Br. J. Pharmac. (1987), **92**, 47–54

Effects of dantrolene on force development in slow- and fast-twitch muscle of euthyroid, hypothyroid, and hyperthyroid rats

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1 The effects of dantrolene on twitch and tetanic force development were determined in soleus and gastrocnemius muscle of euthyroid, hypothyroid, and hyperthyroid rats.

2 Maximum twitch force of the gastrocnemius muscle was significantly more depressed by dantrolene than that of the soleus muscle in euthyroid and hyperthyroid rats. In hypothyroid rats, the effect of dantrolene on maximum twitch force was similar in soleus and gastrocnemius muscle.

3 Maximum tetanic force in soleus and gastrocnemius muscle was less depressed by dantrolene than the twitch force in either thyroid state. The effect of dantrolene on maximum tetanic force increased in both muscles in the direction hypothyroid → euthyroid → hyperthyroid.

4 The results are discussed in terms of an effect of thyroid hormones on Ca^{2+}-cycling during force development, as a result of thyroid hormone-induced proliferation of the sarcoplasmic reticulum.

Introduction

Thyroid hormones markedly increase the rates of contraction and relaxation in skeletal muscle without inducing major changes in the maximum twitch or tetanic force developed (Grossie, 1978; Nicol & Bruce, 1981). This effect is probably due to the stimulating effect of thyroid hormones on the proliferation of the sarcoplasmic reticulum (SR) (Kim *et al.*, 1982; Simonides & van Hardeveld, 1985), leading to an increase in the pool of Ca^{2+} available for mobilization during activation as well as an increased capacity for resequestration of Ca^{2+} from the cytoplasm. The resulting acceleration of the rate of intracellular Ca^{2+} recycling could also, in part, account for the finding that thyroid hormones increase the energy required to produce a certain amount of force or work, thereby reducing the overall energetic efficiency (Everts *et al.*, 1981; Leijendekker *et al.*, 1983; Leijendekker, 1986).

Dantrolene sodium (dantrolene) has been shown to reduce force development in skeletal muscle (Nott & Bowman, 1974; Leslie & Part, 1981) as a result of direct inhibition of Ca^{2+}-release from the SR (van Winkle, 1976) or of interference with excitation-contraction coupling (Putney & Bianchi, 1974). It has also been demonstrated that dantrolene produces a more pronounced effect on maximum twitch and tetanic force in fast- compared to slow-twitch muscle (Nott & Bowman, 1974; Leslie & Part, 1981); this difference could be due to the larger surface of SR in fast-twitch muscle (Luff & Atwood, 1971).

In earlier studies (van Hardeveld & Kassenaar, 1980; van Hardeveld & Clausen, 1984) it was shown that metabolic activity and ^{45}Ca-efflux induced by K^+-depolarization enhanced with increasing thyroid hormone levels, and that these thyroid hormone-dependent effects could be largely or completely abolished by dantrolene. The results implied that at least part of the changes in metabolic activity and Ca^{2+}-kinetics in skeletal muscle induced by alteration of the thyroid state are dependent on a dantrolene-sensitive part of the Ca^{2+}-release mechanism, operating in the SR. To explore this idea further we investigated the effects of dantrolene on twitch and tetanic force development in hypothyroid, hyperthyroid and euthyroid rats. Since the stimulating effect of thyroid hormones on the proliferation of the SR depends on the fibre composition of skeletal muscle (Kim *et al.*, Simonides & van Hardeveld, 1985), a representative slow-twitch (soleus) and fast-twitch (gastrocnemius) muscle were used in this study.

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Methods

Animals

Male Wistar rats (12–14 weeks old) were divided into three groups: two experimental (hypothyroid and hyperthyroid) groups, and one control (euthyroid) group. One group was put on a low-iodine diet and given distilled water. After one week, the animals received an intraperitoneal injection of 750 μCi^{131}I. The animals were used 5 weeks after the injection. It has been shown that within 7 days after an injection of ^{131}I neither T_3 (3,3′,5-triiodothyronine) nor T_4 (3,3′,5,5′-tetraiodothyronine) is detectable in the plasma, and that the basal oxygen consumption of the hind-limb muscles is reduced by 50% 3 weeks after an injection of ^{131}I (van Hardeveld & Kassenaar, 1978). Another group of rats received a daily subcutaneous injection of 20 μg T_3 per 100 g body weight for 20 days. It has been shown that the oxygen consumption of the hind-limb muscles increases by 25% after 14 days T_3 treatment (van Hardeveld & Kassenaar, 1977). Control animals were weight-matched with the experimental animals. At the time of use the mean weight of the animals was: hypothyroid 246 ± 12 g ($n = 9$), euthyroid: 248 ± 10 g ($n = 9$), and hyperthyroid: 248 ± 20 g ($n = 9$). The weight of the soleus and gastrocnemius muscles did not differ between control and experimental animals.

Muscle preparation, stimulation and force development

The rats were anaesthetized by an intraperitoneal injection of pentobarbitone sodium (Nembutal, Abbott) (5 mg per 100 g body weight). The trachea was cannulated, but the rats were allowed to breathe spontaneously. A cannula was inserted in the jugular vein, to allow the injection of subsequent doses of dantrolene. The skin was removed from one leg, and the soleus or the gastrocnemius muscle was prepared for indirect stimulation through the sciatic nerve, as described by Everts *et al.* (1981). In experiments with the soleus muscle, the tendon connecting the gastrocnemius muscle to the calcaneus was sectioned, and this muscle was partly reflected to allow access to the soleus muscle. In experiments in which gastrocnemius was stimulated the soleus muscle was removed. Supramaximal pulses of 0.1 ms duration were obtained from a pulse generator (Grass S48). Isometric force was measured by a force displacement transducer (Grass FTO 3) and recorded by a chart recorder (Gould 2200) calibrated with standard weights. Force was expressed in Newtons (N). Relaxation times were read from fast-chart recordings (100 or 200 mm s^{-1}). Half-relaxation time ($t_{\frac{1}{2}}$R) was defined as the time (in ms) for decay of active force from the peak of the twitch or the plateau of the tetanus to one half of the active force at the optimal length. The rate of rise of tetanic force was calculated as dF_{max}/dt in N s^{-1} according to Drachmann & Johnston (1973). The experiments were carried out at 37°C in a thermostatic chamber, and the muscles were covered with saline-soaked gauze to prevent drying out. A needle thermistor was inserted to the nearest non-stimulated muscle to check the muscle temperature during the experiment.

Experimental series

After adjustment of the optimal length, peak forces of a single twitch (0.4 Hz) and a single tetanus (400 ms, 100 Hz for soleus and 130 Hz for gastrocnemius muscle) were measured to test force development of the preparation, and 5 min rest was given. Pilot experiments with the soleus muscle (stimulated at 10 Hz; 6 pulse trains of 1 s duration per min) and the gastrocnemius muscle (stimulated continuously at 0.4 Hz) showed that 0.5–1.0 ml of the solvent used to dissolve dantrolene (5% mannitol containing 0.3% ammonia) did not influence force. The effect of one dose of dantrolene (0.2 mg per 100 g body weight) on force had reached its maximum within 2 min, and the force then remained at this reduced level for at least 30 min. The effect of two doses of 0.1 mg per 100 g body weight, given with an interval of 15 min, was similar to that after one dose of 0.2 mg per 100 g body weight, implying that it is reasonable to calculate the effect of the cumulative dose of dantrolene from all doses given in a 30 min period.

The following stimulation protocols were followed: (a) Soleus muscle: the muscle was stimulated for 7 min at 10 Hz (6 pulse trains of 1 s duration per min) to obtain a constant level of force and relaxation rate. Thereafter, every 30 s a 500 ms pulse train (except for 0.4 Hz) was applied at the following frequencies: 0.4 Hz (3 s), 5 Hz, 10 Hz, 25 Hz, 50 Hz and 100 Hz. After 1 min rest, the series of stimuli was repeated. These measurements were done to obtain a complete force-frequency curve. After another minute of rest, the muscle was stimulated at 0.4 Hz, and the first dose of dantrolene (0.05 mg per 100 g body weight) was given. When the maximum effect on force at 0.4 Hz was reached, the force at the other frequencies (5, 10, 25, 50, and 100 Hz) was determined. Subsequent doses of dantrolene (0.05, 0.10, 0.20, 0.40 and 0.80 mg per 100 g body weight) were injected every 4 min, and after each dose the force-frequency relation was repeated. Dantrolene was dissolved to a final concentration of 1 to 2 mg ml^{-1}. (b) Gastrocnemius muscle; since the gastrocnemius muscle will show signs of fatigue during the determination of repeated force-frequency curves, the effect of successive doses dantrolene was only determined at 0.4 Hz (twitch), and 130 Hz (tetanus). In pilot experiments it was established that twitches and tetani (500 ms) could be measured every 2 min at least 10 times with only a slight loss of force. After the measurement of 3 control series (twitches and tetani) the gastrocnemius muscle was stimulated at 0.4 Hz, and the first dose of dantrolene (0.025 mg per 100 g body weight) was injected. When the effect had reached its maximum value, tetanic force was determined. After 1 min rest, the muscles were stimulated at 0.4 Hz and the second dose of dantrolene (0.025 mg per 100 g body weight) was given. Subsequent doses of dantrolene (0.05, 0.10, 0.20, 0.40, and 0.80 mg per 100 g body weight) were given every 3 min, and after each dose the force at the two frequencies was determined.

Chemicals

Dantrolene sodium (Dantrium; Norwich Benelux, Utrecht, The Netherlands) was dissolved by gentle heating in 5% mannitol containing 0.3% ammonia. T_3 (3,3'5-triiodo-L-thyronine) was obtained from Sigma Chemicals, St. Louis, MO, U.S.A.

Calculations and statistics

The effect of dantrolene on force development was taken as the quotient of the difference between the initial force minus force after dantrolene divided by the initial force. In gastrocnemius muscle the data were corrected for a slight decrease in force during the experiment. For each muscle (soleus or gastrocnemius) and condition (twitch or tetanus) tested, the maximum effect of dantrolene and the dose giving 50% of the maximum response (ID_{50}) were calculated by linear regression analysis of an Eadie-Hofstee plot (Zivin & Waud, 1982).

All data are expressed as means with their standard deviations (s.d.). Significance of differences between group means was calculated by use of Student's two-tailed t test for non-paired observations.

Results

Effects of thyroid status on contractile properties

The effects of thyroid status on the contractile properties of rat soleus and gastrocnemius muscle are summarized in Table 1. Whereas maximum twitch force did not change with hypo- or hyperthyroidism in soleus muscle, twitch force of gastrocnemius muscle showed a decrease in the hyperthyroid state ($P < 0.001$) and a slight but statistically insignificant increase in the hypothyroid state. Maximum tetanic force was not affected by the thyroid state in either muscle. Therefore, the twitch/tetanus ratio paralleled the changes in twitch force seen in hypo- or hyperthyroidism. In soleus muscle, the twitch/tetanus ratio was similar in hypo-, hyper- and euthyroid rats, whereas in gastrocnemius muscle a significant decrease was observed in the hyperthyroid rats ($P < 0.01$) and a significant increase was observed in the hypothyroid rats ($P < 0.01$). Half-relaxation time ($t_{\frac{1}{2}}R$) was shortened in hyperthyroidism and prolonged in hypothyroidism in both muscles, although these effects were much more pronounced in soleus compared to gastrocnemius muscle. This was particularly evident during a tetanus where the relative changes in $t_{\frac{1}{2}}R$ induced by hypo- or hyperthyroidism were nearly twice as great in the soleus compared to the gastrocnemius muscle.

Table 1 Effects of thyroid status on the contractile properties of rat soleus and gastrocnemius muscle

	Twitch force (N)	*Tetanic force* (N)	*Twitch/tetanus ratio*	*Twitch $t_{\frac{1}{2}}R$* (ms)	*Tetanic $t_{\frac{1}{2}}R$* (ms)
Soleus muscle					
Hypothyroid	0.46 ± 0.06	2.26 ± 0.11	0.21 ± 0.02	102 ± 9**	161 ± 6**
Euthyroid	0.45 ± 0.06	2.23 ± 0.18	0.20 ± 0.02	46 ± 7	72 ± 7
Hyperthyroid	0.48 ± 0.09	2.45 ± 0.21	0.20 ± 0.02	24 ± 4**	36 ± 3**
Gastrocnemius muscle					
Hypothyroid	3.45 ± 0.34	12.9 ± 0.5	0.27 ± 0.03*	14 ± 1*	44 ± 6**
Euthyroid	3.08 ± 0.10	13.9 ± 1.1	0.22 ± 0.02	10 ± 2	26 ± 2
Hyperthyroid	2.48 ± 0.19**	13.9 ± 1.5	0.18 ± 0.02*	10 ± 2	19 ± 2*

The data represent means ± s.d. of 4–5 experiments in each group with soleus or gastrocnemius muscle. Hypothyroidism was induced by an intraperitoneal injection of ^{131}I (0.75 mCi) and the rats were used 5 weeks later. Hyperthyroidism was induced by daily injections of T_3 (20 μg per 100 g body weight) for 20 days. The soleus and gastrocnemius muscle were stimulated indirectly through the sciatic nerve. Twitch force was measured at a stimulation freqency of 0.4 Hz for both muscles, tetanic force was measured at 100 Hz for soleus and 130 Hz for gastrocnemius muscle. *$P < 0.01$ and **$P < 0.001$ for hypo- or hyperthyroid compared to euthyroid.

The effect of thyroid status on the force-frequency relationship was determined in soleus muscle (Figure 1). As maximum tetanic force did not vary with thyroid status, the peak forces measured at frequencies between 0.4 Hz and 100 Hz were expressed relative to maximum tetanic force. Hypothyroidism induced a shift to the left in the force-frequency curve as evidenced by the significantly higher force developed at 10 Hz ($P < 0.001$) and 25 Hz ($P < 0.05$) compared with euthyroid rats. Conversely, hyperthyroidism induced a shift to the right in the force-frequency curve which was indicated by the significantly lower force developed at 25 Hz ($P < 0.02$) and 50 Hz ($P < 0.05$) compared with euthyroid controls.

Effects of dantrolene on maximum twitch force

The effects of dantrolene on twitch force of soleus and gastrocnemius muscles in the different thyroid states are presented in Figure 2. The maximum effects of dantrolene, as calculated by linear regression analysis from Eadie-Hofstee plots and the doses giving 50% of the maximum effect (ID_{50}) are given in Table 2.

In soleus muscle, there was no significant difference between hypo-, hyper- and euthyroid rats in the response to dantrolene at any dose of the drug tested (Figure 2a). The maximum effect of dantrolene amounted to 74%, 75% and 66% for hypo-, eu-, and hyperthyroid rats, respectively (Table 2). The reduction of twitch force was in each group accompanied by a significant reduction of $t_{\frac{1}{2}}R$ which was 33 ± 7 ms in hypothyroid rats (n = 5), 20 ± 4 ms in euthyroid rats (n = 3) and 4 ± 1 ms in hyperthyroid rats (n = 4) (hypothyroid and hyperthyroid compared to euthyroid, $P < 0.05$).

The reduction of twitch force in gastrocnemius muscle varied between 76% and 90% for hypothyroid and hyperthyroid rats, respectively (Figure 2b, Table 2) ($P < 0.05$). The inhibition of twitch force in hypothyroid rats was also significantly less than in euth-

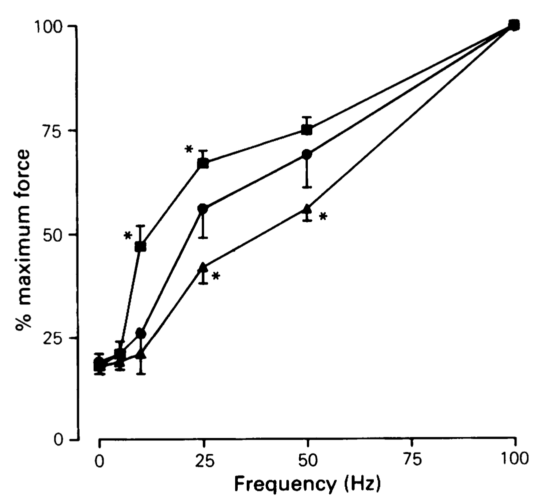

Figure 1 Effects of thyroid status, (■) hypothyroid, (●) euthyroid and (▲) hyperthyroid, on force-frequency relationship in rat soleus muscle. Hypo- and hyperthyroidism were induced as described in the legend to Table 1. The data represent means of 4–5 experiments in each group and vertical lines show s.d. *$P < 0.05$ hypo- or hyperthyroid compared with euthyroid.

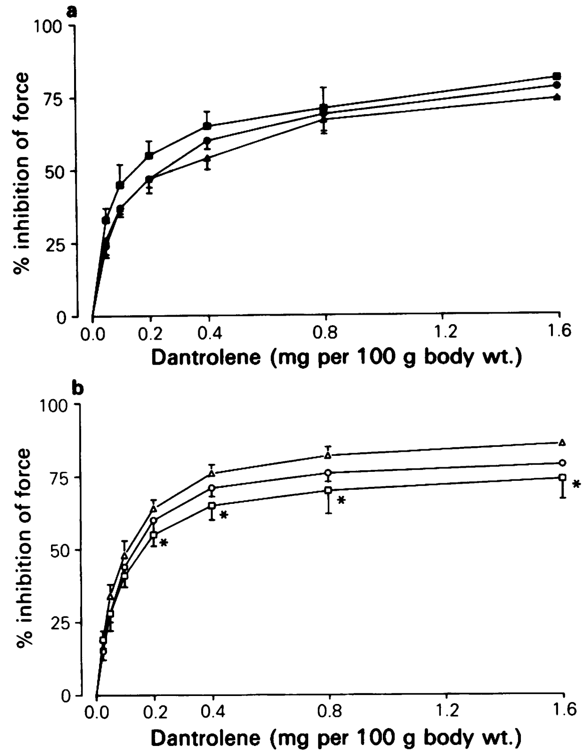

Figure 2 Effects of dantrolene on maximum twitch force in (a) soleus and (b) gastrocnemius muscle in (■, □) hypothyroid, (●, ○) euthyroid and (▲, △) hyperthyroid rats. Hypo- and hyperthyroidism were induced as described in the legend to Table 1. The results represent means of 4–5 experiments in each group and vertical lines show s.d. Points without s.d. were determined in one or two animals. The doses of dantrolene were injected through a canula in the jugular vein. The total amount of dantrolene injected is presented cumulatively on the horizontal axis. Both muscles were stimulated at a frequency of 0.4 Hz. *$P < 0.05$, hypothyroid compared with euthyroid.

Table 2 Maximum inhibition of twitch and tetanic force by dantrolene and ID_{50} values for soleus and gastrocnemius muscle of hypo-, hyper- and euthyroid rats

	Soleus muscle		*Gastrocnemius muscle*	
	Maximum inhibition (%)	*ID_{50}* (mg per 100 g body wt)	*Maximum inhibition* (%)	*ID_{50}* (mg per 100 g body wt)
Twitch				
Hypothyroid	74 ± 9	0.07 ± 0.01*	76 ± 9*	0.08 ± 0.02
Euthyroid	75 ± 7	0.10 ± 0.02	88 ± 1†	0.10 ± 0.02
Hyperthyroid	66 ± 7	0.07 ± 0.02	90 ± 3†	0.09 ± 0.02
Tetanus				
Hypothyroid	33 ± 7	0.06 ± 0.02	28 ± 3*	0.13 ± 0.04†
Euthyroid	33 ± 2	0.08 ± 0.02	39 ± 5	0.14 ± 0.02†
Hyperthyroid	53 ± 6*	0.08 ± 0.02	54 ± 6*	0.18 ± 0.07†

The data represent means ± s.d. of 4–5 experiments in each group with soleus or with gastrocnemius muscle. Data were calculated by linear regression analysis of an Eadie-Hofstee plot based on the values presented in Figures 2 and 3.
*$P < 0.05$ for hypo- or hyperthyroid compared to euthyroid.
†$P < 0.02$ for gastrocnemius muscle compared to soleus muscle.

yroid rats (88%) ($P < 0.05$). In view of the short $t_{\frac{1}{2}}R$ in gastrocnemius muscle and the large reduction of twitch force, $t_{\frac{1}{2}}R$ after dantrolene could not be determined in this muscle.

Comparison of soleus with gastrocnemius muscle (Table 2) showed that maximum twitch force of the gastrocnemius muscle was more depressed by dantrolene compared to that of the soleus in euthyroid rats (88 ± 1% compared to 75 ± 7%, $P < 0.02$). This difference between the two muscles was also found in the hyperthyroid state (90 ± 3% compared to 67 ± 7%, $P < 0.02$) but it was absent in the hypothyroid state (76 ± 9% compared to 74 ± 9%). ID_{50} values did not differ between the soleus and gastrocnemius muscle in either thyroid state.

Effects of dantrolene on maximum tetanic force

The mean curves for the depression of tetanic force by dantrolene are presented in Figure 3a (soleus) and b (gastrocnemius). The calculated values for the maximum inhibition of tetanic force by dantrolene and ID_{50} values are given in Table 2. At the highest dose of dantrolene tested, tetanic force in soleus muscle was inhibited to the same extent in euthyroid (33 ± 2%) and hypothyroid (33 ± 7%) rats. The reduction of force in hyperthyroid rats (53 ± 6%) was, however, significantly larger than in euthyroid rats ($P < 0.05$).

The effect of dantrolene on force development of the soleus muscle was also tested at intermediate frequencies (5–50 Hz). This showed that the effect of dantrolene was dependent on the frequency of stimulation in either thyroid state (Figure 4). The effect of dantrolene decreased by 50% at a frequency of 30 Hz in hypothyroid rats, at 45 Hz in euthyroid rats, and at 65 Hz in hyperthyroid rats.

The maximum inhibition of tetanic force in gastrocnemius muscle of euthyroid rats (39 ± 5%) was slightly but not significantly higher compared with that in soleus muscle (33 ± 2%). The reduction of tetanic force in gastrocnemius muscle of hypo-, and hyperthyroid rats was significantly different from that in the euthyroid state, i.e. 28 ± 3% in hypothyroid rats ($P < 0.05$) and 54 ± 6% in hyperthyroid rats ($P < 0.05$). Neither in hypothyroid nor in hyperthyroid rats did the depression of tetanic force by dantrolene show a significant difference between soleus and gastrocnemius muscle. In contrast to what was found during a twitch, ID_{50} values during a tetanus differed significantly between soleus and gastrocnemius muscle (Table 2). The ID_{50} was 75–125% higher in gastrocnemius than in soleus muscle for euthyroid, hypothyroid, and hyperthyroid rats.

The effect of dantrolene on maximum tetanic force was accompanied by a large reduction of the rate of tetanic force development. Table 3 shows that the rate of rise in tetanic force before the administration of dantrolene was significantly lower in soleus muscle of hypothyroid rats ($P < 0.05$) and significantly higher in soleus muscle of hyperthyroid rats ($P < 0.05$) as compared to euthyroid controls. In gastrocnemius muscles the rate of rise in tetanic force was unaffected by the thyroid status. Dantrolene reduced the rate of tetanic force development by 40% in soleus and by 67% in gastrocnemius muscle of euthyroid rats. The rate of force development in soleus muscle was

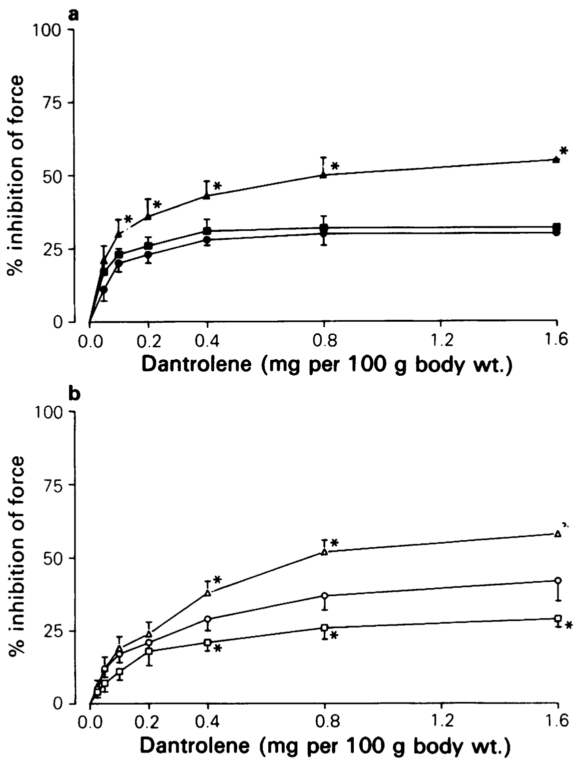

Figure 3 Effects of dantrolene on maximum tetanic force in (a) soleus and (b) gastrocnemius muscle in (■, □) hypothyroid, (●, ○) euthyroid and (▲, △) hyperthyroid rats. The results represent mean of 4–5 experiments in each group and vertical lines show s.d. Points without s.d. were determined in one or two animals. The soleus muscle was stimulated at a frequency of 100 Hz and the gastrocnemius muscle at 130 Hz. $^*P<0.05$, hypo- or hyperthyroid compared with euthyroid.

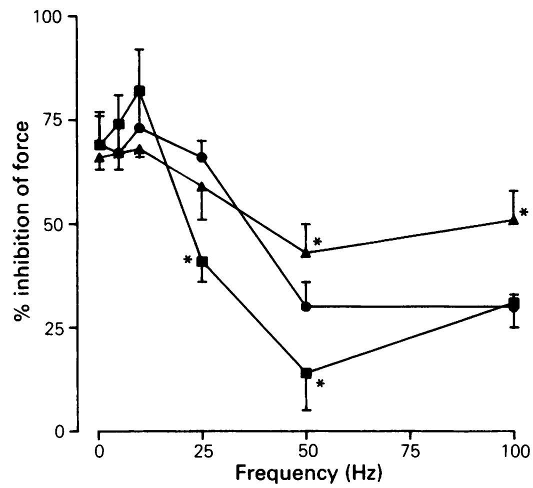

Figure 4 Effects of dantrolene on force at various frequencies of stimulation in soleus muscle in (■) hypothyroid, (●) euthyroid and (▲) hyperthyroid rats. Hypo- and hyperthyroidism were induced as described in legend to Table 1. The results represent means of 4–5 experiments in each group and vertical lines show s.d. The total dose of dantrolene was 0.8 mg per 100 g body weight. $^*P<0.05$ hypo- or hyperthyroid compared with euthyroid.

progressively inhibited by dantrolene in the direction hypothyroid → hyperthyroid and this was similar in gastrocnemius muscle, although less pronounced.

In contrast to the large effect of dantrolene on the rate of rise of tetanic force, it did not affect the rate of relaxation during a tetanus in soleus or gastrocnemius muscle in either thyroid state, indicating that dantrolene does not alter the rate of Ca^{2+} uptake into the SR.

Discussion

The present results confirm and extend the observations that thyroid hormones induce large variations in the rate of contraction and relaxation in skeletal muscle without major changes in the maximum force development (Grossie, 1978; Fitts *et al.*, 1980; 1984; Nicol & Bruce, 1981). The changes in half-relaxation time induced by hypo-, and hyperthyroidism were of the same order of magnitude as the changes in the maximum rate of Ca^{2+} uptake into the SR (Kim *et al.*, 1982, Simonides & van Hardeveld, 1985). In addition, these authors also demonstrated that the effects of thyroid hormones on the rate and capacity for Ca^{2+} accumulation were more pronounced in slow- compared to fast-twitch muscle.

In agreement with previous results (Nott & Bowman, 1974; Leslie & Part, 1981) it was observed that twitch contractions of the soleus muscle were less affected by dantrolene than those of the gastrocnemius muscle in euthyroid rats. The difference was, however, not large and the effects of hypo- and hyperthyroidism were variable.

During high frequency stimulation, the maximum effect of dantrolene on force development did not differ between soleus and gastrocnemius muscle in either thyroid state. It was observed in both muscles that the maximum inhibitory effect of dantrolene on tetanic force increased in the direction hypothyroid →

Table 3 Effects of dantrolene on the rate of tetanic force development in rat soleus and gastrocnemius muscle

	Soleus muscle			*Gastrocnemius muscle*		
	Before dantrolene	*After dantrolene*	Δ	*Before dantrolene*	*After dantrolene*	Δ
Hypothyroid	26 ± 3*	16 ± 2	11 ± 2*	305 ± 16	136 ± 7*	169 ± 17*
Euthyroid	33 ± 2	17 ± 1	16 ± 2	297 ± 16	95 ± 17	198 ± 17
Hyperthyroid	40 ± 5*	17 ± 3	24 ± 5*	314 ± 29	82 ± 16	235 ± 13*

The rate of tetanic force development is expressed in Ns^{-1}. The results were obtained in the experiments described in Figure 3 and are given as means ± s.d. of 4–5 experiments in each group. The values in columns 'After dantrolene' are those measured after the last but one dose (0.4 mg per 100 g body weight). *$P<0.05$ for hypo- or hyperthyroid compared to euthyroid.

euthyroid → hyperthyroid. Similarly, dantrolene induced a progressive reduction in the rate of rise of tetanic force in the direction hypothyroid → hyperthyroid, which provides additional evidence for the idea that the inhibitory effect of dantrolene on the net Ca^{2+} release from the SR enhances with increasing thyroid hormone levels.

From the force-frequency curves presented in Figure 1 it can be deduced that force and probably the cytosolic calcium concentration rise faster in the direction hyperthyroid → hypothyroid with increasing frequencies of stimulation. This could be ascribed to an increase in the Ca^{2+} uptake activity as indicated by the decrease in half-relaxation time in the reverse direction i.e. hypothyroid → hyperthyroid. This implies that during repetitive stimulation, the hyperthyroid rats resequester a larger fraction of the amount of calcium released into the cytosol than hypothyroid rats. From this it follows that during high frequency stimulation the fraction of Ca^{2+} released into the cytosol which participates in the cycling between the SR and cytosol increases in the direction hypothyroid → hyperthyroid. Since dantrolene reduces the amount of Ca^{2+} released into the cytosol (Putney & Bianchi, 1974; van Winkle, 1976) and does not interfere with Ca^{2+} uptake (Desmedt & Hainaut, 1979) an increasing effect of the drug should be expected in the same direction.

Evidence for a reduction of Ca^{2+} cycling and, as a consequence, ATP consumption in hypothyroidism was obtained in experiments where the tension-independent heat was measured in mouse soleus and extensor digitorum longus (EDL) muscles during isometric tetani (Leijendekker, 1986). After thyroid hormone depletion, the tension-independent heat was reduced by 50 and 30% in soleus and EDL muscle, respectively (Leijendekker, 1986).

Nevertheless, the argument developed above, probably gives an incomplete explanation for our observations, since dantrolene had more or less similar effects on force development in soleus and gastrocnemius muscle during high-frequency stimulation, whereas these muscles differ considerably with respect to SR volume and Ca^{2+} uptake activity (Luff & Atwood, 1971; Kim *et al.*, 1982; Simonides & van Hardeveld, 1985). However, the ID_{50} value was twice as high in gastrocnemius compared with soleus muscle. This could point to a higher sensitivity of the soleus muscle to dantrolene, but it is also possible that the soleus muscle is more accessible to dantrolene than the gastrocnemius muscle because of its more extensive vascularization. It has been shown that dantrolene exerts its effect on excitation-contraction coupling and that its site of action resides in the terminal cisternae, i.e. that part of the SR which forms tight junctions with the T-tubules (Putney & Bianchi, 1974; Ikemoto *et al.*, 1986). Recently, it has been demonstrated that the difference in terminal cisternae volume between soleus and EDL muscles from rats is not accompanied by a similar difference in T-tubule surface area or T-tubule volume (Dulhunty *et al.*, 1986). Moreover, it was shown in the same study, that the effects of thyroid hormone treatment on the terminal cisternae volume were quantitatively similar to those found on whole SR, whereas the T-tubule surface area (absolute or relative) increased by 100% in both soleus and EDL muscle, resulting in a disproportionately large amount of T-tubule membranes (Dulhunty *et al.*, 1986). To what extent these findings are relevant to the differential effects of dantrolene on maximum force development in rats with varying thyroid hormone levels, remains to be established.

The authors wish to thank Marian Zuidwijk for skilled technical assistance and Lis Skjøt for expert secretarial assistance. This study was supported in part by the Netherlands Organization for the Advancement of Pure Research.

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(Received November 18, 1986.
Revised April 7, 1987.
Accepted April 29, 1987.