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The action of dantrolene sodium on individual fast and slow motor units of the rat anaesthetized with urethane

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1 Rats, anaesthetized with urethane, were injected intravenously with dantrolene sodium in a vehicle of 5% mannitol taken to pH 10 with NaOH.

2 The muscle relaxant action of dantrolene sodium was measured from the contractions of individual motor units of the extensor digitorum longus (EDL), soleus (SOL) and segmental tail (ST) muscles. Data were also collected from their parent whole muscle preparations.

3 The depressant action of dantrolene sodium on the percentage-normalized amplitude of contraction of the individual motor units was greater than its effect on the whole muscle twitch amplitude, in all three muscles.

4 The twitch amplitude of fast contracting motor units was significantly more reduced ($P < 0.001$) by dantrolene sodium than was that of slow contracting motor units.

5 Dantrolene sodium reduced the contraction time of all motor units. The effect of the drug on half-relaxation time varied within and between groups of motor units studied.

6 The drug was confirmed to have a greater depressant action on the twitch contraction than on the fused tetanus of whole muscle. This was true also for single motor units.

7 With tetanic stimulation the effect of dantrolene sodium was also dependent upon the motor unit type, fast or slow. A maximum depression of contractile tension occurred at a stimulation frequency of 64 Hz for fast EDL motor units whereas the maximum depression for ST slow units, the slowest units tested, was at a stimulation frequency of 14 Hz.

Introduction

The muscle relaxant drug dantrolene sodium (dantrium, Eaton Laboratories, UK) has been shown to have a greater depressant action on fast muscle twitches than on slow muscle twitches in both the cat (Bowman *et al.*, 1979) and the rat (Leslie & Part, 1981a). Both these investigations were performed upon whole muscle contractions, whereas in this present paper we describe similar experiments performed upon individual motor units of rat muscle. We believe that this approach is necessary for two main reasons: firstly, even in a supposedly homogeneous fast or slow muscle there is in fact a histochemical mixture of muscle fibre types and secondly, the control of muscle contraction occurs at the level of individual motor units rather than whole muscles. Therefore, studying the action of dantrolene sodium on whole muscles may not necessarily give true information as to the action of the drug in the intact animal.

Motor units from the segmental tail (ST) muscles were studied (see Andrew & Part, 1972) because these muscles provide the opportunity to study two quite distinct populations of fast and slow units within the same muscle. In addition units from the previously studied muscles, fast extensor digitorum longus (EDL) and soleus (SOL) were investigated.

A preliminary account of some of our results has been given (Leslie & Part, 1981b) as have those of Murthy *et al.* (1980), from their experiments on the cat.

Methods

The experiments were carried out on 36 rats (Sprague Dawley, 200 to 300 g). Surgical anaesthesia was induced with trilene and maintained with intraperitoneal injections of urethane (BDH, 30% w/v, 1.5 g kg^{-1} body weight). Body temperature was monitored rectally and held between 35 and 37°C throughout the experiment. The right femoral or jugular vein was cannulated for intravenous injection

of the drug. In several experiments, the carotid artery was also cannulated, for the recording of blood pressure. Standard dissection techniques were used to expose the hind limb or tail muscle together with its nerve supply and to free maximally the distal tendon. Once prepared the hind limb or tail was passed through a hole in the wall of a perspex bath, then made leak proof by raising skin flaps and tying them tightly to a flange surrounding the entry port to the bath. In each preparation care was taken to stabilize the proximal tendon of the muscle whilst the distal tendon, now cut from its insertion, was attached to the force transducer (Devices 2ST05, 0–100 g). Thereafter the bath was filled with pre-warmed liquid paraffin maintained at 35 to 37°C by warm water circulating through a second chamber in the base of the bath (see Andrew & Part, 1972; Leslie & Part, 1981a). In addition to the techniques used for recording whole muscle tension, the contractions of individual motor units were recorded by means of the standard technique of stimulating functionally single fibre filaments dissected out from the distal portion of the cut ventral root (Andrew & Part, 1972).

In most experiments the muscle contractions were analysed by means of a Sharp MZ80K microcomputer interfaced with the voltage output of the tension transducer by means of an A/D converter (3D, Digital, Design and Development, UK). For reading twitch contractions, an intersample interval of 1 ms was used, whereas for reading the tetanic contractions an interval of 5 ms was used. The microcomputer was also used to generate the stimulus pulses and generally to run the experimental protocol. Twitch stimulations within the protocol were given once every ten to fifteen seconds; tetanic stimulations for 300 ms, at frequencies from 14 to 258 Hz, were never given more often than once per minute, but mostly at two to three minute intervals.

The usual experimental procedure involved isolating two motor units and recording both their twitch and tetanic contraction properties and the twitch properties of the whole muscle. Our tension transducer did not have a sufficient range to be able to record tensions from that of motor unit twitch, after dantrolene sodium, right up to the full tetanic tension of the whole muscle and therefore, the whole muscle tetanic contractions were not recorded. The recordings were taken before, during and after the intravenous injection of dantrolene sodium.

The solution of dantrolene sodium was prepared immediately before intravenous injection as follows: 4 mg of hydrated compound were first dissolved in 3 ml of NaOH solution at pH 10, thereafter 1 ml of 20% w/v mannitol solution was added; this, now isotonic, solution contained 1 mg of drug per ml. In control experiments in which this vehicle was injected alone, it was found to be without effect on the muscle contraction. In all experiments the drug was injected at a dosage of 5 mg kg^{-1} body weight, a dose known to have a maximal effect on muscle contraction (Leslie & Part, 1981a), whilst leaving the animal breathing spontaneously.

Statistical analysis

Student's paired or unpaired t test was used to determine if differences between means were significant; P values of 0.05 or less were taken as being statistically significant.

Drugs

The following drugs were used: trilene (trichloroethylene Ph. Eur. ICI); urethane (ethyl carbamate, BDH); dantrolene sodium (a gift from Norwich Eaton Ltd, Woking, Surrey)

Results

The results obtained from a total of 36 experiments have been presented in two major divisions: (I) results from lateral segmental tail muscles; (II) results from the hind limb muscles – EDL and SOL. Within each of these major divisions, sections have been allocated to groups of observations on (i) whole muscle twitches, (ii) motor unit twitches, (iii) comparisons between whole muscle and motor unit twitches, and (iv) tetanic tensions generated by fast and slow motor units.

(I) *Lateral segmental tail muscles*

Studies were made in a total of 21 preparations located between caudal vertebrae Cd9 and Cd14.

(i) *Observations on whole muscle twitch* Figure 1 shows data for whole muscle twitch tensions, contraction times and half-relaxation times before and after a single intravenous injection of dantrolene sodium (5 mg kg^{-1}), which caused a marked reduction in twitch tension from a control mean of 57.9 ± 12.7 mN to 20.3 ± 6.7 mN ($\pm$s.d.); in terms of percentage change, the amplitude of the whole muscle twitch tension was diminished by some $65 \pm 8.7\%$ ($\pm$s.d.). Contraction times were also diminished from 17.8 ± 2.5 ms to 12.7 ± 2.2 ms (mean $\pm$ s.d.)—a mean reduction of 29%. The considerable spread of the control values of half-relaxation times, from 13.4 ms to 42.1 ms, was not unexpected since this parameter is dependent on the proportion of slow contracting motor units present in a given muscle (see Figure 1). Within our sample the number of slow units ranged

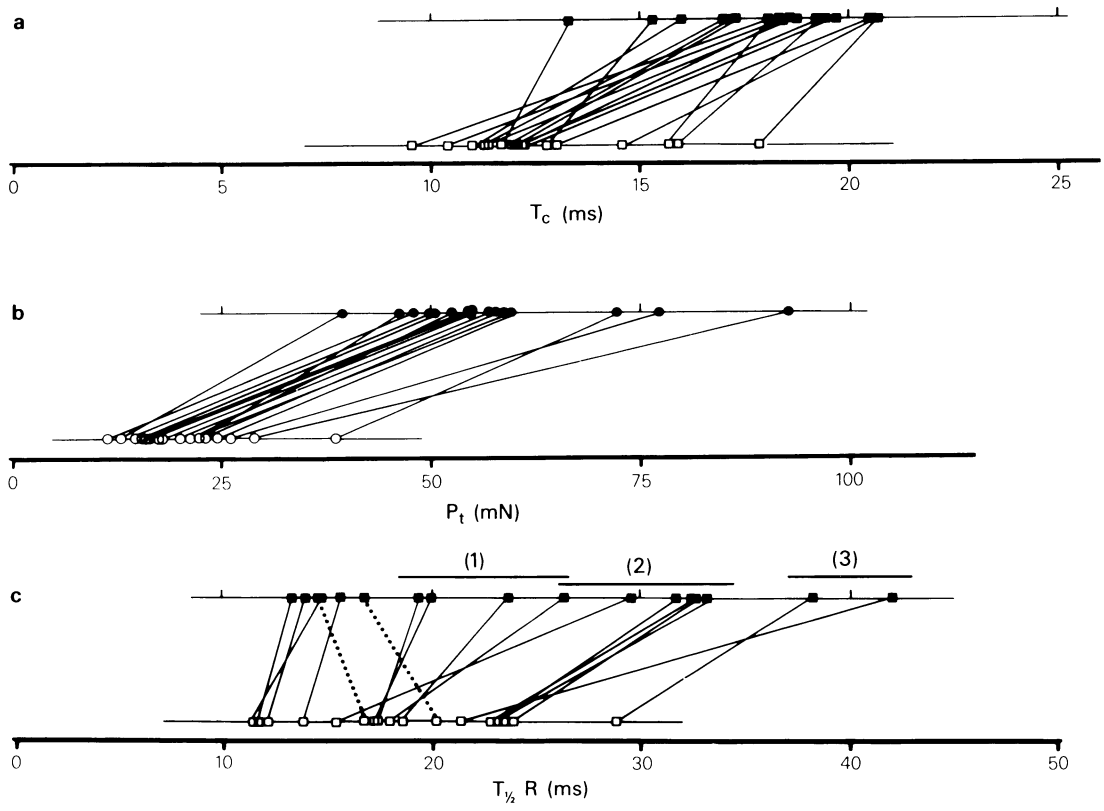

Figure 1 Effect of a single intravenous injection of dantrolene sodium (5 mg kg^{-1} body weight) on the contraction time (T_c) (a) twitch tension (P_t) (b) and half relaxation time ($T_{½}R$) (c) of lateral segmental tail muscles, maintained at 35–37°C and stimulated once every ten to fifteen seconds. The filled symbols on the upper line of each panel were the control data: the open symbols were the data obtained in the presence of the drug; corresponding points in control and experimental data have been joined by a line. The majority of values for T_c, P_t and $T_{½}R$ were obviously decreased by the drug's presence; only in two cases (see dotted lines in (c) did the drug cause an increase over control values. The bars, (1), (2) and (3), show the ranges of half relaxation times in whole muscles containing one, two and three slow motor units, respectively.

from zero to three. As seen from Figure 1, in all but 2 of 18 observations, dantrolene sodium caused the half-relaxation time ($T_{½}R$) to be decreased. This decrease was least in those muscles with either zero or one slow motor unit, and greatest in that muscle with three slow motor units present ($T_{½}R$ decreased from 42.1 ms to 21.4 ms).

(ii) *Observations on motor unit twitches* A total of 27 motor units were studied in 14 preparations. Of the 27 units, 14 were fast contracting, 11 slow contracting and two intermediate. Not all of the units yielded full sets of data since there were occasions when the functionally single fibres, isolated in the ventral roots, did not survive the several hours necessary for the gathering of information. Even so, the displays of absolute data in Figure 2, together with their collected statistics in Table 1, contain much detail regarding the effect of dantrolene sodium on contraction time (T_c), twitch tension (P_t) and half-relaxation time ($T_{½}R$) of motor units in lateral segmental muscles of the rat's tail.

The control data show that, apart from an occasional occurrence of an intermediate type of motor unit, there exist within these muscles distinct populations of fast and slow motor units. While contraction (and/or half-relaxation) times separated clearly the two populations, slight overlap existed between the ranges of twitch tensions for the fast and slow units; this latter fact notwithstanding, the general finding was for the slow contracting units to generate small tensions and for the fast units to generate large twitch tensions. These various observations reaffirm clearly the previous findings of Steg (1964) and Andrew &

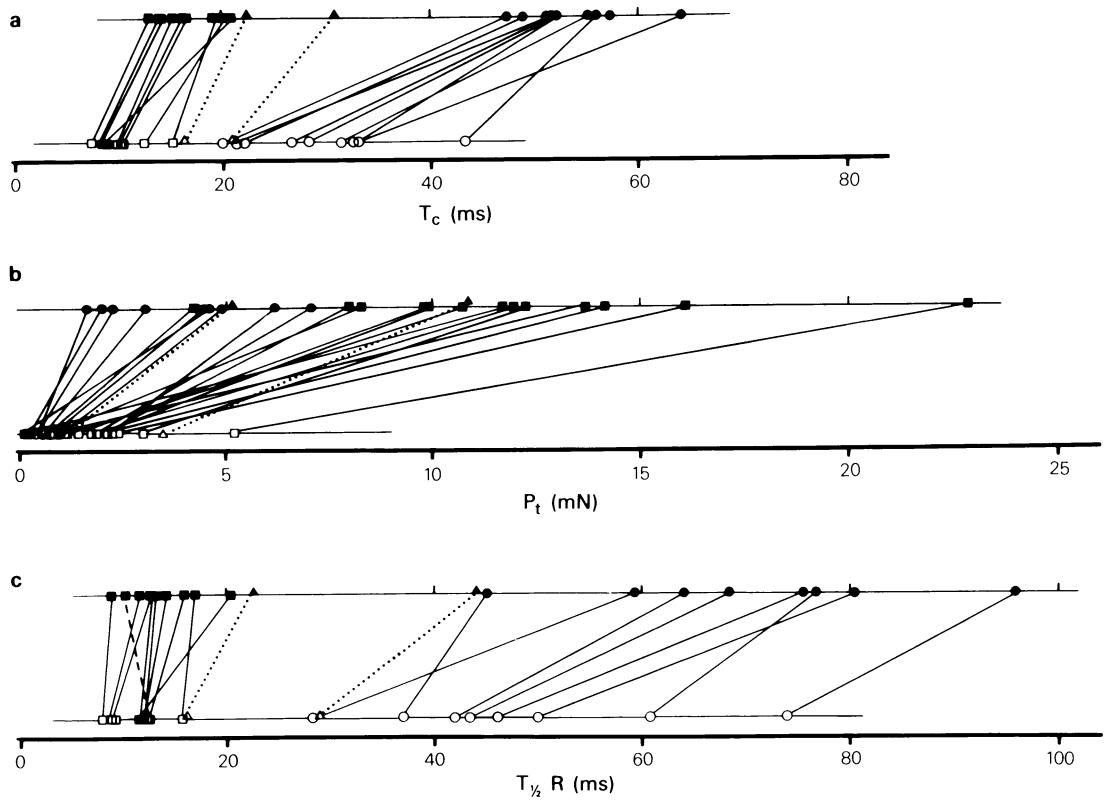

Figure 2 Effect of dantrolene sodium on the twitch parameters, T_c (a), P_t (b) and $T_{\frac{1}{2}}R$ (c), of fast, slow and intermediate type motor units in lateral segmental tail muscles. The closed symbols ((■), fast, (●), slow, (▲), intermediate) represent control values; the open symbols after dantrolene sodium 5 mg kg^{-1}. The dotted lines join up data points for the two intermediate type motor units sampled in this study. The broken line within the third ($T_{\frac{1}{2}}R$) panel indicates the minority observation of one, out of a total of sixty three, when the drug caused an increase from the control.

Part (1972). Indeed, the mean statistics for the present data agree closely with those of Andrew & Part (1972) [see Table 1, columns (i) and (v)]

Figure 2 shows that dantrolene sodium caused reductions in each of the three specified parameters (T_c, P_t and $T_{\frac{1}{2}}R$) in all but one observation when the half-relaxation time was increased. Clearly the largest reductions (shifts to the left in Figure 2) in absolute values occurred in contraction and half-relaxation times of slow units and in active tension of many of the fast contracting units. With the sample populations of fast and slow units taken together, significant correlations existed between the control value before giving the drug and the absolute difference caused by the drug. For example, the correlation coefficient between the reduction in contraction time and the original contraction time was 0.914 ($P<0.01$). The correlation coefficients for similar relationships with twitch tension and with half-relaxation time were 0.983 ($P<0.01$) and 0.898 ($P<0.01$) respectively. Whereas these relationships existed over the total sample population, correlations within the single samples (fast or slow) were not significantly different in all but three cases. These cases were (i) twitch tension in slow units ($r=0.94$, $n=10$, $P<0.01$), (ii) twitch tension in fast units ($r=0.966$, $n=13$, $P<0.01$), and (iii) half-relaxation times in fast units ($r=0.708$, $n=9$, $P<0.05$).

Student's t tests for paired or unpaired data (as necessary), were performed on the data summarized in columns (i) and (ii) of Table 1. These tests clearly showed that the changes effected by dantrolene sodium were statistically significant for all three parameters in both samples of fast and slow motor units. Whereas the absolute changes in twitch tensions of fast and slow motor units were distinctly different, it can be seen from Table 1, column (iv) that the normalized percentage changes were little

Table 1 The effect of intravenous dantrolene sodium (DaNa) (5 mg kg^{-1} body weight) on the twitch parameters of fast, slow and intermediate type motor units in segmental tail muscles of the rat.

	(i) *Before DaNa*	(ii) *After DaNa*	(iii) *Absolute change*	(iv) *% change*	(v) *Data from Andrew & Part (1972)*
Fast units					
T_c	16.1± 3.1 (13)	10.7± 2.1 (10)	6.1±2.0 (10),	36.3± 8.4 (10),	16.2± 1.9 (21)
	——$P<0.001$——				
P_t	11.8± 4.3 (13)	1.7± 1.3 (13)	10.1±3.5 (13),	85.6± 7.7 (13),	13.7± 6.9 (21)
	——$P<0.001$——				
$T_{\frac{1}{2}}R$	13.5± 3.0 (13)	11.8± 2.4 (9)	2.7±2.5 (9),	17.4±11.8 (9),	13.2± 3.4 (21)
	——$P<0.02$——			$P<0.025$	
Slow units					
T_c	51.8± 6.5 (10)	30.0± 6.4 (9)	23.5±5.3 (9),	44.2±10.1 (9),	50.9± 7.1 (8)
	——$P<0.001$——				
P_t	4.1± 1.7 (10)	0.9± 0.7 (10)	3.1±1.2 (10)	78.5± 8.9 (10),	3.3± 1.7 (8)
	——$P<0.001$——				
$T_{\frac{1}{2}}R$	67.3±15.3 (10)	47.9±13.3 (8)	23.1±7.5 (8),	32.7±10.9 (8),	78.0±21.3 (8)
	——$P<0.02$——				
Intermediate units					
T_c (a)	23.8	16.1	7.7	32.4	
T_c (b)	30.8	20.7	10.1	32.8	
P_t (a)	10.6	3.4	7.2	67.9	
P_t (b)	4.9	1.1	3.8	77.6	
$T_{\frac{1}{2}}R$ (a)	22.9	16.8	6.1	26.6	
$T_{\frac{1}{2}}R$ (b)	44.7	28.4	16.3	36.5	

The results are given as mean ± standard deviation: numbers of observations in parentheses, except for the intermediate units when only two (a and b) were studied.
Significant differences where they exist between means in unpaired *t* tests are shown together with the related *P* value.
The data in column (v) are included for ready comparison with the control data in our present study (see column (i))
T_c, contraction time (ms); P_t, twitch tension (mN); $T_{\frac{1}{2}}R$, half relaxation time (ms).

different for T_c and P_t, indeed the differences in their means (using the unpaired *t* tests) were not statistically significant (see below, however, for P_t). The only significant difference existed between the mean percentage reductions in half-relaxation time.

(iii) *A comparison of the reductions of twitch tension in whole muscle and motor units* In 11 of the 14 tail muscle preparations it was possible to compare the normalized reductions in twitch tension for two motor units and the muscle in which they were contained. Thus in one experiment we observed that dantrolene sodium reduced the whole muscle twitch tension by 66%, a fast motor unit by 86% and a slow unit by 80%. This combination of muscle with one fast and one slow unit was obtained in six other preparations. Other combinations studied were: muscle with two fast units (two experiments), muscle with one slow and one intermediate unit (two experiments). The data from these 11 experiments are shown in Figure 3. The trend is clearly for the whole muscle twitch to be less reduced than that of the motor units and for the fast units to be more reduced than the slow units. The mean reductions (± s.d.) for whole muscles, fast units and slow units were 65±8.9%, 85±7.4% and 80±8.5% respectively. Paired *t* tests showed that in all combinations there were significant ($P<0.001$) differences between means from whole muscle, fast and slow motor units.

Prolonged observations were made (over 5 to 8 h) in three of the above experiments. These observations showed that the rates of recovery for the normalized twitches of whole muscle and motor units were similar.

(iv) *Observations on the tetanic tensions generated by motor units in lateral segmental tail muscles* Data in this section refer to 12 motor units (six fast, six slow) investigated in the course of 8 experiments. During these experiments each unit was activated at a number of stimulation frequencies and the maximum tension which developed measured before and after dantrolene sodium. These data are presented collectively in Table 2.

The maximum tensions developed by the slow units, stimulated at 258 Hz, ranged from 9.4 to

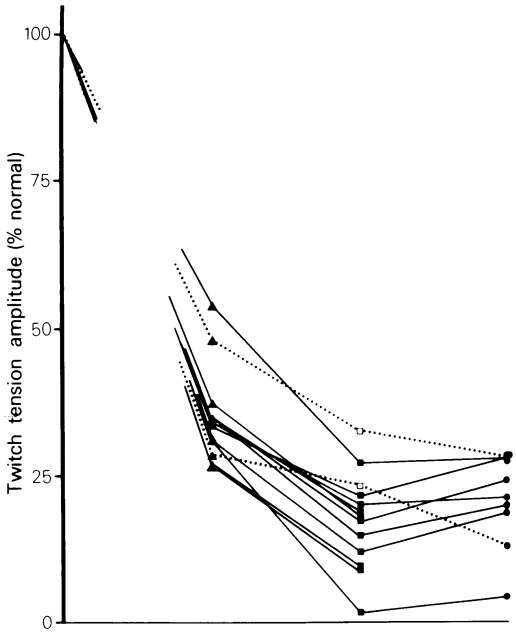

Figure 3 Effect of dantrolene sodium (5 mg kg^{-1}) on the twitch amplitude of whole muscle (▲) and fast (■), intermediate (□) and slow (●) motor units in the segmental tail musculature of the rat. Corresponding data from a whole muscle and two units within it are joined together; solid lines take in fast units, dotted lines the intermediate units. The data illustrate the least depression of twitch tension in whole muscle, most depression in fast units, with the slow units on average some 4% less depressed than the fast units. Highly significant differences ($P < 0.001$) exist between the depression of twitch amplitude from whole muscle and those from both fast and slow motor units: a highly significant difference also exists between the paired data from fast and slow units.

34.2 mN with a mean of 18.2 ± 10 mN (± s.d.). The corresponding data for the six fast units were 89.5 to 112.5 mN and 98.6 ± 8.8 mN respectively. Fusion frequencies for the slow contracting motor units were about 25 Hz and for the fast units between 75 and 100 Hz. Dantrolene sodium caused loss of tetanic tension which was dependent both on the applied frequency of stimulation and the fusion frequency for the type of motor unit being investigated. Below fusion frequency (say 25 and 75 Hz for slow and fast contracting units respectively), loss of tension was large, in some cases almost total, but less so above these critical frequencies. For example, the mean tensions developed by fast units stimulated at 14 Hz before and after dantrolene sodium were 11.9 and 1.1 mN respectively, a fall of some 91%, while at 258 Hz the corresponding fall was only 16%. To emphasize the importance of fusion frequency as a turning point in the drug's effect on tetanic tension, the original data may be reworked as follows: consider absolute loss of tension at each frequency as a percentage of maximum possible tension generated; for example, in Table 2, the mean loss in the fast units stimulated at 14 Hz is 10.8 mN, this is 11% of the mean maximum control of 98.6 mN. On this calculation basis it can be clearly shown that the drug effects an overall maximum percentage loss of tension in any ST motor unit at or about its fusion frequency. This characteristic, though presented differently, can also be seen to exist in the data from hind limb motor units (see Figure 6).

(II) *Hind limb muscles*

Preparations were made in 15 rats to obtain observations from 5 extensor digitorum longus (EDL) and 10 soleus (SOL) muscles.

Table 2 A statistical summary of tetanic tensions (mN) developed by fast and slow tail motor units indirectly stimulated through a series of frequencies, both before and after intravenous dantrolene sodium (DaNa) (5 mg kg^{-1} body weight)

		Stimulation frequency (Hz)					
		14	*31*	*64*	*87*	*129*	*258*
Fast units	Before (mN)	11.9 ± 1.9	16.4 ± 2.9	80.3 ± 7.1	93.2 ± 7.1	96.1 ± 7.7	98.6 ± 8.8
	After DaNa (mN)	1.1 ± 0.9	1.2 ± 0.9	3.6 ± 2.1	25.3 ± 18.7	58.1 ± 29.8	83.2 ± 14.3 (n = 3)
	Reduction (%)	91	93	96	73	40	16
Slow units	Before (mN)	9.3 ± 4.2	16.0 ± 8.2	17.6 ± 9.3	18.8 ± 9.3	18.5 ± 9.7	18.2 ± 10.0
	After DaNa (mN)	1.0 ± 0.6	5.7 ± 4.3	13.2 ± 9.3 (n = 5)	15.8 ± 10.6 (n = 5)	18.1 ± 11.9 (n = 4)	18.8 ± 11.3 (n = 4)
	Reduction (%)	89	64	26	16	2	–

The results are given as mean ± standard deviation: unless indicated otherwise the numbers of observations were 6, for both samples of fast and slow motor units.
The percentage reductions are mean data only.

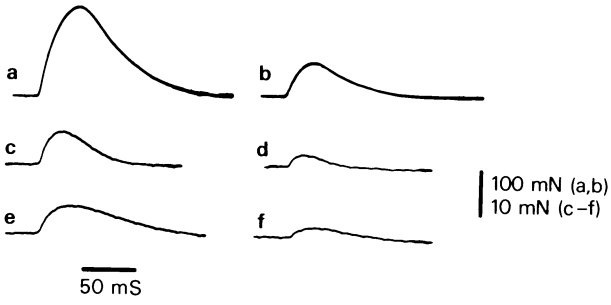

Figure 4 Twitch tension before (left) and after (right) a single intravenous injection of dantrolene sodium (5 mg kg^{-1}). The recordings (all from a single soleus muscle) are: whole muscle (a, b); an intermediate type motor unit, relatively infrequently found (c, d); and a slow contracting unit (e, f).

(i) *Observations on whole muscle twitches* A single intravenous injection of dantrolene sodium (5 mg kg^{-1}) effected reductions in contraction times, half-relaxation times and peak tensions developed by both slow (SOL) (see Figure 4) and fast (EDL) muscles. The statistics for these observations are shown in Table 3. These reductions between mean values before and after the drug were statistically significant for all but those related to the contraction and half-relaxation times for the EDL muscle, where the observations were from a minimum of three. Absolute changes brought about by the drug's action on individual parameters bore a relationship to their control values if the data from SOL and EDL were treated as a single group. Thus the absolute change in contraction time against control value yielded a correlation coefficient of 0.953, significant at $P<0.001$. The corresponding r values for the data on whole muscle twitch tension and half-relaxation time were 0.906 and 0.899 respectively, both significant at $P<0.001$. Similar analysis within the single groups, of 10 SOL and 5 EDL, showed less rigid relationships. Indeed significant relationships between absolute reduction in a parameter against its control value were found only in SOL contraction times ($r=0.8$, $P<0.01$) and in twitch tension amplitudes for SOL and EDL ($r=0.89$ and 0.91 respectively, $P<0.05$ for both sets of data). When normalized in percentage terms, the various changes caused by the drug were as follows: contraction times for EDL and SOL were decreased by 33±11.7% and 32±10.9% (mean±s.d.) respectively; twitch tension amplitude

Table 3 The effect of intravenous dantrolene sodium (DaNa) (5 mg kg^{-1} body weight) on the twitch parameters of whole muscle and motor unit contraction in extensor digitorum longus (EDL) and soleus (SOL) muscles of the rat

		Before	*After DaNa*	*Change*	*Percentage change*
EDL					
Muscle	T_c (ms)	10.7± 2.1 (5)	7.1± 1.3 (3)	3.7± 2.2 (3)	33±11.7 (3)
	P_t (mN	232.7±25.6 (5)	72.6±20.6 (5)	160.0±24.4 (5)	69± 5.0 (5)
		—$P<0.001$—			$P<0.05$
	$T_{\frac{1}{2}}R$ (ms)	11.7± 3.3 (5)	10.9± 2.8 (3)	3.56± 6.00 (3)	1.0±15.4 (3)
Motor units	T_c (ms)	10.7± 2.2 (10)	7.2± 4.3 (8)	4.2±4.3 (8)	37±36.4 (8)
		—$P<0.05$—			
	P_t (mN)	10.2± 7.5 (10)	1.6± 1.0 (10)	8.6± 6.7 (10)	80± 9.0 (10)
		—$P<0.01$—			
	$T_{\frac{1}{2}}R$ (ms)	12.4± 3.0 (10)	6.9± 3.4 (9)	5.0± 3.8 (9)	41±32.2 (9)
		—$P<0.01$—			
SPLEUS					
Muscle	T_c (ms)	41.3± 6.1 (10)	26.9± 4.1 (10)	14.5± 3.2 (10)	32±10.9 (10)
		—$P<0.001$—			
	P_t (mN)	195.4±17.4 (9)	67.4±10.9 (9)	128.0±16.3 (10)	66± 5.5 (10)
		—$P<0.001$—			$P<0.01$
	$T_{\frac{1}{2}}R$ (ms)	33.5± 5.2 (10)	26.0± 4.5 (10)	7.5± 1.9 (10)	22± 4.4 (10)
		—$P<0.01$—			
Motor units	T_c (ms)	34.3± 9.9 (13)	23.2± 7.1 (11)	9.9± 5.3 (11)	28±14.7 (11)
		—$P<0.001$—			
	P_t (mN)	9.1± 1.8 (13)	2.3± 0.6 (13)	6.8± 1.8 (13)	74± 7.2 (13)
		—$P<0.001$—			
	$T_{\frac{1}{2}}R$ (ms)	42.3±12.8 (13)	30.2± 9.0 (13)	12.0± 5.3 (13)	27± 8.3 (13)
		—$P<0.02$—			

The results are given as mean±standard deviation: numbers of observations in parentheses. Significant differences between means have, where they exist, been indicated together with the relevant P value.
T_c, contraction time (ms); P_t, twitch tension (mN); $T_{\frac{1}{2}}R$, half relaxation time (ms).

was reduced by about 69% and 66% respectively for EDL and SOL, a marked difference occurred between the percentage change in half-relaxation time of SOL and EDL—the former changing by $22 \pm 4.4\%$, the latter by only $1 \pm 15.4\%$. The latter statistic for EDL, a mean change of only 1% in its half-relaxation time for the whole muscle twitch, should not obscure the fact that actual data ranged from a 10.8% reduction to a 13.7% increase in half-relaxation time.

(ii) *Observations from EDL and SOL motor unit twitches* A total of 23 motor units were studied, 10 in EDL (5 preparations) and 13 in SOL (10 experiments). Yet again the overall results showed that the drug reduced all three parameters (T_c, P_t and T_tR) of individual motor units both in SOL (see Figure 4) and EDL. However, two divergences from this trend did occur, both were in motor units of EDL: one showed an increased contraction time (12.8 ms as opposed to 10.5 ms (control value)); the other an increased half-relaxation time from 9.4 ms to 11.2 ms. These data were included in the statistical analyses, the summaries of which are shown for all units in Table 3. For both muscles there were significant differences in the means for each of the measurements made, before and after giving the drug.

Correlation coefficients between the absolute change brought about by the drug's effect and the corresponding control value were calculated. Using the collected data from all 23 motor units (EDL and SOL together), there were significant correlation coefficients for each of the sets of data associated with contraction time ($r = 0.735$, $P < 0.01$), twitch tension amplitude ($r = 0.971$, $P < 0.001$) and half-relaxation time ($r = 0.873$, $P < 0.001$). Comparable analysis of data from within the single group (EDL or SOL) showed that there were still significant relationships for those data from SOL (T_c, $r = 0.729$, $P < 0.05$; P_t, $r = 0.938$, $P < 0.001$; T_tR, $r = 0.935$, $P < 0.001$), but that only the data relating to twitch tensions of EDL motor units maintained a significant correlation coefficient ($r = 0.994$, $P < 0.001$).

(iii) *Comparisons of the reductions of twitch tension in whole muscles and motor units* Normalized percentage data were analysed, where permissible, using Student's *t* tests, so as to compare the mean changes effected at the level of whole muscle and motor unit. These tests clearly showed that motor units, both in SOL and EDL, had their twitch tension amplitudes depressed more significantly in percentage terms than those of their parent muscles. For example, EDL units were depressed some $80 \pm 9.0\%$ (mean $\pm$ s.d.) while the whole muscles were depressed by only $69\% \pm 5.0\%$. This finding, that the motor units were relatively more affected than the muscle overall, is in exact agreement with the data for fast and slow motor units in lateral segmental muscles. In this series of hind limb experiments, no significant difference was found between the mean percentage changes in whole muscle twitch tension amplitudes of EDL and SOL, nor was there a significant difference between the mean percentage changes of twitch tensions in EDL and SOL motor units ($79.8 \pm 9.0\%$, $n = 10$, as against $74.3 \pm 7.2\%$, $n = 13$).

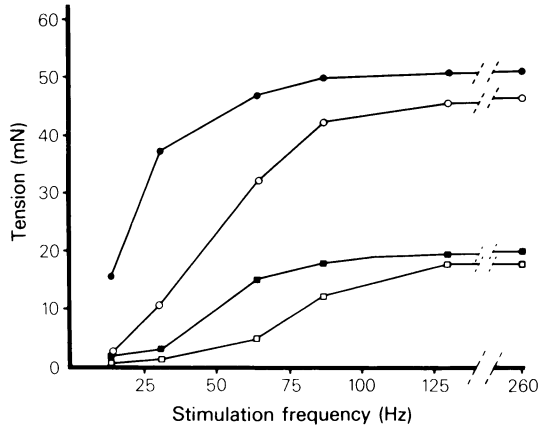

Figure 5 Illustrates in absolute values (mN) the average tetanic tensions generated by 6 SOL (circles) and 4 EDL (squares) motor units, indirectly stimulated for 300 ms through a series of frequencies from 14 to 258 Hz. Closed symbols are for data taken before, open symbols after, dantrolene sodium (5 mg kg^{-1}).

(iv) *Observations on tetanic tensions generated by fast (EDL) and slow (SOL) motor units in rat hind limb muscles* The maximum tetanic tension developed by the SOL units ($n = 6$), stimulated at 258 Hz, ranged from 34.4 to 66.4 mN with a mean of 51.0 ± 13.4 mN (s.d.); the data for the EDL units ($n = 4$) were 9.7 to 31.0 mN and 19.9 ± 12.0 mN respectively. These stastics and further data are illustrated in Figure 5, which shows that dantrolene sodium causes a loss of tetanic tension, the actual amplitude of which is dependent both on the applied stimulation frequency and the type of motor unit to which it is applied. Thus, for example, the mean percentage depressions for SOL units ranged from $87 \pm 3.8\%$ (mean $\pm$ s.d.) at 14 Hz to $9 \pm 3.1\%$ (mean $\pm$ s.d.) at 258 Hz, with the maximum loss occurring at 14 Hz. The mean percentage depressions for EDL were some 79, 64 and 61% at stimulation frequencies of 14, 31 and 64 Hz respectively, whilst at 87, 129 and 258 Hz they were 28%, 7% and 10%.

Discussion

Whole muscle experiments have shown beyond doubt the muscle relaxant properties of dantrolene sodium (Ellis *et al.*, 1973; Nott & Bowman, 1974; Ellis *et al.*, 1976; Bowman *et al.*, 1979; Mai & Pedersen, 1979; Leslie & Part, 1981a). However, as mentioned in the Introduction, there are limitations to the whole muscle approach. It is only by studying individual motor units that it is possible to be certain of investigating the action of the drug on muscle fibres of the same histochemical type (Stein & Padykula, 1962; Kugelberg, 1973; Schmalbruch & Kamieniecka, 1975; Pullen, 1977). In addition, normal muscle contraction is based upon the activity of individual motor units, with the central nervous system having control of muscles solely at the level of individual motor units.

Our present observations from whole muscle preparations, both in control experiments and in the drug's presence, generally agreed well with previously published data for EDL and SOL (Close, 1967; Leslie & Part, 1981a). The control data from segmental tail (ST) whole muscles also agreed with previous data (Steg, 1964; Andrew & Part, 1972) and our present experiments showed these heterogeneous muscles to behave similarly to EDL and SOL when dantrolene sodium was given. For example the ST whole muscle twitch was depressed on average by 65%, a value little different from the 66% for SOL or the 69% for EDL in this series of experiments.

From Table 3 it can be noted that there was no significant difference between these mean values of normalized twitch depressions from EDL and SOL. This is in contrast to previous results from fast and slow whole muscles (Bowman *et al.*, 1979; Leslie & Part, 1981a). The present results from these muscles were not obtained, however, as paired data from a single animal. Given this latter fact plus the scatter of data and the numbers in our samples, this lack of a significant difference between experimental means is not therefore surprising. Using our paired data from the ST motor units, however, we have shown that fast and slow motor units within the same muscle yield a significant difference between their normalized amplitudes of twitch depression. This shows without doubt that dantrolene sodium does depress the twitch amplitude of fast muscle fibres more than slowly contracting muscle fibres.

The significant difference in twitch depression between fast and slow muscle fibres may reflect the drug's effect on the amount of Ca^{2+} made available from the sarcoplasmic reticulum (SR), the response of the contractile components to the released Ca^{2+}, or both. While it is known that dantrolene sodium inhibits the release of Ca^{2+} from the SR (van Winkle, 1976), comparative data between fast and slow muscles on their rates and absolute amounts of Ca^{2+} release are lacking. However, it is known from experiments with skinned fibres that there are characteristic differences between the slow- and fast-twitch muscle fibres, such as a lower $[Ca^{2+}]$ threshold for activation, in slow fibres (Stephenson & Williams, 1981).

Dantrolene sodium-induced depression of twitch tension is greater for individual motor units than for the parent muscle (see Figure 3 and Table 3). It might be expected that the motor unit depressions would lie on either side of that of the parent muscle. The explanation of this finding probably lies in changes in the form of the active state and its interaction with the series elastic component. Further, the contribution of the parallel elastic component must become greater when considering the activity of a single motor unit within an otherwise inactive whole muscle. Our experiments were not in any way designed to investigate these points. Rapid stretch experiments (Hill, 1949) which in effect remove the distorting effect of the series elastic component might shed some light on this matter. In addition the timecourse of the twitches is worth considering. The form of the whole muscle twitch must depend upon temporal summation of motor unit twitches. The dantrolene sodium-induced changes in contraction time and half-relaxation time of motor units were more widely scattered about their means than were those of the whole muscles (see Table 3). Thus it is possible that the integration of these twitches with scattered time courses produces a lesser loss of tension of the whole muscle, as compared with its effects on the motor units.

It is generally accepted from whole muscle experiments that dantrolene sodium has a greater depressant action on the twitch contraction than on the fused tetanus (Ellis & Carpenter, 1972; Putney & Bianchi, 1974; Bowman *et al.*, 1979; Leslie & Part, 1981a). Our results from the motor unit studies are in agreement with this observation. Further, the present work shows that at the level of the motor unit, the depression of tetanic tension by dantrolene sodium is dependent on the frequency of the stimulus and on the type of muscle fibre stimulated and is greatest at those frequencies which produce contractions in the untreated muscles that are just beginning to fuse. The subtlety of action of dantrolene sodium at the unitary level, the interactions of units (with their orders of recruitment, their different responses to frequencies of stimulation as commanded by the central nervous system) make for a complex situation, which may be compounded by the pathophysiological states of spasticity against which the drug is used therapeutically in such diverse conditions as stroke, cerebral

palsy and multiple sclerosis. It is concluded that in using the drug as a muscle relaxant, the role played by the nervous system is paramount and that the activity of the nervous system in altered physiological states may work against the action of the drug and give it lesser therapeutic properties as a muscle relaxant than might seem the case from its pharmacology.

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