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KINETIC STUDIES OF Ca^{2+} RELEASE FROM SARCOPLASMIC RETICULUM OF NORMAL AND MALIGNANT HYPERTHERMIA SUSCEPTIBLE PIG MUSCLES

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The time-course of Ca^{2+} release from sarcoplasmic reticulum isolated from muscles of normal pigs and those of pigs susceptible to malignant hyperthermia were investigated using stopped-flow spectrophotometry and arsenazo III as a Ca^{2+} indicator. Several methods were used to trigger Ca^{2+} release: (a) addition of halothane (e.g., 0.2 mM); (b) an increase of extravesicular Ca^{2+} concentration ($[Ca_0^{2+}]$); (c) a combination of (a) and (b), and (d) replacement of ions (potassium gluconate with choline chloride) to produce membrane depolarization. The initial rates of Ca^{2+} release induced by either halothane or Ca^{2+} alone, or both, are at least 70% higher in malignant hyperthermic sarcoplasmic reticulum than in normal. The amount of Ca^{2+} released by halothane at low $[Ca_0^{2+}]$ in malignant hyperthermic sarcoplasmic reticulum is about twice as large as in normal sarcoplasmic reticulum. Membrane depolarization led to biphasic Ca^{2+} release in both malignant hyperthermic and normal sarcoplasmic reticulum, the rate constant of the rapid phase of Ca^{2+} release induced by membrane depolarization being significantly higher in malignant hyperthermic sarcoplasmic reticulum ($k = 83\ s^{-1}$) than in normal ($k = 37\ s^{-1}$). Thus, all types of Ca^{2+} release investigated (a, b, c and d) have higher rates in malignant hyperthermic sarcoplasmic reticulum than normal sarcoplasmic reticulum. These results suggest that the putative Ca^{2+} release channels located in the sarcoplasmic reticulum are altered in malignant hyperthermic sarcoplasmic reticulum.

Introduction

It is generally believed that malignant hyperthermia is a genetic disorder involving abnormal intracellular Ca^{2+} movements in muscle cells in response to certain anesthetic drugs such as halothane. Abnormalities of muscle function are reflected in a rapid increase in body temperature, severe respiratory and metabolic acidosis, and muscle rigidity [1–6]. It was suggested that the abnormality in malignant hyperthermic muscle lies distal to the postjunctional membrane [7–9]. Possible sites are (a) sarcolemma, (b) transverse-tubule, (c) transverse-tubule/sarcoplasmic reticulum coupling, and (d) sarcoplasmic reticulum. Of these membranes, sarcoplasmic reticulum plays the primary role in the regulation of the free Ca^{2+} concentration in muscle cells [10–17]. Therefore, studies on the malignant hyperthermic muscle sarcoplasmic reticulum are essential to elucidate the etiology of the disease.

Previous data using pig and human sarco-

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Abbreviation: Mes, 2-(*N*-morpholino)ethanesulfonic acid.

plasmic reticulum have been contradictory [18–25]. It has been reported that the rate and the capacity of Ca^{2+} uptake are unchanged [5] or decreased [21,22]. Some work on Ca^{2+} release in malignant hyperthermic sarcoplasmic reticulum has been reported. Malignant hyperthermic sarcoplasmic reticulum has a higher spontaneous Ca^{2+} release [22] and a higher rate of Ca^{2+}-induced Ca^{2+} release [26]. Recent studies with isolated pig sarcoplasmic reticulum have suggested that the malignant hyperthermic sarcoplasmic reticulum has an enhanced Ca^{2+} release induced by Ca^{2+} [27,28], caffeine [28] and halothane [27].

Recently, we reported a stopped-flow spectrophotometric method for the determination of various kinetic parameters of Ca^{2+} release induced by various triggering methods [29]: (a) increasing extravesicular $[Ca^{2+}]$ ($[Ca_0^{2+}]$) from about 0.1 μM to several micromolar [30–33]; (b) addition of drugs such as caffeine, quercetin, and halothane at various $[Ca_0^{2+}]$ [34–38]; and (c) substitution of permeable cations for impermeable ones (depolarization-induced Ca^{2+} release) [39–43]. The aim of this study was to resolve the kinetic characteristics of (Ca^{2+} + halothane)-induced and depolarization-induced Ca^{2+} release in malignant hyperthermia susceptible pig sarcoplasmic reticulum with the use of high resolution kinetic techniques. The results show that all types of Ca^{2+} release investigated occur at higher rates in malignant hyperthermic sarcoplasmic reticulum than normal, suggesting that the putative Ca^{2+} release channels located in the sarcoplasmic reticulum are altered in malignant hyperthermic sarcoplasmic reticulum.

Materials and Methods

Care and screening of pigs for malignant hyperthermic trait

Pure-bred Poland China pigs were used as experimental animals. The initial breeding stock animals were tested for malignant hyperthermic susceptibility by a muscle contracture test in vitro (caffeine dose-contracture response in the absence and presence of halothane). In vivo tests as described below were carried out. For normal controls, we used cross-bred Poland China and Yorkshire pigs screened similarly. The animals were kept in our institutional farm in accordance with the guidelines of the National Research Council (DHH publication, NIH 78-23, revised 1978).

The animals were anesthetized with 2% halothane, N_2O and O_2 by inhalation and the trachea was incubated. During induction and maintainence of anesthesia, heart rate, rhythm, respiratory rate, rectal temperature and hind limb stiffness were monitored at 5 min intervals. After 1 h of anesthesia, if there was no positive response, 1 mg/kg of succinylcholine was administered intravenously. If no positive response was seen, a second dose was given 30 min later. A positive response was considered to be an elevation in rectal temperature by 1.5 °C in 25 min and/or marked stiffness of the hind limbs. If positive signs were elicited, the animals were cooled with surface cooling, the anesthesia was discontinued, and dantrolene sodium (2 mg/kg) was administered intravenously. If no positive signs were elicited the anesthesia was terminated 30 min after the second dose of succinylcholine, and the animal was allowed to recover. From 4 to 6 weeks after the anesthesia challenge, the pigs ($n = 14$) were anesthetized with intravenous thiopental, intubated and ventilated with oxygen. After the loss of the lid reflex, the animal was bled from the neck, the back muscles were quickly excised and placed into ice for further procedures.

Preparation of sarcoplasmic reticulum

A fraction containing sarcoplasmic reticulum was prepared by a differential centrifugation method [27] from pig back muscle (primarily fast twitch muscle). About 200 g muscle were homogenized in a Waring blender with 4 vol. of a solution containing 2.5 mM NaOH/20 mM Mes (pH 6.8) for 2 min (20 s × 6). During the homogenization the pH was adjusted to 6.8 with NaOH as required. The suspension was centrifuged at 10000 × g for 3 min in a GSA rotor (Sorvall). The supernatant was filtered through four layers of cheese-cloth and then Whatman filter papers (No. 4). After readjusting the pH to 6.8, the filtrate was centrifuged again at 17000 × g for 25 min. The pellets were suspended in a solution containing 0.15 M KCl and 20 mM Mes (pH 6.8) and centrifuged again at 17000 × g for 25 min. The pellets were resuspended in the same buffer and the final protein concentration was adjusted to 20–30

mg/ml and stored at 0–4°C. Samples were used within 5 h of preparation because Ca^{2+} uptake and release values did not remain stable beyond this time.

Ca^{2+} loading

Sarcoplasmic reticulum vesicles were loaded by ATP-dependent Ca^{2+} accumulation in 0.15 M KCl, 1.6 mg protein per ml, 50 μM $CaCl_2$, 20 mM Mes (pH 6.8), 9 μM arsenazo III, 0.5 mM MgATP, with or without an ATP regenerating system (2.5 mM phospho*enol*pyruvate and 10 units/ml pyruvate kinase) (solution A) at 27°C for 1–2 min. The endogenous Ca^{2+} was determined by titration using arsenazo III as described previously [29]. The means ± S.E. of the amounts of endogenous Ca^{2+} in normal and malignant hyperthermic sarcoplasmic reticulum are 61.9 ± 9.2 ($n = 5$) and 63.9 ± 3.2 ($n = 5$) nmol Ca^{2+} per mg protein, respectively.

Ca^{2+} release

Ca^{2+} and halothane-induced Ca^{2+} release. For the actively loaded sarcoplasmic reticulum, when the extravesicular concentration of Ca^{2+} became nearly zero in solution A, one part of solution containing 0.15 M KCl, 20 mM Mes (pH 6.8), and 9 μM arsenazo III and appropriate amounts of Ca^{2+} or 0.2 mM halothane, or both (solution B), was mixed with one part of solution A in order to trigger the release of accumulated Ca^{2+}. Changes in the extravesicular concentration of Ca^{2+} were monitored by the difference in the absorbance of 9 μM arsenazo III at 700 and 650 nm with the use of a stopped-flow system (see below).

Depolarization-induced Ca^{2+} release. Sarcoplasmic reticulum vesicles were loaded with Ca^{2+} as described above except that 0.15 M potassium gluconate was used in place of KCl. One part of solution A was mixed with one part of solution B containing 0.15 M choline chloride, 20 mM Mes (pH 6.8) and 9 μM arsenazo III.

Stopped-flow assay

A multiple-channel stopped-flow spectrophotometer system was used to study the kinetics of (Ca^{2+} + halothane)-induced Ca^{2+} release under various reaction conditions described in the figure legends. The system consists of (i) a rotating air turbine containing eight different interference filters, (ii) a turbine-demodulator including an A-log A converter, (iii) a Durrum stopped-flow system (Model D-110) and (iv) a PDP 11-03 computer. Changes in the difference of absorbance at 700 nm and 650 nm were recorded, and signal averaged. Kinetic parameters (A and k) were calculated by single exponential iterative computer fitting, $y = A[1 - \exp(-kt)] + A_0$ [29]. For depolarization-induced Ca^{2+} release, a dual-beam stopped-flow spectrophotometer system with a 680–650 nm wavelength pair was used [44] and the size and the rate constant of depolarization-induced Ca^{2+} release were calculated by a manual analysis of the plotted curves as follows. Three initial phases of the Ca^{2+} release (viz., (1) lag phase; (2) rapid Ca^{2+} release; (3) plateau phase) were fitted by three sets of lines. Intersection of these lines produced two points: one at (x_1, y_1) and the other at (x_2, y_2). The size of Ca^{2+} release and time span of phase were calculated from the $y_2 - y_1$ and $x_2 - x_1$ values, respectively. The rate constant of rapid Ca^{2+} release (k) was approximated as $2(\ln 2)/(x_2 - x_1)$, assuming that $t_{1/2} = (x_2 - x_1)/2$.

Miscellaneous

Arsenazo III (contaminating $Ca^{2+} < 0.04$ μmol/mg) was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents used were of analytical grade. Protein concentrations were determined by the method of Lowry et al. [45] using bovine serum albumin as a standard.

Normal and malignant hyperthermic groups were compared using Student's t-test.

Results

Contents of sarcoplasmic reticulum and transverse-tubule membranes in normal and malignant hyperthermic muscles

The yields of sarcoplasmic reticulum from normal and malignant hyperthermia susceptible pig muscles were 1.11 ± 0.06 ($n = 4$) and 1.05 ± 0.06 ($n = 5$) mg/g muscle, respectively.

In order to investigate the content of transverse-tubules the cholesterol contents in normal and malignant hyperthermic sarcoplasmic reticulum preparations were determined. Since trans-

verse-tubules are enriched in cholesterol (350 μg cholesterol per mg protein) compared with sarcoplasmic reticulum (cf. Ref. 46), the cholesterol content was used as a measure of the transverse-tubule content. There was no significant difference between normal and malignant hyperthermic sarcoplasmic reticulum, 39.1 ± 3.4 (mean $\pm$ S.E.) ($n = 7$) and 38.3 ± 1.1 ($n = 5$) μg/mg protein, respectively. This suggests that the transverse-tubule content of malignant hyperthermic sarcoplasmic reticulum preparation is about the same as that of normal preparation.

The kinetics of Ca^{2+} release

Normal and malignant hyperthermic sarcoplasmic reticulum preparations were incubated in the presence of MgATP and an ATP-regenerating system. Vesicles accumulated about 100 nmol Ca^{2+} per mg protein, a sufficient Ca^{2+}-loading level to produce Ca^{2+} release for both normal and malignant hyperthermic sarcoplasmic reticulum (cf. Ref. 27). In agreement with the report of Ohnishi et al. [27], there is no significant difference in the Ca^{2+} uptake capacity between normal and malignant hyperthermic sarcoplasmic reticulum.

In Ca^{2+} release function, however, there is a significant difference between normal and malignant hyperthermic sarcoplasmic reticulum. When Ca^{2+} release is induced by halothane alone (Fig. 1 A, C), both the amount of Ca^{2+} release (A) and

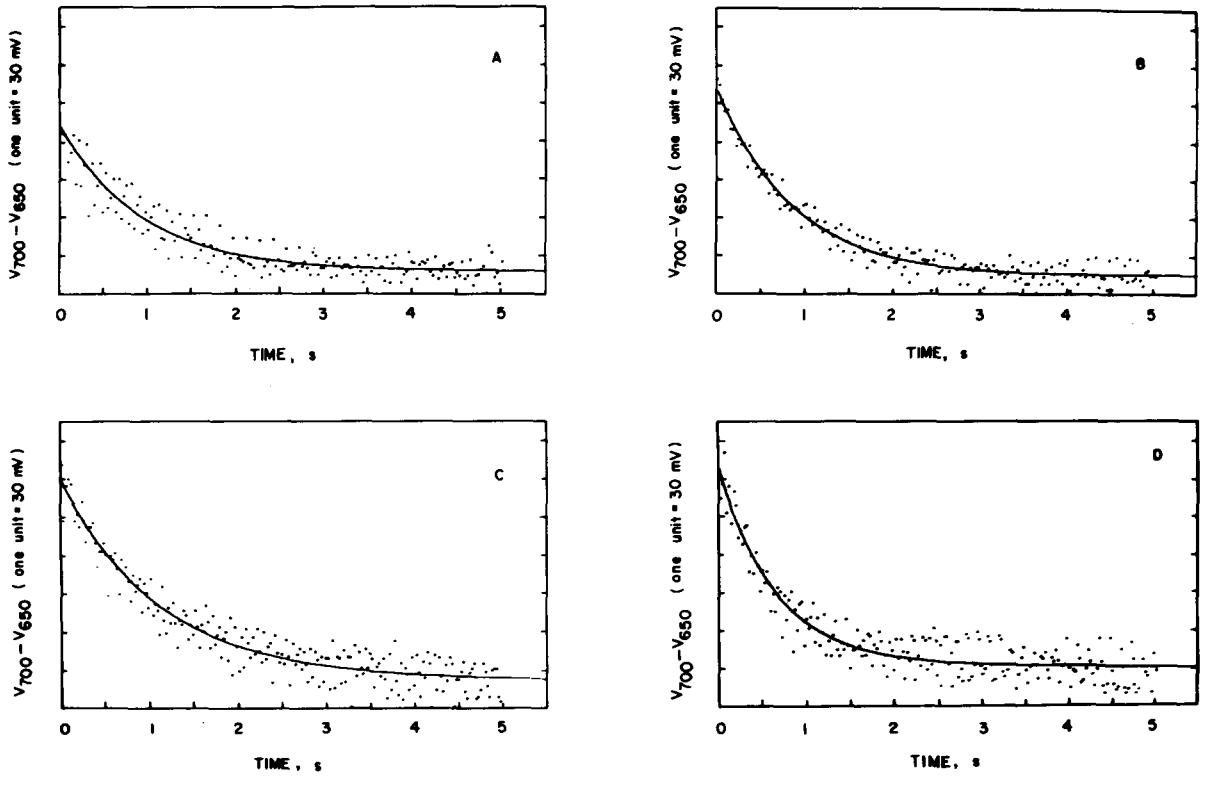

Fig. 1. Stopped-flow spectrophotometric recording of Ca^{2+} release induced by 0.2 mM halothane in the presence or in the absence of 2 μM Ca^{2+}. (A) and (B) Normal sarcoplasmic reticulum. (C) and (D) Malignant hyperthermic sarcoplasmic reticulum. For active Ca^{2+} loading, 0.5 mM MgATP was added to a solution containing 0.15 M KCl, 1.6 mg/ml sarcoplasmic reticulum, 9 μM arsenazo III, 50 μM $CaCl_2$, 2.5 mM phospho*enol*pyruvate, 10 units/ml pyruvate kinase and 20 mM Mes (pH 6.8) at 27 °C and then loaded in the syringe A of a Durrum stopped-flow apparatus. At the steady state of Ca^{2+} uptake, at which time the extravesicular concentration of Ca^{2+} was nearly zero (5–15 min), Ca^{2+} release was triggered by mixing the contents of syringe A and syringe B of a Durrum stopped-flow apparatus (1 : 1, v/v). The syringe B solution contained 0.15 M KCl, 20 mM Mes (pH 6.8), 9 μM arsenazo III and 0.2 mM halothane in the presence (B and D) or in the absence (A and C) of 2 μM Ca^{2+}. $V_{700} - V_{650} = 10$ mV corresponds to either 1.0 nmol Ca^{2+} per mg sarcoplasmic reticulum (A, C) or 1.5 nmol Ca^{2+} per mg sarcoplasmic reticulum (B, D).

the rate constant (k) are larger in malignant hyperthermic sarcoplasmic reticulum than in normal ($P < 0.05$). If Ca^{2+} release is induced by both halothane and Ca^{2+}, there is no appreciable difference between malignant hyperthermic and normal sarcoplasmic reticulum in the amount of Ca^{2+} release, whereas the rate of Ca^{2+} release ($A \cdot k$) is much higher in malignant hyperthermic sarcoplasmic reticulum than in normal (e.g., $P < 0.05$ at $pCa = 5.7$) (Fig. 1 B, D).

Fig. 2 illustrates that the A and k values of halothane-induced Ca^{2+} release vary with $[Ca_0^{2+}]$ both in normal (circle) and malignant hyperthermic sarcoplasmic reticulum (triangle). The A and k values of Ca^{2+} release induced by various $[Ca_0^{2+}]$ of normal (circle) and malignant hyperthermic sarcoplasmic reticulum (triangle) are plotted in Fig. 3. As seen from a comparison between Figs. 2 and 3, the pCa_0-dependance remains roughly the same under the influence of halothane, suggesting

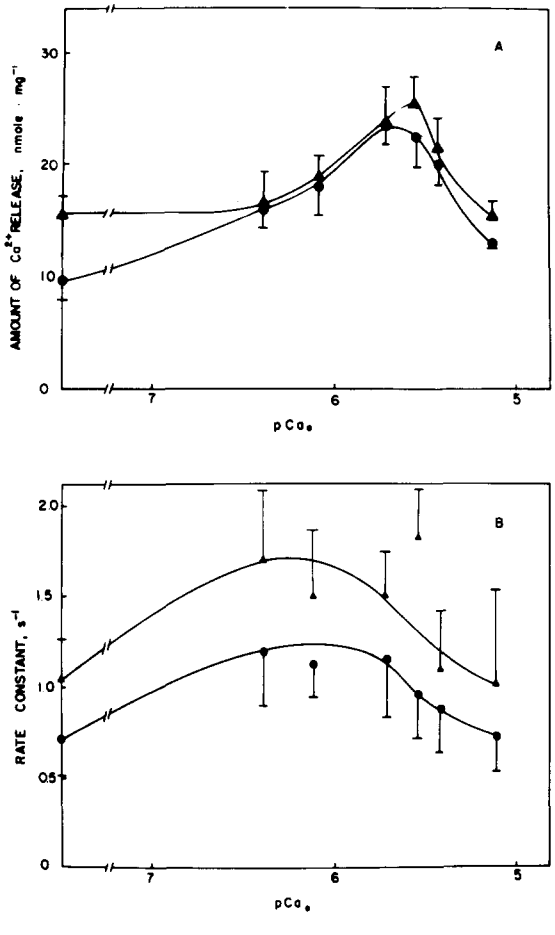

Fig. 2. The $[Ca_0^{2+}]$-dependence of the amounts and the rates of halothane-induced Ca^{2+} release from normal and malignant hyperthermic sarcoplasmic reticulum. Active Ca^{2+} loading was done as described in the legend to Fig. 1. At the steady state of Ca^{2+} uptake, one part of syringe A solution (see legend to Fig. 1) was mixed with one part of syringe B solution containing various concentrations of Ca^{2+} with 0.2 mM halothane. The amounts (A) and the rate constants (B) of Ca^{2+} release from normal (●) and malignant hyperthermic (▲) sarcoplasmic reticulum were calculated as described in Materials and Methods.

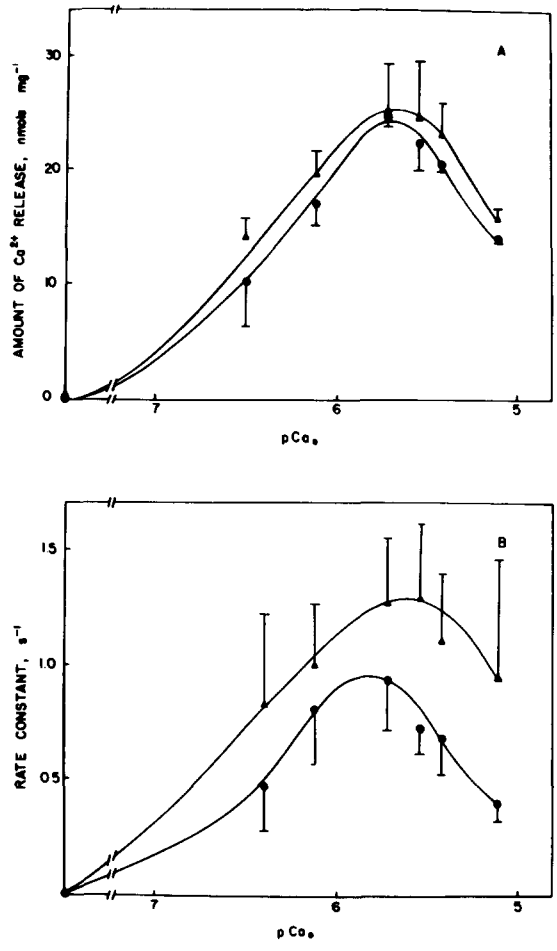

Fig. 3. The $[Ca_0^{2+}]$-dependence of Ca^{2+}-induced Ca^{2+} release in normal and malignant hyperthermic sarcoplasmic reticulum. At the steady state of Ca^{2+} uptake, one part of syringe A solution (see the legend to Fig. 1) was mixed with one part of syringe B solution containing various concentrations of Ca^{2+}. The amounts (A) and the rate constants (k) of Ca^{2+} release from normal (●) and malignant hyperthermic (▲) sarcoplasmic reticulum were calculated as described in Materials and Methods.

that halothane potentiates Ca^{2+}-induced Ca^{2+} release in the same way as caffeine and quercetin [29]. It is interesting that the A values of Ca^{2+}-induced Ca^{2+} release of normal and malignant hyperthermic sarcoplasmic reticulum (Fig. 3) are indistinguishable over the whole $[Ca_0^{2+}]$ range, whereas the k value of malignant hyperthermic sarcoplasmic reticulum is larger than that of normal sarcoplasmic reticulum (e.g., $P < 0.05$ at pCa = 5.7) (Fig. 3).

We investigated the effect of several different combinations of ionic replacement in attempts to induce depolarization-induced Ca^{2+} release. It was found that the previously used method for induction of membrane depolarization (replacement of KCl with choline chloride, Ref. 29) produces significant levels of light-scattering changes as well as Ca^{2+} release. However, replacement of both cations and anions (e.g., replacement of potassium gluconate with choline chloride) leads to rapid Ca^{2+} release without producing appreciable light-scattering changes [47]. Fig. 4 and Table I represent the traces and the kinetic parameters of depolarization-induced Ca^{2+} release of normal (Fig. 4A) and malignant hyperthermic sarcoplasmic reticulum (Fig. 4B) induced by the latter method. As seen here, the time-course of Ca^{2+} release exhibits several distinguishable phases. In the first phase (0–45 ms), there is virtually no Ca^{2+} release; in the second phase, there is rapid release of a small amount of Ca^{2+} (see A_1 and k_1, Table I). In the third phase, a larger amount of Ca^{2+} is released at a lower rate (see A_2 and k_2, Table I). The rate

TABLE I

DEPOLARIZATION-INDUCED Ca^{2+} RELEASE FROM SARCOPLASMIC RETICULUM OF NORMAL AND MALIGNANT HYPERTHERMIA SUSCEPTIBLE PIG

The experiment was carried out as described in the legend to Fig. 4. The kinetic parameters (A_1, A_2: nmol Ca^{2+}/mg; k_1, k_2: (s^{-1}) were calculated as described in Materials and Methods. Date represent means ± S.E. for two determinations.

	A_1	k_1	A_2	k_2
Normal	4.8 ± 0.8	37.3 ± 9.0	12.9 ± 9.0	1.3 ± 0.4
Malignant hyperthermic	6.9 ± 0.3 [a]	82.7 ± 9.8 [b]	37.5 ± 2.2 [a]	1.4 ± 0.4

[a] $P < 0.1$.
[b] $P < 0.05$.

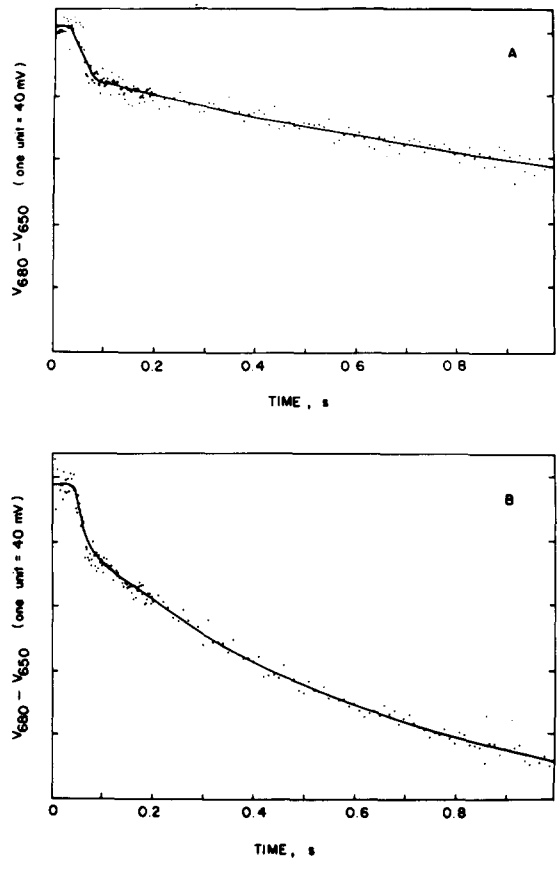

Fig. 4. The time-courses of Ca^{2+} release from normal and malignant hyperthermic sarcoplasmic reticulum induced by choline chloride replacement of potassium gluconate. (A) Normal sarcoplasmic reticulum. (B) Malignant hyperthermic sarcoplasmic reticulum. For active Ca^{2+} loading, potassium gluconate vesicles were incubated in a reaction solution containing 1.6 mg/ml sarcoplasmic reticulum, 200 μM $CaCl_2$, 0.15 M potassium gluconate, 0.5 mM MgATP, 2.5 mM phospho*enol*pyruvate, 10 units/ml pyruvate kinase, 9 μM arsenazo III, and 20 mM Mes (pH 6.8) for about 5 min at 27 °C. The reaction solution was placed in syringe A of a Durrum multimixing apparatus. At the steady state of Ca^{2+} uptake, at which time the extravesicular concentration of Ca^{2+} became nearly zero (5–12 min), the content of syringe A was mixed with syringe B solution containing 0.15 M choline chloride, 9 μM arsenazo III and 20 mM Mes (pH 6.8) to induce Ca^{2+} release. Changes in $V_{680} - V_{650}$ were recorded with a dual beam stopped-flow spectrophotometer. $V_{680} - V_{650} = 10$ mV corresponds to 5.6 nmol Ca^{2+} per mg sarcolasmic reticulum.

constant of the rapid phase (k_1) in malignant hyperthermic sarcoplasmic reticulum was significantly larger than that in normal. The amount of Ca^{2+} released in the second slow phase (A_2) of

malignant hyperthermic sarcoplasmic reticulum was also significantly larger than the corresponding values of normal sarcoplasmic reticulum.

Discussion

This paper demonstrates that malignant hyperthermia susceptible pig sarcoplasmic reticulum differs from normal in several kinetic characteristics of Ca^{2+} release. A summary of the observations is shown in Table II. When the Ca^{2+}-release time-courses are analysed and the two parameters of the exponential (viz. the size of Ca^{2+} release, A; and the rate constant, k) are compared, the values of k are significantly higher in malignant hyperthermic sarcoplasmic reticulum than in normal for all triggering methods investigated here. However, the difference in the values of A is not always significant. According to recent reports [27,28], the extent of intravesicular Ca^{2+} loading required for induction of Ca^{2+} release is much less in malignant hyperthermic sarcoplasmic reticulum than normal, whereas at a sufficient high Ca^{2+} loading (at least 100 nmol Ca^{2+} per mg sarcoplasmic reticulum) the amount of Ca^{2+} release (A) is about the same for normal and malignant hyperthermic sarcoplasmic reticulum. The present study confirms this, and further indicates that even at saturating levels of Ca^{2+} loading there are distinct differences in the rate constant of Ca^{2+} release (k) between normal and malignant hyperthermic sarcoplasmic reticulum.

TABLE II

THE RELATIVE INDICES OF THE KINETIC PARAMETERS (A, k, Ak) TO COMPARE THE Ca^{2+} RELEASE FUNCTIONS OF NORMAL AND MALIGNANT HYPERTHERMIA SUSCEPTIBLE PIG SARCOPLASMIC RETICULUM

The ratio and the standard errors were calculated from the data originating from four different preparations of each normal and malignant hyperthermic sarcoplasmic reticulum. Reagents added were: Ca^{2+}, 1 μM; halothane, 200 μM. $R_X = X_{\text{malignant hyperthermic}} / X_{\text{normal}}$ where X is the selected parameter (A, k or Ak).

	Ca^{2+}	Halothane	Ca^{2+} + halothane
R_A	1.2±0.1	1.7±0.2	1.2±0.2
R_k	1.6±0.5	1.7±0.3	1.5±0.4
R_{Ak}	1.7±0.4	2.6±0.5	1.8±0.8

It is widely recognized on the basis of the experiments with skinned muscle fiber preparations [30–32,39] and isolated sarcoplasmic reticulum [33,35,40–43] that Ca^{2+}-induced Ca^{2+} release and depolarization-induced Ca^{2+} release are clearly distinguishable by various criteria: e.g., different sensitivities to different agents [48], different $[Ca_0^{2+}]$-dependence and different rate constants of Ca^{2+} release [29]. Thus, it is necessary to consider at least two different mechanisms for triggering Ca^{2+} release. However, these different types of Ca^{2+} release may be carried out through common channels, since they share several common properties of Ca^{2+} efflux kinetics: e.g., inhibition by high $[Ca_0^{2+}]$ (e.g., 20 μM), low concentrations of Ruthenium red (e.g., 1 μM or less), and spontaneous attenuation of Ca^{2+} release [29,49].

It is interesting in this context that the rate constants (k) of all types of Ca^{2+} release are much higher in malignant hyperthermic sarcoplasmic reticulum than normal. Since higher values of the rate constant of Ca^{2+} release presumably represent either wider opening or higher population of the channel, one of the likely candidates for a molecular component responsible for the altered functions in the malignant hyperthermic sarcoplasmic reticulum is the Ca^{2+} channel through which release takes place.

One of the most important results described here is the finding that replacement of ions (e.g., potassium gluconate with choline chloride) led to Ca^{2+} release at the rates (40–80 s^{-1}) which are on the same order as those expected for Ca^{2+} release in vivo (28–139 s^{-1}) [29]. Recently, we found that the previously used method for induction of membrane depolarization (cation replacement alone) produces a light scattering response as well. The method described here (changes of both cations and anions) not only circumvents the light scattering problem, but also leads to more rapid and larger amounts of Ca^{2+} release. The rapid Ca^{2+} release is triggered via depolarization of transverse tubule membrane as evidenced by the fact that depolarization-induced Ca^{2+} release is reversibly inhibited by dissociation of the transverse-tubule/sarcoplasmic reticulum complex [47]. Therefore, the depolarization-induced Ca^{2+} release described here appears to represent an in vitro model of

physiological coupling between transverse-tubule and sarcoplasmic reticulum. As clearly seen from the present study, the rate constant and the amount of the transverse-tubule-mediated rapid Ca^{2+} release are much higher in malignant hyperthermic sarcoplasmic reticulum than in normal. However, the content of transverse-tubules, which are responsible for triggering the rapid Ca^{2+} release, appears to be the same in normal and malignant hyperthermic sarcoplasmic reticulum. The most likely explanation for this finding is again that the putative Ca^{2+} channels rather than triggering mechanisms are altered in malignant hyperthermic sarcoplasmic reticulum.

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