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A COMPARISON OF THE EFFECTS OF SODIUM THIOCYANATE AND DANTROLENE SODIUM ON A MAMMALIAN ISOLATED SKELETAL MUSCLE

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1 A combination of electrical and pharmacological stimulation has been used to compare the effects of sodium thiocyanate and dantrolene sodium on the excitation-contraction coupling (ECC) mechanism of the mouse soleus muscle.

2 Thiocyanate prolonged the 'active state' after electrical stimulation and increased the response to 8 mM caffeine and 80 mM KCl. Dantrolene had an opposite effect to thiocyanate on all the indices studied.

3 It is concluded that the mechanism of action of dantrolene is by inhibition of the release of the calcium ions involved in the ECC mechanism, probably at the level of the transverse tubules.

Introduction

Dantrolene sodium is a recently discovered skeletal muscle relaxant (Snyder, 1967). It has already been found useful in the treatment of skeletal muscle spasticity (Dykes, 1975), and is thought to have a unique mechanism of action by interfering with the excitation-contraction coupling (ECC) mechanism.

There is considerable interest in elucidating in detail its exact mode of action. Firstly, it would seem to have a precise action on a single step of the ECC process, probably by interfering with the calcium release mechanism which couples the sarcolemmal action potential to the contractile process (Ellis & Carpenter, 1972; Hainaut & Desmedt, 1974; Putney & Bianchi, 1974). It therefore provides a potentially useful tool for studying this mechanism, about which at present very little is known. Secondly, it has been found to be successful in the treatment of the syndrome of malignant hyperpyrexia occurring under general anaesthesia (Harrison, 1975). This syndrome has been shown to be caused by an abnormal sensitivity of skeletal muscle to various drugs, but particularly general anaesthetic agents (Moulds & Denborough, 1974). Exposure of isolated malignant hyperpyrexia muscle from either susceptible humans (Moulds & Denborough, 1974) or pigs (Anderson & Jones, 1976) to halothane produces an abnormal contracture, probably by an excessive release of the calcium ions involved in the ECC mechanism and dantrolene has been shown to prevent this abnormal contracture (Anderson & Jones, 1976). Therefore a more detailed knowledge of its mechanisms of action would also almost certainly help to elucidate the underlying defect in muscle predisposing to malignant hyperpyrexia.

Many agents are known to influence the ECC mechanism in skeletal muscle, but probably most is known about the twitch potentiating effect of the lyotropic anions (Sandow, 1965). Like dantrolene, they probably also act on a single step of the ECC process and prolong the 'active state' during which calcium ions are released. Therefore, in order to learn more about the mechanism of action of dantrolene, a detailed comparison has been made between its effects on mammalian isolated skeletal muscle and those of the most potent of the lyotropic anions, the thiocyanate ion.

Methods

The soleus muscles from young adult Balb/c mice of either sex were used. Each tendon was tied with black linen thread and attached to two small rings and the muscle suspended vertically in a 20 ml tissue bath. It was maintained at 37°C by a Churchill pump and the bathing fluid had the following composition (mM): Na^+ 137.4, K^+ 5.4, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 131.6, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, HCO_3^- 15.0 and glucose 11.5. O_2 containing 5% CO_2 was bubbled through the solution which then had a pH between 7.2 and 7.4.

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Isometric tension was measured with a Statham force transducer and recorded on a Devices high speed recorder. The resting tension was adjusted to give the maximum twitch and was usually 2–3 grams. Curare, 25 μg/ml, was sometimes added to the bath solution, but it had no effect on the responses being tested so was usually omitted. Electrical stimulation was performed through two thin platinum foil electrodes placed in contact with each end of the muscle preparation, using a Grass S44 stimulator set at supramaximal voltage (usually 30 V) and with pulses of 0.5 ms duration.

Dantrolene sodium was suspended as a slurry in 0.1 N NaOH in a concentration of 1 mg/ml and the appropriate amount added to the bath solution to give a final concentration of 3 μg/ml (approx. 10.5 μmol/litre). Caffeine (Sigma) was dissolved in the bath solution (not containing calcium) to make a concentration of 100 mM and the appropriate volume added to the bath to give a final concentration of 8 mM. Potassium chloride (KCl) was made up as a stock solution of 2 M and the appropriate volume added to the bath to give a final concentration of 80 mM.

Results

In order to study the ECC mechanism as closely as possible, a combination of electrical and pharmacological tests was used. The following measurements were therefore made on normal solei, and on solei exposed for at least 10 min to either dantrolene 3 μg/ml or NaCNS 20 mM: twitch height, tetanus height, the half-life of the exponential phase of the relaxation from a tetanus, the frequencies required to produce a tetanus, the height and time to peak of the contracture produced by 80 mM KCl, and the height of the contracture produced after 10 min by 8 mM caffeine.

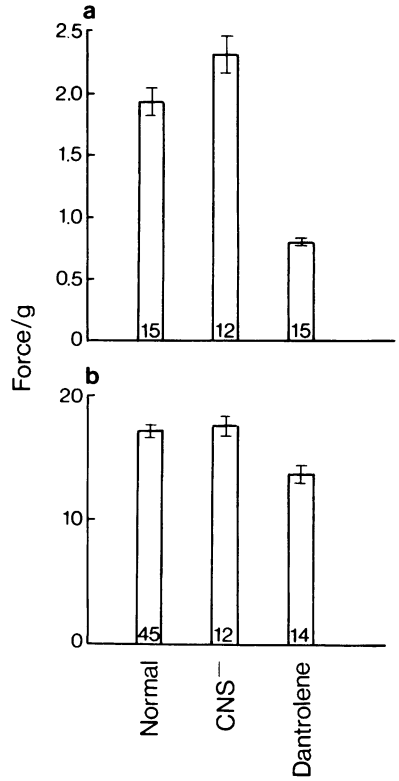

Figure 1 The effect of dantrolene and thiocyanate (CNS^-) on (a) the twitch and (b) maximum tetanus of the mouse isolated soleus muscle. The numbers of solei tested in each case are shown at the base of the column, and error bars are s.e. mean.

Table 1 Twitch height, maximum tetanus height and relaxation half-life in normal solei, solei exposed to NaCNS (20 mM) and solei exposed to dantrolene sodium (3 μg/ml)

	Normal solei	*Solei in NaCNS*	*Solei in dantrolene*
Twitch height (g)	1.93 ± 0.11 (15)	2.32 ± 0.15 $P < 0.05$ (12)	0.80 ± 0.03 $P < 0.001$ (15)
Tetanus height (g)	16.11 ± 0.44 (45)	16.49 ± 0.79 NS (12)	13.61 ± 0.71 $P < 0.005$ (14)
Tetanus relaxation $T\frac{1}{2}$ (ms)	16.72 ± 0.44 (45)	16.56 ± 0.73 NS (14)	16.66 ± 0.75 NS (12)

Results are mean ± s.e. in each case. Number of solei tested in each case are given in parentheses. *P* values are result of statistical analysis compared with normal solei (unpaired Student's *t* test) in each case.

(a) *Electrical stimulation*

A comparison between the effects of dantrolene and NaCNS on the height of the twitch and a tetanus is given in Table 1 and shown diagrammatically in Figure 1. It can be seen that, as has been previously reported for amphibian muscle (Ellis & Carpenter, 1972), in this mammalian muscle preparation dantrolene had a greater inhibitory effect on the twitch than on the tetanus, and also thiocyanate enhanced the twitch whilst having no effect on the tetanus. Table 1 also shows that despite their marked and opposite effects on the twitch height, neither of these agents had a significant effect on the time course of the relaxation from a tetanus.

It was found that when the force developed (as a percentage of the maximum) by the muscle was plotted against the logarithm of the frequency of electrical stimulation, then a sigmoid curve typical of a pharmacological dose-response curve was obtained. As shown in Figure 2, this curve was shifted markedly to the right (i.e. much higher frequencies of stimulation were required for tetanic tension to develop) by dantrolene, and was shifted to the left (i.e. tetanic tension developed at lower frequencies) by NaCNS.

(b) *Pharmacological stimulation*

The addition of either KCl (80 mM) or caffeine (8 mM) to the bath solution produced in normal solei typical contractures, illustrations of which are shown in Figure 3. The comparison between the effects of dantrolene and of NaCNS on these contractures is given in Table 2, and typical examples are illustrated in Figure 3. It can be seen that dantrolene inhibited both the contracture produced by KCl (80 mM) and that produced by caffeine (8 mM), while conversely NaCNS potentiated both of the contractures.

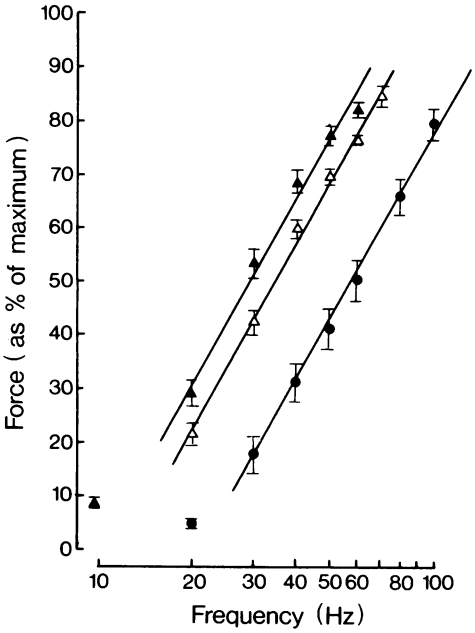

Figure 2 Plots of the force developed by the muscle (as a percentage of the maximum) against the logarithm of the frequency of electrical stimulation. Each point is the mean of measurements made on twelve normal solei (△), twelve solei in NaCNS 20 mM (▲) and five solei in dantrolene sodium 3 μg/ml (●). Vertical lines show s.e. mean.

Discussion

The results described in this paper show that dantrolene has an effect on a mammalian isolated muscle preparation similar to that which it has been

Table 2 Contractures produced by KCl (80 mM) or caffeine (8 mM) in normal solei, solei exposed to NaCNS (20 mM), and solei exposed to dantrolene sodium (3 μg/ml)

		Normal solei	*Solei in NaCNS*	*Solei in dantrolene*
KCl 80 mM	Height of contracture (g)	3.75 ±0.13 (10)	9.21±0.34 $P<0.001$ (5)	2.00±0.24 $P<0.001$ (4)
	Time to peak of contracture (s)	24.6±2.5 (10)	67.0±4.26 $P<0.001$ (4)	42.0±2.55 $P<0.001$ (5)
Caffeine 8 mM	Height of contracture (g)	2.08±0.45 (10)	3.40±0.59 $P<0.01$ (5)	0.81±0.13 $P<0.05$ (5)

Results are mean ± s.e. mean in each case. Number of solei tested in each case are given in parentheses. P values are result of statistical analysis compared with normal solei (unpaired Student's t test) in each case.

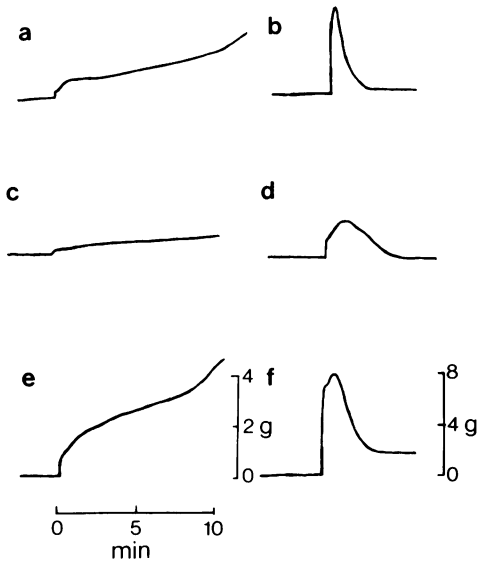

Figure 3 Examples of contractures obtained by the addition of caffeine 8 mM (left hand column) or KCl 80 mM (right hand column) to normal solei (a, b), solei in dantrolene sodium 3 μg/ml (c, d) and solei in NaCNS 20 mM (e, f). The horizontal time scale for all contractures is as shown for (e), but note that the vertical force scale (in g) is as shown for (e) in all the contractures except (f), where the scale is halved.

reported to exert on frog isolated muscle – i.e. it causes a marked reduction in twitch height but a much smaller reduction in the height of a tetanus. More importantly however, dantrolene has been shown to have directly opposite effects from another agent which influences the ECC mechanism, the thiocyanate ion.

The lyotropic anions are generally considered to have a specific action on the ECC mechanism by prolonging the 'active state' produced by the sarcolemmal action potential during which calcium ions are released into the myoplasm. This action is almost certainly exerted superficially on the sarcolemma, and it has been suggested that it is mediated by a change in the electrical field surrounding the T-tubules by virtue of the greater adsorption of the lyotropic anions to the sarcolemmal membrane (Bianchi, 1968; Chapman, 1969).

The effects of the thiocyanate ion described in this paper are compatible with this mechanism, and they will be considered in turn.

An agent which increased the amount of calcium released by each action potential would be expected to enhance a single twitch, but not a maximal tetanus, the height of which is probably determined by the limits of the contractile elements themselves rather than the availability of calcium ions. The relaxation from a tetanus is also probably determined either by the rate of re-uptake of the activating calcium ions, or more likely by the actual rate of disassociation of the actomyosin crossbridges. Whichever of these mechanisms is responsible, neither would be expected to be influenced by an agent which merely increased the amount of calcium released by a single action potential.

The frequency of stimulation required to produce a tetanus is more complex, potentially being a function of at least three and probably more different processes, these being the amount of calcium released by the repetitive stimuli, the rate of re-uptake of that calcium by the sarcoplasmic reticulum, and the rate of disassociation of the actomyosin crossbridges. However, if an agent increased the amount of calcium released per impulse without affecting any of the other processes, then the muscle would develop tension at a lower frequency of stimulation and that is what was found. Furthermore, the 'frequency-response' curve in the presence of thiocyanate was parallel to the normal curve also suggesting that the presence of the thiocyanate caused a constant amount of extra calcium to be released by each impulse.

The mechanism of the contracture produced by 80 mM KCl is by an artificial and persistent depolarization of the sarcolemmal membrane, thus simulating the transient depolarization produced by an action potential. Any agent which changed the electrical properties of the membrane such that more calcium ions were released by depolarization would therefore also be expected to cause potentiation of the contracture produced by 80 mM KCl.

However, the contracture produced by caffeine (8 mM), is more complex than that produced by KCl (80 mM). It is independent of the membrane polarity and has been shown to be due in part to a release of the calcium ions involved in ECC coupling and in part to an inhibition of their re-uptake by the sarcoplasmic reticulum (Weber & Herz, 1968). The major source of the calcium ions causing a caffeine contracture is generally considered to be from the sarcoplasmic reticulum and not the sarcolemma. But the lyotropic anions have also been found to enhance the caffeine contracture in frog muscle (Foulks, Perry & Sanders, 1971), and the enhancement of the caffeine contracture produced by thiocyanate in this study was relatively modest. It may therefore well have been due to an enhancement of the sarcolemmal contribution to the calcium release.

The above discussion shows that the effects of sodium thiocyanate are compatible with an enhancement of the release of calcium ions early in the ECC process, and probably at the transverse tubular level. A similar argument suggests that the effects of dantrolene, being the opposite of those of thiocyanate, are compatible with an inhibition of the release of the calcium ions involved in the ECC process. Some

direct evidence to support this conclusion is provided by the work of Putney & Bianchi (1974) and Hainaut & Desmedt (1974) who found that dantrolene had no effect on the resting influx of Ca into frog muscle, but decreased the influx associated with a twitch.

It is therefore concluded that in a mammalian muscle dantrolene acts on the ECC process by reducing the amount of calcium released by the sarcolemmal action potential. Moreover the inhibition probably occurs early in the coupling process, most probably at the site of the transverse tubules.

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