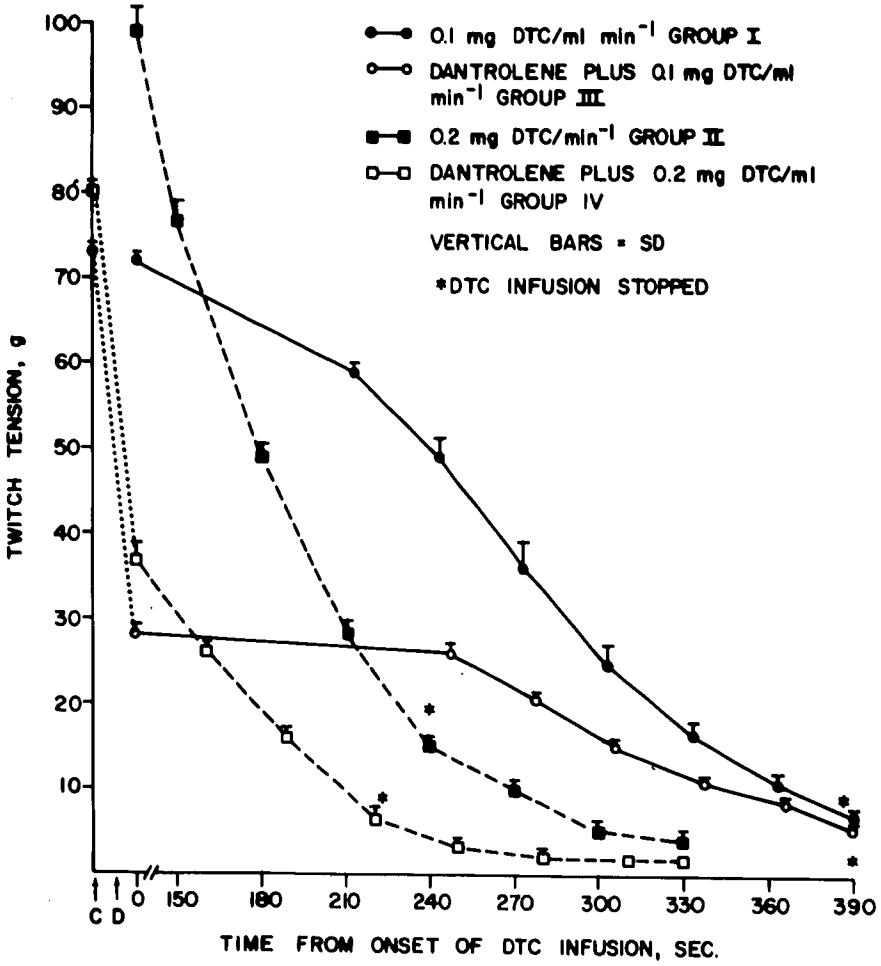

FIG. 1. Effect of dantrolene on change in twitch tension, g, produced by *d*-tubocurarine (*d*Tc). C = control twitch amplitude before drug administration; D = dantrolene effect; 0 = initiation of *d*Tc infusion. Each point is the mean of three observations.

cent and 80 per cent, respectively. However, differences among these four residual blocks were not statistically significant.

The response to neostigmine was appreciably less in the dantrolene-treated animals. Administration of neostigmine resulted in almost complete reversal of twitch depression in Group I, while twitch heights of Group II animals surpassed initial control levels (table 1). In contrast, dantrolene-treated groups had significantly lower levels of recovery after neostigmine, achieving only 52 per cent and 53 per cent of control twitch heights for Groups III and IV, respectively (table 1 and fig. 3).

Although Group III animals achieved only partial recovery, their final twitch height values, when expressed as percentage of block, were significantly higher than twitch heights observed after the initial dantrolene-induced block (52 *vs.* 61 per cent). The same changes were not seen for the Group IV animals.

Discussion

In this study, muscle paralysis (measured by absolute twitch tension in g) produced by infusion of *d*Tc was initially augmented by previous dantrolene administration. These results may be explained by separate sites of action for *d*Tc and dantrolene. The effect of *d*Tc is at acetylcholine receptors, while dantrolene is thought to act more distally on the excitation–contraction coupling mechanism.[4-6] The difference between dose–response curves (relative to absolute twitch height) for *d*Tc versus dantrolene-plus-*d*Tc is not indicative of any synergistic effect. The result of this experiment is best explained by the two drugs acting in an additive manner through a tandem nature of their sites of action.

In patients taking therapeutic dantrolene orally, 64 per cent twitch depression of the adductor pollicis muscle, a block slightly greater than that achieved in the present investigation, has been reported to occur.[8] From a clinical viewpoint, prior dantrolene administration is predicted to have little effect on the dose of *d*Tc necessary for surgical relaxation or endotracheal intubation, since a high degree of twitch depression is needed to achieve these goals. This statement is supported by the asymptotic association of the dose–response curves (fig. 1). Although dantrolene initially

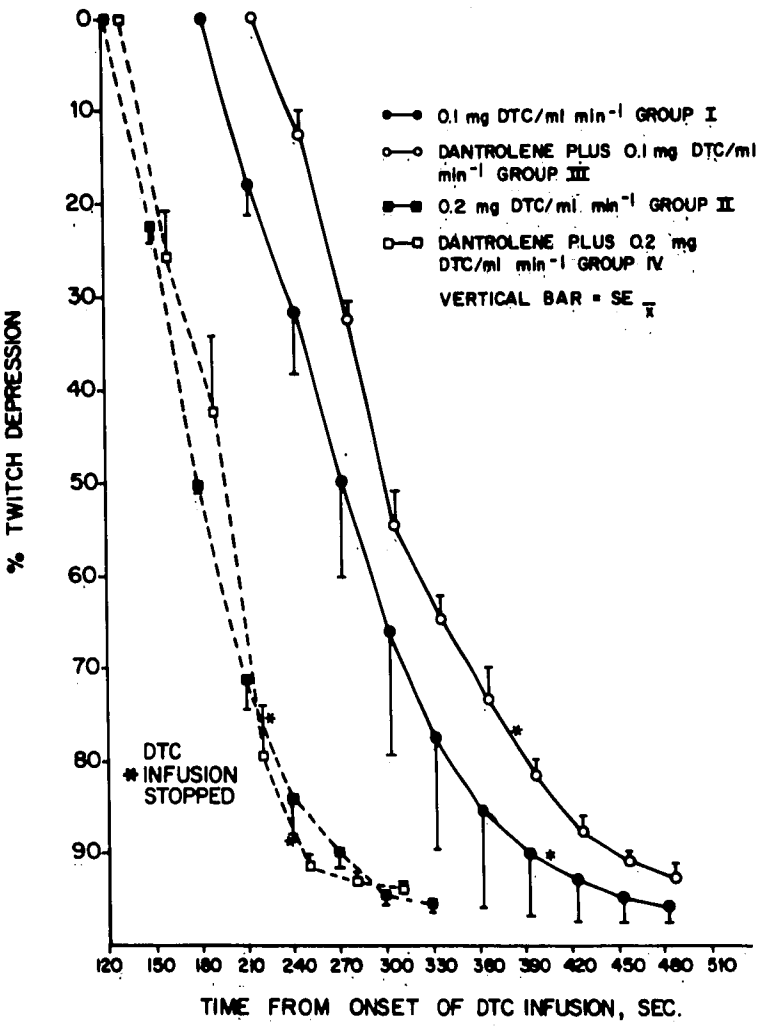

FIG. 2. Effect of dantrolene on percentage change in twitch tension from control twitch height produced by *d*-tubocurarine (*d*Tc). Control twitch height is that amplitude just prior to *d*Tc infusion.

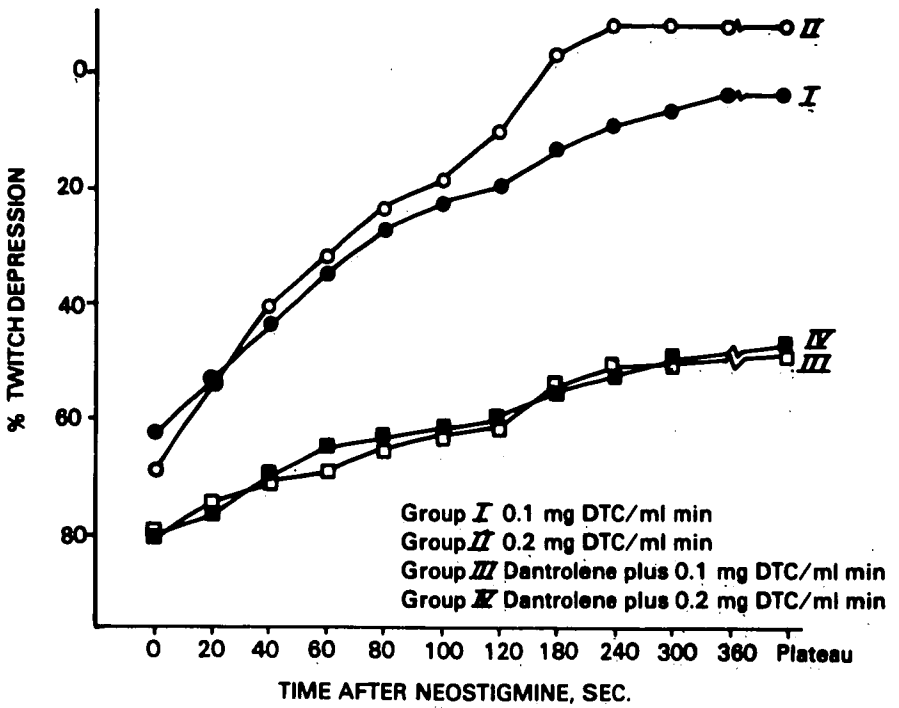

FIG. 3. Neostigmine antagonism of *d*-tubocurarine with and without dantrolene. Without dantrolene, twitch had spontaneously recovered to 63 per cent; with dantrolene, only to 81 per cent. A change in percentage twitch depression was observed after bolus injection of neostigmine.

alters the absolute dose response to *d*Tc, practical differences disappear near 95 per cent twitch block.

A previous study concluded that dantrolene had no effect on the *d*Tc dose response *in vitro*.[3] This conclusion was reached by observing percentage twitch depression as a function of *d*Tc concentration in the presence and absence of dantrolene, using as control amplitude the twitch height just prior to *d*Tc administration. A similar result was obtained in this study when the data were analyzed in the same manner (fig. 2). However, when the data are presented in a different way (fig. 1), the opposite conclusion can be drawn. We propose that dantrolene does alter the *d*Tc dose response, and that the two drugs are pharmacologically independent.

The results of neostigmine antagonism indicate that *d*Tc, but not dantrolene, effects were reversed by the dose of neostigmine administered. All groups showed spontaneous twitch recovery as a function of time after maximal depression of *d*Tc. After neostigmine reversal, Group III animals recovered muscle function slightly greater than their post-dantrolene plateaus, but a block of approximately 50 per cent remained.

Although dantrolene has not produced respiratory depression in animals,[2,10] people receiving the drug may complain of weakness.[11] The clinician is cautioned to evaluate patients receiving orally administered dantrolene carefully during recovery from anesthesia. Residual dantrolene block might contribute to the development of respiratory inadequacy.

We conclude that patients being treated with orally administered dantrolene preoperatively will have an altered *d*Tc dose response, but that the dose of *d*Tc necessary to achieve surgical relaxation will not be significantly changed, nor will reversal of *d*Tc-induced block with neostigmine be impeded.

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