Dantrolene does not block calcium pulse-induced calcium release from a putative calcium channel in sarcoplasmic reticulum from malignant hyperthermia and normal pig muscle

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Calcium pulse additions to isolated SR membranes can cause a reversible efflux of calcium. The threshold level of calcium loading at which calcium efflux occurs is lower for SR membranes isolated from malignant hyperthermia susceptible (MHS) swine. Dantrolene, a unique muscle relaxant, had no effect on threshold calcium load, amounts and rates of calcium release from SR isolated from control and MHS skeletal muscle. It is concluded that the putative calcium channel through which this calcium pulse-induced calcium release mechanism occurs is not affected by dantrolene under these experimental conditions.

Sarcoplasmic reticulum *Ca release* *Caffeine* *Malignant hyperthermia* *Halothane* *Dantrolene*

1. INTRODUCTION

A recent report [1] provided indirect evidence to conclude that dantrolene inhibited halothane-induced calcium release from SR isolated from skeletal muscle of pigs genetically predisposed to malignant hyperthermia. We have also examined the calcium-induced calcium release from a putative calcium channel in a heavy SR fraction from rabbit muscle [2]. Further, we have previously reported an abnormality of calcium efflux from this calcium channel in the SR from skeletal muscle of genetically predisposed malignant hyperthermia susceptible (MHS) pigs [3]. The basis for this abnormality lies primarily in the lower calcium threshold for calcium pulse-induced calcium release [3]. Here we have measured the effect of dantrolene on this mechanism in SR from control and MHS porcine skeletal muscle.

2. MATERIALS AND METHODS

Three purebred MH-susceptible Poland China pigs and 3 control pigs were used to obtain gracilis muscle biopsies. The control pigs were 2 purebred Poland China littermates (unrelated to MH pigs) and one cross-bred pig. Each animal's susceptibility to MH was assessed by in vitro muscle contracture testing [4] and by a thorough anesthetic challenge [5]. The MH-susceptible animals were phenotype H [5] and the controls were phenotype N (negatives). Gracilis muscle was obtained while each animal was anesthetized with thiopental and spontaneously breathing 100% oxygen through an orotracheal tube. Muscle fascicles were excised and contracture tested as in [4]. The remainder of the gracilis muscle, ~30 g, was surgically removed in bulk, placed on an ice block and minced for homogenization and centrifugation as in [3]. Only a heavy SR fraction (8–12000 ± *g*) was used in this study. Calcium release from the SR was induced by the calcium pulse technique in [2,3]. The threshold for calcium release is defined as the cumulative calcium pulses (2 μM each) at which calcium efflux occurred. In addition to threshold, rate and amount of calcium released were measured. Calcium uptake and release by the SR was measured in a dual wavelength (650–700

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nanometers) spectrophotometer utilizing arsenazo III as a calcium indicator. The experimental conditions for calcium pulse-induced calcium release in the cuvette were KCl, 150 mM; histidine, 20 mM (pH 6.8); NaN_3, 5 mM; arsenazo III, 16 μM; Mg-ATP, 1 mM and SR, 0.5 mg/ml. The total cuvette volume of 1 ml was maintained at 30°C. Calcium was added as 2 μM pulses (0.5 μl) as in [3]. Dantrolene was dissolved in tetrahydrofurfuryl alcohol (THFA) at a stock concentration of 0.1 M. Serial dilutions of stock dantrolene in THFA were made such that a 0.5 μl addition to the cuvette would provide a final concentration of 6.25, 12.5, 25, or 50 μM dantrolene. For solvent control, 0.5 μl THFA was tested. Dantrolene or THFA was added to the cuvette immediately (~30 s) before calcium pulse additions were initiated.

3. RESULTS

Gracilis skeletal muscle of the MHS pigs responded in a characteristically abnormal manner to the in vitro application of halothane and caffeine [4,5]. An isometric contracture response to 3% halothane ($\bar{x}$ = 1.15 g) is characteristic of a phenotype H genetic predisposition [5] (fig.1). In contrast, muscle from control pigs had no contracture response to 3% halothane, but instead only a potentiation of the electrically evoked twitch was observed (fig.1). Similarly, MHS pig muscle had abnormal sensitivity to the contracture producing effect of caffeine applied in vitro. The threshold caffeine concentration for contracture was 2 mM for MHS muscle and 8 mM for controls (fig.2). The average caffeine concentration producing 1 g isometric contracture was calculated from the dose–response curve and this value was 3.7 ± 1.7 for MHS and 11.9 ± 2.3 for controls.

The heavy SR fraction isolated from the MHS pig muscle was also abnormally different from the heavy SR isolated from control muscle. As reported [3], the threshold for calcium pulse-induced calcium release is lower for SR from MHS ($\bar{x}$ = 17.3 ± 8.3 nmol Ca/mg SR) than that for controls ($\bar{x}$ = 86.2 ± 18 nmol Ca/mg SR). The amount of calcium released was greater for controls than for MHS SR fractions wherease no difference was observed for rate of Ca^{2+} release between the two types of SR (table 1).

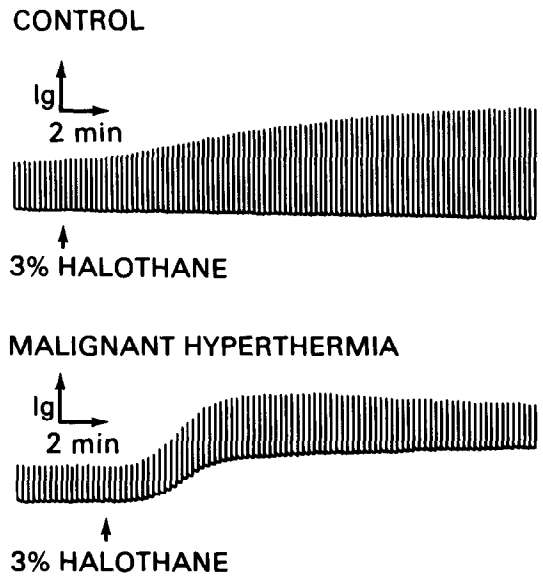

Fig.1. The effect of 3% halothane on control and malignant hyperthermia susceptible skeletal muscle in vitro. Halothane 3% (v/v) was initiated at arrows. In upper panel, control muscle responded with twitch potentiation while malignant hyperthermia muscle (lower panel) has a contracture response.

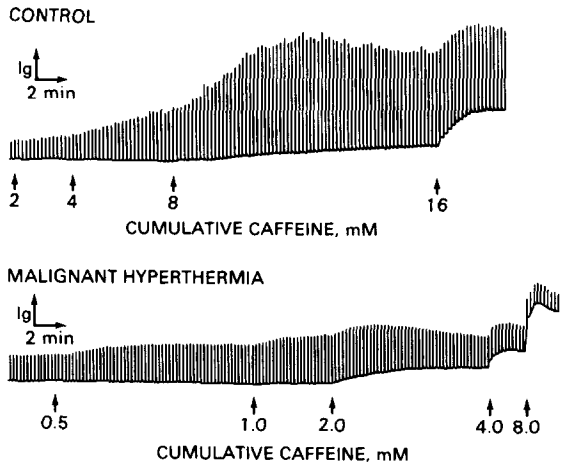

Fig.2. Contracture response of control (upper panel) and malignant hyperthermia susceptible (lower panel) pig skeletal muscle to caffeine. Contracture, upward shift in baseline, is initiated at 8 mM caffeine in control muscle and at 2 mM caffeine in malignant hyperthermia muscle.

Table 1

Effect of dantrolene on calcium pulse-induced Ca^{2+} release from MH and control sarcoplasmic reticulum

		Control	THFA[a]	Dantrolene (μM)			
				6.25	12.5	25	50
Threshold	C[b]	86.2 ± 18	80.8 ± 18	81.2 ± 20	75.4 ± 15	80.0 ± 23	80.7 ± 21
nmol Ca^{2+}/mg SR	MH[c]	17.3 ± 8	14.0 ± 9	12.7 ± 11	14.0 ± 13	13.3 ± 10	11.3 ± 9
Amount	C	13.0 ± 12	12.3 ± 13	17.1 ± 16	11.3 ± 11	12.2 ± 12	12.5 ± 12
nmol Ca^{2+}/mg SR	MH	1.3 ± 0.4	2.4 ± 1.7	2.6 ± 1.5	2.6 ± 1.7	2.4 ± 1.1	2.1 ± 1.1
Rate	C	1.5 ± 2	1.97 ± 2	2.99 ± 4	1.87 ± 2	2.0 ± 2	2.18 ± 3
nmol $Ca^{2+} \cdot s^{-1} \cdot mg^{-1}$	MH	1.19 ± 0.5	1.47 ± 1.0	1.48 ± 0.8	2.32 ± 2	1.82 ± 0.9	1.10 ± 0.6

[a] THFA is 0.5 μl of dantrolene solvent (tetrahydrofurfuryl alcohol), the volume for each concentration of dantrolene studied

[b] C, HSR preparations from 3 control pig muscle

[c] MH, HSR preparations from 3 MH-susceptible pig muscle

Each value is the mean ± SD

The solvent for dantrolene, THFA at a concentration of 5 μM slightly decreased threshold and increased rate and amount of Ca^{2+} release from MH-SR (table 1). Dantrolene, at concentrations ranging from 6.25 to 50 μM, did not significantly alter the threshold, rate or amount of calcium release observed for the THFA-treated MH-SR membranes (table 1). Likewise, dantrolene did not significantly change the threshold, rate and amount for calcium pulse-induced calcium release from the values observed in THFA-treated control SR membranes (table 1).

4. DISCUSSION

Malignant hyperthermia is a catastrophic, hypermetabolic syndrome occurring in genetically predisposed man and animals [6]. It is usually associated with the clinical use of halogenated anesthetic agents and the muscle relaxant succinylcholine. The primary defect appears to exist in skeletal muscle and an idiopathic increase in myoplasmic Ca^{2+} is postulated as the primary etiologic event. The abnormal in vitro contracture response of MHS skeletal muscle to caffeine and to halothane demonstrate the myogenic basis for MH. Although the association between caffeine's contracture producing effects on skeletal muscle and its effects on calcium uptake and release by the SR have been recognized for some time [7], it is only recently that a pharmacogenetic defect has been described for SR isolated from MHS skeletal muscle [3]. In that study [3], a defective calcium release mechanism was identified and the putative calcium channel for these events had abnormal sensitivity to caffeine.

Here, dantrolene, a unique, directly acting muscle relaxant, had no observable effect on the threshold for calcium pulse-induced calcium release nor did it effect the rate and amounts of calcium released. This lack of dantrolene effect was the same for calcium release mechanisms in both control and MH-SR fractions. Dantrolene is the therapeutic and prophylactic agent for MH and yet it does not block the abnormal calcium pulse-induced calcium release from the MH-SR membrane. Several explanations can be given for this apparent discrepancy. It has been proposed that one or more steps in the electromechanical coupling pathway of MH skeletal muscle may be defective [8,9]. Dantrolene may be acting at some site in the electromechanical coupling pathway that is either proximal or distal to the abnormal calcium channel and thereby blocking the abnormal calcium release. It is also possible that more than one defective site exists in MH skeletal muscle and the abnormal calcium efflux represents only one defect. Although several sites of action for dantrolene on skeletal muscle have been suggested [8,10], the exact site(s) remain(s) unknown. A

previous study showed that 20 μM dantrolene decreased the amount of calcium released by halothane effects on a heavy SR fraction [1]. This study did not describe the solvent used for the poorly water-soluble dantrolene nor did it state if the SR studied was from control or MH pig muscle. A number of serious inconsistencies in this report [1] draws concern. For example, Mg concentrations varied from 0.5 to 10 mM in the experiments reported without Mg effects being studied and in fig.4 of that study it states that 0–40 μM dantrolene was studied and only one dantrolene dose is shown. These experimental inconsistencies make it difficult to draw conclusions from this previous investigation.

Here, no dantrolene effect on calcium pulse-induced calcium release was observed. Since this mechanism appears to represent one possible defect in the SR membrane from MHS skeletal muscle, the exact site of action of dantrolene to block muscle contraction and the MH syndrome is yet to be determined.

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