

# Abnormality in Calcium Release from Skeletal Sarcoplasmic Reticulum of Pigs Susceptible to Malignant Hyperthermia

THOMAS E. NELSON, *Department of Anesthesiology, The University of Texas Medical Branch, Galveston, Texas 77550*

**ABSTRACT** Two fractions of sarcoplasmic reticulum, one light (LSR) and one heavy (HSR), were isolated from gracilis muscle of control and malignant hyperthermia (MH)-susceptible pigs. Part of the gracilis muscle biopsy was used to compare the contracture sensitivity of the muscle to the calcium-releasing effects of caffeine on isolated SR membranes. Gracilis muscle of MH pigs was more sensitive to the contracture-producing effects of caffeine than control pig muscle. The caffeine dose-cumulative contracture response curve for MH muscle was shifted left of that for controls.

The amount of caffeine-induced calcium released from SR is a function of the amount of calcium preload and this did not differ between LSR of MH and control muscle. When LSR fractions were optimally loaded with calcium for caffeine-induced calcium release, no difference in calcium-releasing effects of varying caffeine doses was observed between MH and control LSR. At calcium preloads below optimal, the MH-LSR appeared to be more sensitive to caffeine-induced calcium release. The HSR fractions could not be loaded with calcium in a manner similar to the LSR fractions because of an apparent calcium-induced calcium release phenomenon. Therefore, calcium threshold for calcium-induced calcium release was compared between MH and control HSR fraction. The effect of caffeine on the calcium-induced calcium release was also studied. The average calcium concentration threshold for calcium-induced calcium release was markedly lower for MH vs. control HSR; 20 vs. 63 nmol  $\text{Ca}^{2+}$ /mg, respectively. Caffeine decreased the threshold for calcium-induced calcium release more in the MH than in control HSR. Under all conditions studied, the amount of calcium released did not differ

between the two groups. Ruthenium red increased the threshold calcium concentration for calcium-induced calcium release while it reduced the amount of calcium released. Increasing concentrations of  $\text{Mg}^{2+}$  increased the  $\text{Ca}^{2+}$  threshold for release and the amount of  $\text{Ca}^{2+}$  released but did not significantly affect rate of  $\text{Ca}^{2+}$  release. Results of the study suggest a defect in the mechanisms causing calcium release from SR in MH-affected muscle.

## INTRODUCTION

Malignant hyperthermia (MH)<sup>1</sup> is a life threatening hypermetabolic syndrome occurring in genetically predisposed man and swine (1-3). In man, MH is usually associated with the administration of certain anesthetic agents (4, 5); whereas in swine the MH syndrome can also be triggered by stress alone (6, 7). The primary defect associated with MH susceptibility in man and pigs appears to be located in skeletal muscle (8-10). Abnormal metabolic (8, 11, 12) and contracture (13, 14) responses of skeletal muscle from MH-susceptible man and swine have produced a working hypothesis that abnormal increases in myoplasmic calcium ion concentration stimulate the hypermetabolism and skeletal muscle rigidity observed with the MH syndrome.

Additional support for a primary lesion in skeletal muscle of MH-affected man and pigs is the fact that dantrolene, a unique skeletal muscle relaxant is an effective prophylactic and therapeutic agent for MH in man (15) and pigs (16). The exact site of action of dantrolene is unknown but it does act directly on skeletal muscle (17) and diminishes the amount of calcium

Received for publication 22 September 1982 and in revised form 18 May 1983.

<sup>1</sup> Abbreviations used in this paper: HSR, heavy SR; LSR, light SR; MH, malignant hyperthermia; SR, sarcoplasmic reticulum.

released to the myoplasm (18–20). Also, the abnormal *in vitro* metabolic and contracture responses of MH muscle can be abolished or diminished by lowering the calcium concentration of the muscle bath (21).

These studies have provided direct evidence to support the theory that MH is a consequence of abnormal calcium regulation in affected skeletal muscle. The sarcoplasmic reticulum (SR) is the primary calcium-regulating membrane in skeletal muscle and represents a likely target for a defect in MH skeletal muscle. Most studies on SR from MH muscle have measured calcium fluxes into isolated SR vesicles and the effects of drugs and physical perturbations on this process (22–27). A direct demonstration of a defect in calcium binding or uptake by SR from MH muscle, or an abnormal effect of drugs on this process, has not been reported.

Studies on calcium efflux from SR have been limited by the lack of suitable systems for studying calcium release (28, 29). An abnormal increase in rate of calcium efflux from mitochondria isolated from MH pig muscle has been reported (30, 31). These studies were performed on muscle obtained from pigs postmortem, after conventional slaughter techniques (30). The stress conditions of slaughter are known to trigger metabolic responses in the muscle similar to those observed in anesthetic-induced MH (32). The effect of these postmortem changes on the function of mitochondria in MH muscle are unknown and therefore the significance of these observations to the etiology of MH remains a question.

Recent studies have reported methods for studying calcium efflux from isolated SR vesicles (33, 34). We have used this new methodology for comparing the biochemical and pharmacological properties of SR from MH susceptible and control pig skeletal muscle.

## METHODS

Two groups of pigs were used in this study. One group of pigs comprised three litters of purebred Poland China pigs bred for MH susceptibility. One litter of MH pigs was used to isolate and study a light fraction (12–48,000 *g*) of SR and MH pigs from the other two litters were used to obtain and study a heavy SR fraction (8–12,000 *g*). For comparative purposes, crossbred pigs were purchased as controls from a commercial farm and paired with the MH pigs in both studies. Isolation of SR was alternated by day between MH and control pigs in order to help eliminate time effects on the results. Each pig was tested for MH susceptibility by muscle contracture testing (35) and by anesthetic challenge. Biopsies of gracilis muscle were obtained from each pig after induction and maintenance of anesthesia with thiopental. Each pig underwent orotracheal intubation and was allowed to spontaneously breathe 100% oxygen. Strips of gracilis muscle were obtained for *in vitro* contracture testing by methods previously described (35). An additional 20 *g* of gracilis muscle was removed for isolation of SR fractions. Immediately after biopsy, the muscle was placed on ice and minced with scissors into small pieces and placed in 35-ml centrifuge

tubes containing 1.4 ml of 20 mM histidine (pH 6.8) per *g* of muscle. The muscle was homogenized 15 s  $\times$  2, setting of 5, using a Polytron homogenizer with a PT 20 generator (Brinkmann Instruments, Inc., Westbury, NY). Histidine, 20 mM was increased to a final ratio of one part muscle to 5 vol histidine buffer and the pH was measured and adjusted to pH 6.8 using 1 M NaOH. The following centrifugation steps were performed using a Sorvall RC5 refrigerated (4°C) centrifuge and SS-34 rotor (DuPont Instruments, Sorvall Biomedical Div., DuPont Co., Newtown, CT). The muscle homogenate was centrifuged at 12,000 *g* (LSR) for 20 min, the resultant supernatant decanted, filtered (Whatman 802 fluted paper, Whatman Chemical Separation, Inc., Clifton, NJ), and extracted with 0.6 M KCl (16 ml of 3.75 M KCl added per milliliter homogenate). The KCl-extracted supernatant was centrifuged at 48,000 *g* for 60 min, the resultant supernatant discarded, and the pellet suspended in 70 ml of 20 mM histidine, 150 mM KCl (pH 6.8) and centrifuged at 48,000 *g* for 45 min. The resultant pellet was suspended in 20 mM histidine, 150 mM KCl to obtain a final protein concentration of  $\sim$ 25 mg SR protein/ml. This SR fraction (i.e., 12–48,000 *g* is referred to as the light (LSR) SR fraction and was isolated from muscle obtained from five MH and seven control crossbred pigs. In a separate series of experiments, a heavy (HSR) fraction was isolated from muscle obtained from 10 MH and 6 control pigs, different from those used for the LSR studies. Methods for isolating the HSR fraction were identical to those described above for the LSR except for the centrifugation methods. After homogenization of muscle for isolation of HSR, the homogenate was first centrifuged at 8,000 *g* for 20 min. The pellet was discarded and the supernatant was filtered and extracted with 0.6 M KCl as described above for the LSR fraction. The 0.6-M KCl extract was centrifuged at 12,000 *g* for 20 min. The supernatant was discarded and the pellet suspended in 70 ml of 20 mM histidine, 150 mM KCl (pH 6.8) and centrifuged for 30 min at 48,000 *g*. The resultant pellet was suspended once more in histidine-KCl to a final protein concentration of  $\sim$ 25 mg/ml. The entire SR isolation and storage procedures were carried out at 4°C. The SR protein concentrations were estimated by a Folin method using crystalline bovine serum albumin as standard.

Changes in calcium concentration during calcium binding and release by the SR were estimated by dual-wavelength spectrophotometry using arsenazo III as calcium indicator. The wavelength pair (650 and 700 nm) was selected to minimize interference from changes in Mg concentrations. Measurement of changes in calcium concentration were made in 750  $\mu$ l solution in a 10-mm pathlength cuvette with the solution continuously stirred by a magnetic stir bar. The amount of calcium released from the SR fractions was estimated by measuring the absorbance change and comparing this to a standard curve. The standard curve was determined by the absorbance changes resulting from calcium additions to the reaction solution containing arsenazo III, 16  $\mu$ M; histidine, 20 mM (pH 6.8); Na<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 5 mM; and KCl, 150 mM. The composition of this solution is identical to that in which the calcium uptake and release studies were performed except that SR, 0.5 mg/ml, and MgATP, 1 mM, were omitted for determination of the standard curve. After correcting for the contaminating calcium concentration of the solution ( $\sim$ 5  $\mu$ M), the absorbance change following the addition of the ionophore A23187 (5  $\mu$ g/ml final concentration) to SR, 0.05 mg/ml was used to estimate the endogenous calcium content of each SR fraction. The total Ca<sup>2+</sup> content of LSR and HSR fractions from four MH-susceptible and four control pigs was determined by atomic absorption spectropho-

tometry. For the LSR and HSR fractions, changes in calcium concentration were measured under the following conditions: SR, 0.5 mg/ml; arsenazo, 16  $\mu$ M; histidine, 20 mM (pH 6.8);  $\text{NaN}_3$ , 5 mM; KCl, 150 mM; and MgATP, 1 mM. Any variation from this method is noted in the text. Calcium binding and release were measured at a temperature of 30°C, maintained by a circulating water bath. All studies comparing SR between MH and control muscle were conducted within 4 h of isolation.

## RESULTS

The gracilis muscle from the MH-susceptible pigs in this study was more sensitive to the contracture-producing effects of caffeine than was muscle from the control pigs (Fig. 1). The isometric contracture-caffeine dose-response curve for MH muscle falls left of that for control muscle and the caffeine threshold concentration for contracture was 1 mM for MH muscle compared with 8 mM for controls (Fig. 1).

To compare the sensitivity of caffeine-induced calcium release from the LSR fraction of MH and control pig muscle, the effect of amount of calcium loading (calcium preload) in the SR on the amount of calcium release by caffeine was first determined (Fig. 2). Optimum calcium preload (i.e., amount of calcium preload that resulted in maximum amount of calcium released by 1 mM caffeine) tended to be lower for SR from MH muscle (mean = 40 nmol calcium/mg SR) compared with the optimum calcium preload for control LSR (mean = 53 nmol/mg SR). However, because of large variation among calcium preloads within pig groups, these mean values are not significantly different (Fig. 2). The minimum calcium preload at which 1 mM caffeine could produce calcium release was significantly ( $P < 0.05$ ) lower for the MH-LSR than for control-LSR (Fig. 2). In one MH-LSR preparation, 1 mM caffeine was able to produce calcium release even in the absence of a calcium preload (Fig. 2).

Determined either by the ionophore equilibration method, or by atomic absorption spectrophotometry, the endogenous calcium content of the LSR fractions from controls did not statistically differ in a significant way from the MH group (Table I). Likewise, the final calcium content (i.e., endogenous  $\text{Ca}^{2+}$  plus preload  $\text{Ca}^{2+}$ ) did not differ between LSR from control and MH muscle (Table I).

Unlike the differences between MH and control *in vitro* muscle contracture responses to caffeine, the LSR fractions from MH and control muscle did not differ in the sensitivity to caffeine-induced calcium release (Fig. 3). Two of five MH-LSR fractions and none of the control-LSR fractions had calcium released at 0.1 mM caffeine and all LSR fractions had calcium released at 0.2 mM caffeine.

Determining the optimum calcium preload for caffeine-induced calcium release in the HSR fractions,

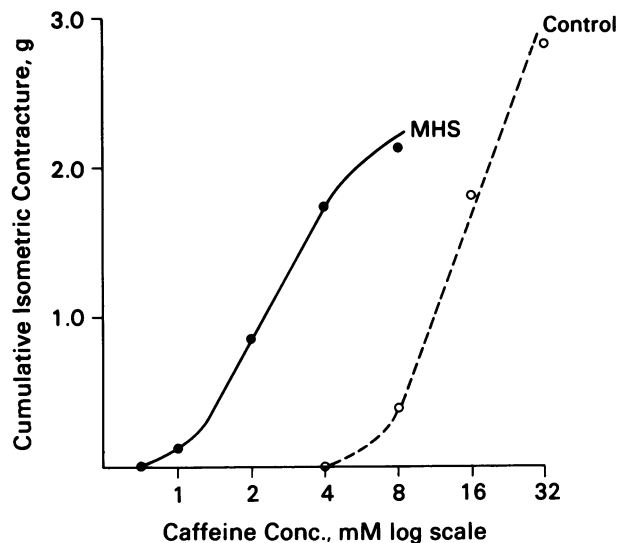


FIGURE 1 Cumulative caffeine dose-isometric contracture responses for gracilis muscle strips from MH-susceptible and control pigs. Each muscle strip was exposed to cumulative caffeine doses shown on the abscissa and the cumulative isometric contracture response was measured.  $n = 7$  MH-susceptible and seven control pigs, two muscle strips tested for each pig.

as was done for the LSR fractions, was not feasible. There were two reasons for this. First, it was discovered that the HSR fractions were susceptible to spontaneous calcium release and that additions of bolus calcium preloads often resulted in calcium release before the calcium preload was totally bound by the SR. Second, the threshold calcium preload for this spontaneous calcium release was considerably lower for MH-HSR fractions than for the control HSR fractions. Therefore, the threshold calcium concentrations that produced an apparent calcium-induced calcium release and the effect of caffeine on this phenomenon in the HSR fractions were studied. Rather than adding the calcium preload as a bolus dose, as done for the LSR studies, low concentration calcium (0.5–2  $\mu$ M) pulses were added consecutively to the cuvette until the phase of rapid calcium release occurred (Fig. 4). The addition of each calcium pulse resulted in a rapid uptake by the HSR fractions such that a change in absorbance signal was barely detectable (Fig. 4). As the amount of calcium accumulated by the HSR fractions increased, the rate of uptake slowed and when a critical amount of calcium was bound, a subsequent calcium pulse resulted in a rapid phase of calcium release (Fig. 4). The calcium threshold for MH-HSR (mean =  $20.1 \pm 14$  nmol calcium/mg SR) was significantly lower ( $P < 0.05$ ) than the calcium threshold concentration for control-HSR fractions (mean =  $62.8 \pm 6$  nmol calcium/mg SR) (Table II). The HSR

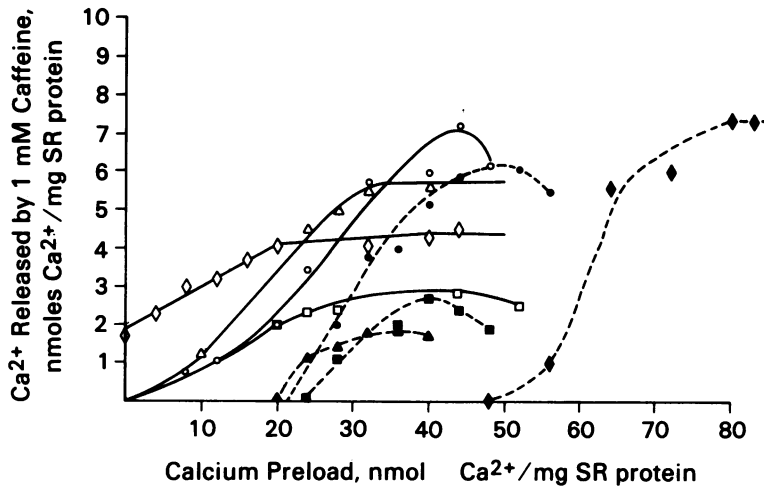


FIGURE 2 The effect of calcium preload on 1 mM caffeine-induced calcium released from SR of MH-susceptible and control muscle. Each calcium preload was either totally or partially taken up by the SR and then 1 mM caffeine added. The amount of calcium released by 1 mM caffeine was determined.  $n = 4$  MH-susceptible pigs (open symbols) and four control pigs (filled symbols).

endogenous calcium content did not differ significantly between MH-susceptible and control sources, excluding this as a possible reason for the lower threshold in MH-HSR (Table I). The total calcium content (endogenous plus preload) was lower ( $P < 0.05$ ) for the MH-HSR than for controls (Table I). The amount of calcium released by the threshold calcium preload

did not differ significantly between control HSR ( $11.7 \pm 6.7$  nmol calcium/mg SR) and MH-HSR ( $12.2 \pm 4.4$  nmol calcium/mg SR) (Table II). The average rate of calcium efflux at the critical calcium preload threshold was greater for MH-HSR compared with controls (Table II), but this difference was not statistically significant.

TABLE I  
Comparison of Endogenous, Preload, and Total Calcium Concentrations for Light and Heavy SR Vesicles from MH-Susceptible and Control Pig Skeletal Muscle

SR Fraction	Pig Group	n	*Endogenous Ca <sup>2+</sup>		Preload Ca <sup>2+</sup>	Total Ca <sup>2+</sup>
			Ionophore	AAS		
<i>nmol/mg</i>						
LSR	MHS	5	66.8±18†		41.8±12	108.6±11
		4	113±60	104±54		
	Control	7	66.6±22		58.0±20	
		4	90.1±44	93.2±23		
HSR	MHS	5	61.5±26		14.1±10§	75.6±30§
		5	46.4±23		26.1±16§	74.9±29§
		4	70.3±16	81.8±13		
	Control	6	64.4±9		62.8±6	127.3±11
		4	72.5±39	78.5±13		

\* Endogenous Ca<sup>2+</sup> was determined in all SR fractions by equilibration using the ionophore A23187. In four other MH and four control SR, total Ca<sup>2+</sup> was also determined by atomic absorption spectrophotometry (AAS).

† Values are means±SD and  $n$  refers to number of animals.

§ Preload and total Ca<sup>2+</sup> are statistically different ( $P < 0.05$ ) when MH-susceptible and control HSR means are compared. No statistically significant differences were observed for other between group mean comparisons.

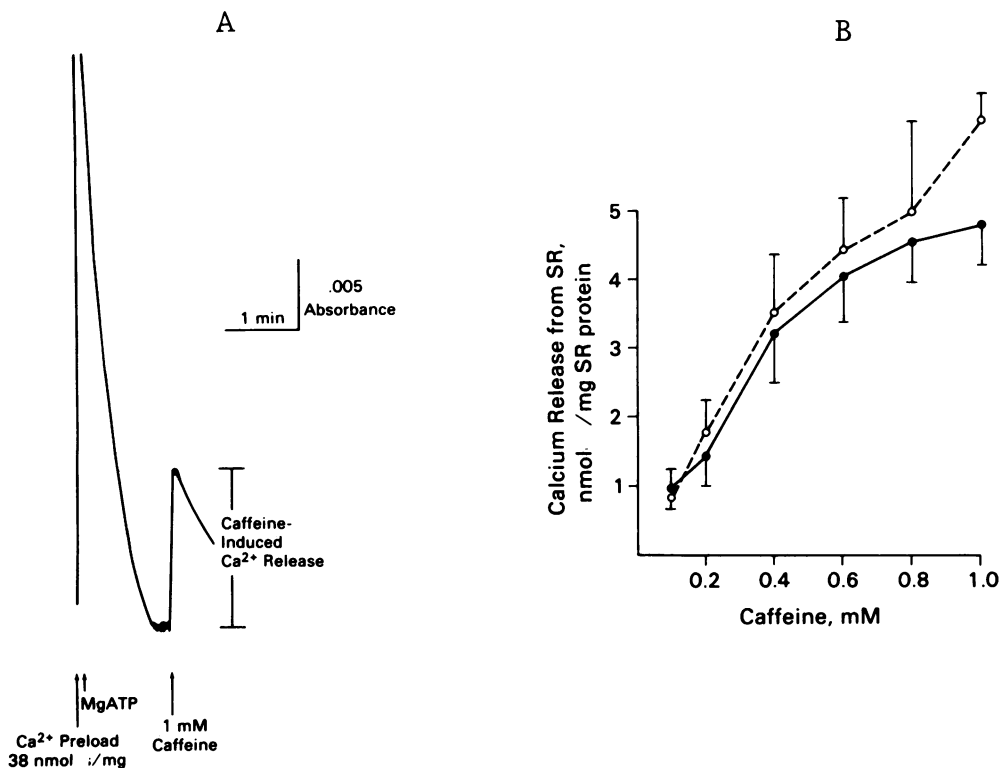


FIGURE 3 (A) Illustration of calcium preload followed by caffeine-induced calcium release. Addition of 38 nmol Ca<sup>2+</sup>/mg preload causes changes in absorbance moving signal upwards, offscale. MgATP addition activates inward pumping of Ca<sup>2+</sup>, reducing the absorbance signal to zero Ca<sup>2+</sup> outside the SR membrane. 1 mM caffeine addition causes Ca<sup>2+</sup> release. (B) Effect of varying caffeine concentrations on Ca<sup>2+</sup> release from SR fractions (MH susceptible, open symbols; control, filled symbols) after each fraction was optimally loaded with Ca<sup>2+</sup>. Conditions: average calcium preload, 40 nmol/mg SR for MH and 53 nmol/mg SR for controls; SR, 0.5 mg/ml; MgATP, 1 mM; KCl, 150 mM; histidine, 20 mM; pH, 6.8; temperature 30°C; n = 5 MH-susceptible and seven control pigs.

The effect of varying concentrations of caffeine on the threshold calcium preload was tested by adding caffeine to the cuvette after the addition of MgATP, but before calcium additions. In both control and MH-HSR fractions, the effect of caffeine was to decrease the threshold concentration of calcium preload for calcium release (Fig. 5). Caffeine affected the calcium preload threshold more in MH-HSR than for controls. The ED<sub>50</sub> caffeine concentration for lowering calcium threshold averaged 0.25 mM for MH-HSR vs. 1.0 mM for control HSR.

Increasing concentrations of ruthenium red (from 10 to 300 × 10<sup>-9</sup> M) increased the calcium concentration threshold for calcium release in both control and MH-HSR (Fig. 6). Ruthenium red in a concentration-dependent manner also decreased the amount and rate of calcium released (data not shown). Increasing total Mg<sup>2+</sup> concentration from 1 to 1.5–2.0 mM increased threshold values by a factor of 1.2 in control

HSR whereas the MH-HSR threshold values were doubled (Table III). In spite of this, threshold values of MH-HSR remained much lower than for controls. Amount of Ca<sup>2+</sup> release was not affected by Mg<sup>2+</sup> in either HSR group. Rate of release tended to decrease with increasing Mg<sup>2+</sup> concentration more so in control than in MH-HSR (Table III).

## DISCUSSION

The gracilis muscle of pigs susceptible to MH has hypersensitivity to the contracture-producing effects of caffeine. When optimally loaded with calcium, the LSR membranes isolated from MH-susceptible gracilis muscle did not differ from controls in the sensitivity to caffeine-induced calcium release. When calcium preload is varied, 1 mM caffeine produces calcium release at lower calcium preloads in MH-LSR membranes than for controls. For example, the average

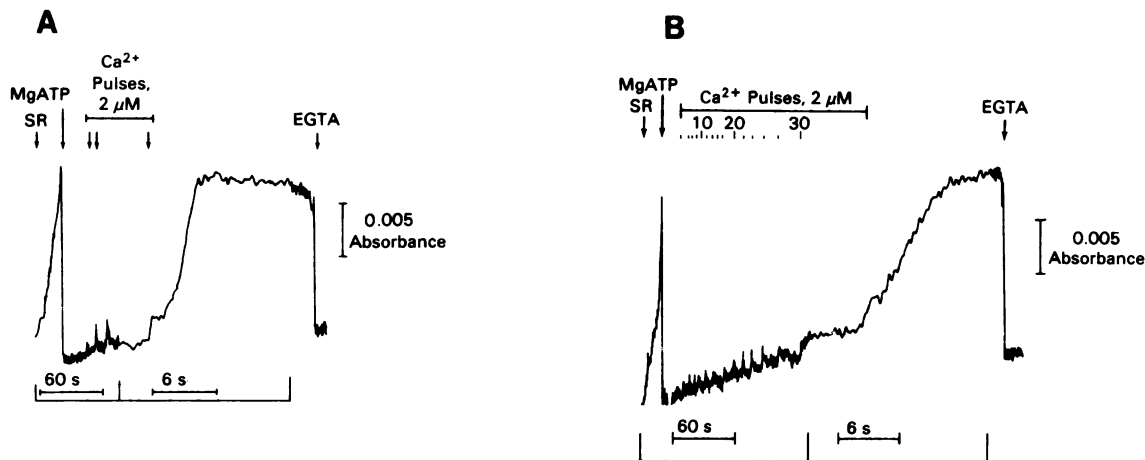


FIGURE 4 (A) Calcium efflux from MH-HSR during  $\text{Ca}^{2+}$  pulse loading. Addition of SR to cuvette produces absorbance change, moving signal upwards. Next, MgATP addition causes removal of contaminating, exogenous  $\text{Ca}^{2+}$  and signal returns to base line, zero  $\text{Ca}^{2+}$  level. Addition of  $2\text{-}\mu\text{M}$   $\text{Ca}^{2+}$  pulse produces a spike signal, measuring added  $\text{Ca}^{2+}$  signal and its rapid removal by  $\text{Ca}^{2+}$  uptake by the SR. A second  $2\text{-}\mu\text{M}$   $\text{Ca}^{2+}$  pulse produces similar effect, and the third  $2\text{-}\mu\text{M}$   $\text{Ca}^{2+}$  pulse produces a signal spike that is not reversed by  $\text{Ca}^{2+}$  uptake but is followed by a rapid phase of  $\text{Ca}^{2+}$  release. In time, the released  $\text{Ca}^{2+}$  could be slowly removed by SR but in above experiment, alteration of  $\text{Ca}^{2+}$  signal by EGTA identifies zero base line. (B) Calcium efflux from control-HSR during  $\text{Ca}^{2+}$  pulse loading. Conditions were identical to those described in A above except that the threshold  $\text{Ca}^{2+}$  preload for  $\text{Ca}^{2+}$  release was  $30\text{ }\mu\text{M}$  ( $60\text{ nmol/mg HSR}$ ).

calcium preload at which  $1\text{ mM}$  caffeine produced release of  $1\text{ nmol calcium/mg LSR}$  was  $10\text{ nmol/mg MH-LSR}$  compared with  $38\text{ nmol/mg control-LSR}$ . From these results it is difficult to ascertain if the MH-LSR membranes are more sensitive to the direct effects of caffeine or if some other mechanism related to calcium loading is indirectly affected by caffeine in the MH-LSR fraction. The LSR endogenous calcium levels were not different between the two groups, excluding this as a possible explanation.

The heavy fraction of SR membranes from MH gracilis muscle did not conform to the calcium preload protocol used for the LSR fractions. The basis for this problem appears to be a function of the calcium-in-

duced calcium release phenomenon (33, 34, 36). The calcium-induced calcium release occurs both in MH and control HSR fractions but the calcium concentra-

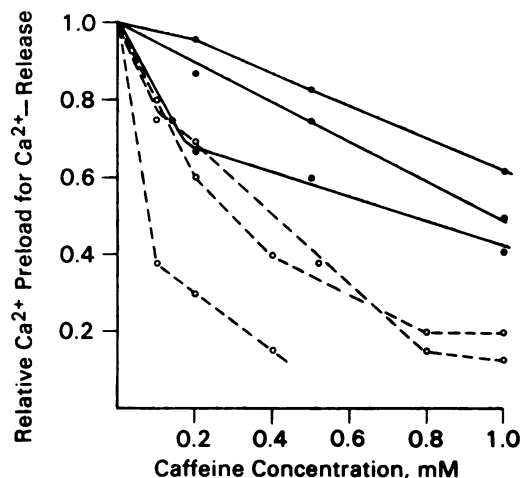


FIGURE 5 The effect of caffeine concentration on the  $\text{Ca}^{2+}$  pulse threshold for  $\text{Ca}^{2+}$  release from HSR. Experimental conditions were as described in Fig. 4, except the appropriate caffeine dose was added after MgATP and before the  $\text{Ca}^{2+}$  pulses. Relative  $\text{Ca}^{2+}$  threshold value of 1.0 represents the  $\text{Ca}^{2+}$  threshold (nanomoles per milligram SR) for  $\text{Ca}^{2+}$  release in the absence of caffeine. Control HSR (solid lines) and MH-HSR (broken lines).

TABLE II  
Characteristics of Calcium Efflux from Skeletal Muscle SR of MH and Control Pigs

Pig group	n	Calcium threshold <i>nmol/mg SR</i>	Calcium release <i>nmol/mg</i>	Calcium release rate <i>nmol/s mg<sup>-1</sup></i>
MH	10	$20.1 \pm 14^*$	$10.7 \pm 6.7$	$4.3 \pm 2.8$
Controls	6	$62.8 \pm 6.3$	$12.1 \pm 4.4$	$2.0 \pm 1.4$

Values are means  $\pm$  SD; values marked with (\*) are significantly different from controls ( $P < 0.05$ ).

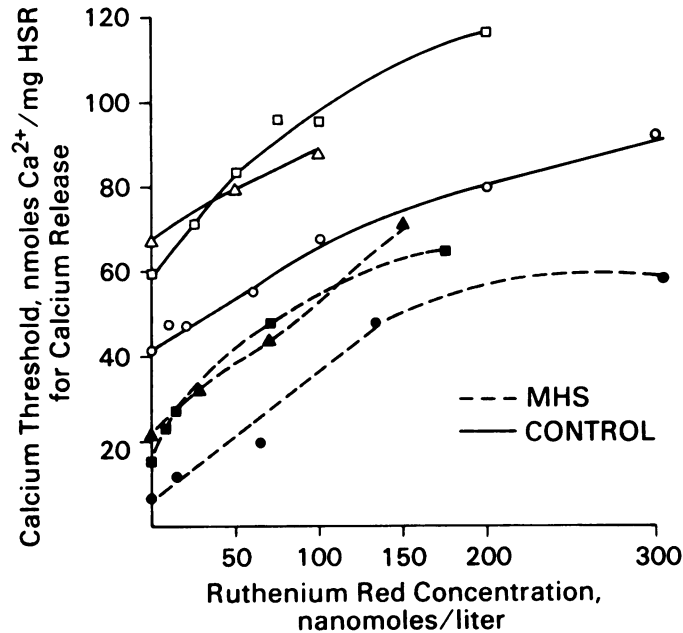


FIGURE 6 The effect of varying concentrations of ruthenium red on the calcium threshold for calcium release from MH and control HSR. Experimental conditions for studying calcium release from HSR are defined in the text. The concentration of ruthenium red indicated was added to the HSR immediately before calcium pulse additions. Each line represents data of HSR isolated from a control (open symbols,  $n = 3$  pigs) or MH (filled symbols,  $n = 3$  pigs) pig.

tion threshold at which calcium release occurred was considerably lower for the MH-HSR membranes. This difference could not be due to varying amounts of endogenous calcium between the two groups since these values were not significantly different. Furthermore, caffeine, which lowers the calcium concentration threshold for calcium release, produced this effect at

much lower concentrations in MH-HSR when compared with controls. This greater sensitivity of MH-HSR to the caffeine effect on calcium release is consistent with the hypersensitivity of MH muscle to the contracture-producing effects of caffeine.

The mechanism(s) responsible for the calcium release phase observed in this study appears to be ab-

TABLE III  
Effects of  $Mg^{2+}$  on Threshold, Amount, and Rates for  $Ca^{2+}$  Efflux from Control and MH Skeletal HSR

*Total $Mg^{2+}$		Threshold	Amount $Ca^{2+}$ released	Rate of $Ca^{2+}$ release
mM		nmol $Ca^{2+}$ /mg SR		nmol/s $mg^{-1}$
1.0	Control	113±26†	15.9±10	1.39±1
	MHS	29±22†	8.8±4	3.9±1
1.5	Control	123±23	15.7±10	0.54±0.36
	MHS	48±33	11.8±8	3.6±2
2.0	Control	138±24	16.7±10	0.61±0.43
	MHS	61±36	14.4±8	4.4±3

MHS, MH susceptible.

\* Of the total  $Mg^{2+}$  concentration, each experiment contained 1 mM  $Mg^{2+}$  as contributed by addition of 1 mM  $MgATP$ .

† Values are means±SD of HSR fractions from three control and three MHS pigs.

normal for the HSR isolated from MH-susceptible skeletal muscle. What is known about the nature of mechanisms involved in calcium release from the SR? Using techniques similar to those of the present study and validating the results by criteria established for skinned muscle fibers, Ohnishi (33) described the nature of this calcium release as a calcium-induced calcium release phenomenon. In that study, calcium-induced calcium release was inhibited by local anesthetics, ruthenium red, and high magnesium concentrations, while caffeine and halothane enhanced the calcium-induced calcium release (33). In other recent studies, Miyamoto and Racker (34, 36) described experiments in which calcium-induced calcium release phenomena were observed. This form of calcium release was also inhibited by ruthenium red and enhanced by caffeine (34, 36). Based on results obtained in their studies, Miyamoto and Racker (34) postulated two calcium channels in the HSR membrane fraction they studied. One calcium channel is described as a ruthenium-red sensitive channel that contains a calcium receptor that upon binding calcium causes the channel to open and allows calcium release to occur. The second calcium channel is a voltage-sensitive channel, opened by an inside negative potential (34).

Based upon the criteria for validating calcium-induced calcium release in skinned muscle fibers and in fragmented SR membranes (28), the calcium release observed in our study may be a calcium-induced calcium release phenomenon. We have observed that the calcium release (*a*) is increased with increasing calcium preload (data not shown for HSR); (*b*) is inhibited in a dose-dependent manner by ruthenium red; (*c*) is partially inhibited by  $Mg^{2+}$ ; and (*d*) is enhanced by caffeine. These observations were made in SR from both normal, control, and MH-susceptible pig skeletal muscle. Therefore, we postulate that an abnormality exists in the mechanism(s) creating calcium release from the HSR fraction isolated from MH skeletal muscle. With increasing doses, ruthenium red increases the calcium preload threshold for calcium release while it decreases the amount of calcium released from MH and control HSR fractions. For the HSR from MH muscle, these ruthenium red effects suggest that the calcium pump and the binding capacity of the HSR are not primarily responsible for the abnormal sensitivity to calcium-induced calcium release. Furthermore, it suggests that the ruthenium red-sensitive calcium channel may represent a defect in the MH-HSR. The basis for this interpretation is the conclusion by previous workers (34, 36) that ruthenium red enhances calcium uptake by blocking calcium-induced calcium release.

Although the threshold calcium preload for calcium-induced calcium release was always lower for MH-

HSR than for controls, the amount of calcium released did not differ between the two sources of HSR. This indicated that the mechanism that stops calcium release (i.e., increased inward calcium pumping and/or closure of calcium channel gate) is not different between MH and control HSR fractions. Although not statistically different in this study, it is possible that rate of calcium release is greater from the MH-HSR. The rate of calcium release is a function of several variables (i.e., rate of inward calcium pumping, number of calcium channels, time of channel open vs. close, etc.), which were not controlled during the measured calcium efflux. Since calcium flux was probably not unidirectional under the present experimental conditions, it is difficult to establish the possible cause for increased rates of calcium release from the MH-HSR.

The discovery of an abnormal release mechanism for calcium from a HSR fraction isolated from MH-susceptible skeletal muscle is compatible with current theories regarding the etiology of MH. Further studies are needed to characterize the physiology and pharmacology of the calcium release mechanisms in normal SR membranes before the exact nature of the membrane defect in SR from MH skeletal muscle can be described.

#### ACKNOWLEDGMENTS

Dr. S. T. Ohnishi provided valuable consultation for initiating these studies; Pat Turk provided excellent technical skill and service and Mary Jane Kelly helped with manuscript preparation.

This work was supported, in part, by National Institutes of Health grant DHHS 5R01 GM 23875-05. Breeding colony maintained at The University of Texas System Cancer Center, Science Park, Veterinary Resources Division, Bastrop, TX 78602, was supported, in part, by grant Ca-16672 from the Division of Cancer Research Resources and Centers, National Institutes of Health.

#### REFERENCES

1. Denborough, M. A., and R. R. H. Lovell. 1960. Anaesthetic deaths in a family. *Lancet*. II:45.
2. Gronert, G. A. 1980. Malignant hyperthermia. *Anesthesiology*. 53:395-423.
3. Jones, E. W., T. E. Nelson, I. L. Anderson, D. D. Kerr, and T. K. Burnap. 1972. Malignant hyperthermia in swine: general characteristics. *Anesthesiology*. 36:42-51.
4. Britt, B. A., and W. Kalow. 1970. Malignant hyperthermia: a statistical review. *Can. Anaesth. Soc. J.* 17:293-315.
5. Denborough, M. A., J. F. A. Forster, R. R. H. Lovell, P. A. Mapleston, and J. D. Villiers. 1962. Anaesthetic deaths in a family. *Br. J. Anaesth.* 34:395-396.
6. Nelson, T. E. 1973. Porcine stress syndrome. In International Symposium on Malignant Hyperthermia. R. A. Gordon, B. A. Britt, and W. Kalow, editors. Charles C. Thomas, Springfield, IL. 191-197.
7. Nelson, T. E., E. W. Jones, R. L. Henrickson, S. N. Falk,



- and D. D. Kerr. 1974. Porcine malignant hyperthermia: observations on the occurrence of pale, soft, exudative musculature among susceptible pigs. *Am. J. Vet. Res.* 35:347-350.
8. Kalow, W., B. A. Britt, M. E. Terreau, and C. Haist. 1970. Metabolic error of muscle metabolism after recovery from malignant hyperthermia. *Lancet.* II:895-898.
  9. Moulds, R. F. W., and M. A. Denborough. 1974. Biochemical basis of malignant hyperpyrexia. *Br. Med. J.* 2:241-244.
  10. Nelson, T. E., E. W. Jones, J. H. Venable, and D. D. Kerr. 1972. Malignant hyperthermia of Poland China swine: studies of a myogenic etiology. *Anesthesiology.* 36:52-56.
  11. Harrison, G. G., S. J. Saunders, J. F. Biebuych, R. Hickman, D. M. Dent, V. Weaver, and J. Terblanche. 1969. Anaesthetic-induced malignant hyperpyrexia and a method for its prediction. *Br. J. Anaesth.* 41:844-854.
  12. Britt, B. A., L. Endrenyi, W. Kalow, and P. L. Peters. 1976. The adenosine triphosphate (ATP)-depletion test: comparison with the caffeine contracture test as a method of diagnosing malignant hyperthermia susceptibility. *Can. Anaesth. Soc. J.* 23:624-635.
  13. Kalow, W., B. A. Britt, and A. Richter. 1977. The caffeine test of isolated human muscle in relation to malignant hyperthermia. *Can. Anaesth. Soc. J.* 24:678-694.
  14. Nelson, T. E., D. M. Bedell, and E. W. Jones. 1975. Porcine malignant hyperthermia: effects of temperature and extracellular calcium concentration on halothane-induced contracture of susceptible skeletal muscle. *Anesthesiology.* 42:301-306.
  15. Kolb, M. E., M. L. Horne, and R. Martz. 1982. Dantrolene in human malignant hyperthermia. *Anesthesiology.* 56:254-262.
  16. Flewelling, E. H., and T. E. Nelson. 1980. Dantrolene dose response in malignant hyperthermia-susceptible (MHS) swine: method to obtain prophylaxis and therapeutics. *Anesthesiology.* 52:303-308.
  17. Ellis, K. O., and J. F. Carpenter. 1972. Studies on the mechanism of action of dantrolene sodium, a skeletal muscle relaxant. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 275:83-94.
  18. Desmedt, J. E., and K. Hainaut. 1977. Inhibition of the intracellular release of calcium by dantrolene in barnacle giant muscle fibres. *J. Physiol. (Lond.).* 265:565-585.
  19. Morgan, K. G., and S. H. Bryant. 1977. The mechanism of action of dantrolene sodium. *J. Pharmacol. Exp. Ther.* 201:138-147.
  20. Hainaut, K., and J. E. Desmedt. 1974. Effect of dantrolene sodium on calcium movements in single muscle fibres. *Nature (Lond.).* 252:728-730.
  21. Nelson, T. E., and A. B. Chausmer. 1981. Calcium content and contracture in isolated muscle of malignant hyperthermia in pigs. *J. Pharmacol. Exp. Ther.* 219:107-112.
  22. Brucker, R. F., C. H. Williams, J. Popinigis, T. L. Galvez, W. J. Vail, and C. A. Taylor. 1973. *In vitro* studies on liver mitochondria and skeletal muscle sarcoplasmic reticulum fragments isolated from hyperpyrexia swine. *In International Symposium on Malignant Hyperthermia.* R. A. Gordon, B. A. Britt, and W. Kalow, editors. Charles C. Thomas, Springfield, IL. 238-270.
  23. Dhalla, N. S., P. V. Sulakhe, N. F. Clinch, G. Wade, and A. Naimark. 1972. Influence of fluothane on calcium accumulation by the heavy microsomal fraction of human skeletal muscle: comparison with a patient with malignant hyperpyrexia. *Biochem. Med.* 6:333-343.
  24. Gronert, G. A., J. J. A. Heffron, and S. R. Taylor. 1979. Skeletal muscle sarcoplasmic reticulum in porcine malignant hyperthermia. *Eur. J. Pharmacol.* 58:179-187.
  25. McIntosh, D. B., M. C. Berman, and J. E. Kench. 1977. Characteristics of sarcoplasmic reticulum from slowly glycolysing and from rapidly glycolysing pig skeletal muscle postmortem. *Biochem. J.* 166:387-398.
  26. Nelson, T. E., and D. E. Bee. 1979. Temperature perturbation studies of sarcoplasmic reticulum from malignant hyperthermia pig muscle. *J. Clin. Invest.* 64:895-901.
  27. Wood, D. S., A. Mozo, and J. H. Wilner. 1979. Malignant hyperthermia: the relation of sarcoplasmic reticulum dysfunction to the pathogenesis of the disease. *Neurology.* 29:557-558 a. (Abstr.)
  28. Endo, M. 1977. Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.* 57:71-108.
  29. MacLennan, D. H., and A. Klip. 1979. Calcium transport and release by sarcoplasmic reticulum: a mini-review. *In Membrane Transduction Mechanisms.* R. A. Cone and J. E. Dowling, editors. Raven Press, New York. 61-75.
  30. Cheah, K. S., and A. M. Cheah. 1976. The trigger for PSE condition in stress-susceptible pigs. *J. Sci. Food Agric.* 27:1137-1144.
  31. Cheah, K. S., and A. M. Cheah. 1978. Calcium movements in skeletal muscle mitochondria of malignant hyperthermic pigs. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 95:307-310.
  32. Lister, D., R. A. Sair, J. A. Will, G. R. Schmidt, R. G. Cassens, W. G. Hoekstra, and E. J. Briskey. 1970. Metabolism of striated muscle of stress-susceptible pigs breathing oxygen or nitrogen. *Am. J. Physiol.* 218:102-107.
  33. Ohnishi, S. T. 1979. Calcium-induced calcium release from fragmented sarcoplasmic reticulum. *J. Biochem.* 86:1147-1150.
  34. Miyamoto, H., and E. Racker. 1982. Mechanism of calcium release from skeletal sarcoplasmic reticulum. *J. Membr. Biol.* 66:193-201.
  35. Nelson, T. E., and E. H. Flewelling. 1979. Malignant hyperthermia: diagnosis, treatment, and investigations of a skeletal muscle lesion. *Tex. Rep. Biol. Med.* 38:105-120.
  36. Miyamoto, H., and E. Racker. 1981. Calcium-induced calcium release at terminal cisternae of skeletal sarcoplasmic reticulum. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 133:235-238.
  37. Nelson, T. E., and E. H. Flewelling. 1979. Rationale for dantrolene vs. procainamide for treatment of malignant hyperthermia. *Anesthesiology.* 50:118-122.