J. Membrane Biol. 54, 73–80 (1980)

The Journal of
Membrane Biology

Chloride-Induced Release of Actively Loaded Calcium from Light and Heavy Sarcoplasmic Reticulum Vesicles

Kevin P. Campbell* and Adil E. Shamoo**

Department of Radiation Biology and Biophysics, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642

Summary. Light and heavy sarcoplasmic reticulum vesicles (LSR, HSR) isolated from rabbit leg muscle have been used in a study of chloride-induced Ca^{2+} release. The biochemical and morphological data indicate that LSR is derived from the longitudinal reticulum and HSR is derived from the terminal cisternae of the sarcoplasmic reticulum. LSR and HSR were both able to accumulate Ca^{2+} in the presence of ATP to amounts greater than 100 nmol Ca^{2+}/mg of protein in less than 1 min. LSR and HSR each had a biphasic time course of Ca^{2+} uptake. The initial uptake was followed by a rapid release, after approximately 1 min, of 30–40% of the accumulated Ca^{2+}, which was then followed by a slower phase of Ca^{2+} accumulation. Ca^{2+} taken up by the SR vesicles could be released from both the LSR and HSR by changing the anion outside the vesicles from methanesulfonate to chloride. Due to the difference in permeability between methanesulfonate and chloride, this change should result in a decreased positivity inside the vesicles with respect to the exterior. It could also result in osmotic swelling of the vesicles. Changing the ionic medium from chloride to methanesulfonate caused no release of Ca^{2+}. The amount of accumulated Ca^{2+} released in 6 sec by changing the anion outside the vesicles from methanesulfonate to chloride was 30–35 nmol/mg membrane protein for LSR and HSR, respectively. Osmotic buffering with 200 mM sucrose caused a slight inhibition of chloride-induced Ca^{2+} release from HSR (17%→15%) but it greatly reduced the release of Ca^{2+} from LSR (32%→15%). The specificity of Ca^{2+} release was measured using SR vesicles which were passively loaded with 10 mM $^{22}Na^{+}$. LSR released five times more $^{22}Na^{+}$ than HSR under same conditions as chloride-induced Ca^{2+} release occurred. Na dantrolene (20 μm) had no effect on the release of Ca^{2+} from LSR but it inhibited the chloride-induced Ca^{2+} release from HSR by more than 50%. Na dantrolene also increased the Ca^{2+} uptake in the HSR by 20% while not affecting LSR Ca^{2+} uptake. Our results indicate the presence of a chloride-induced, Na dantrolene inhibited, Ca^{2+} release from HSR, which is not due to osmotic swelling.

It is estimated that more than a quarter of the Ca^{2+} present in the sarcoplasmic reticulum is released by a single stimulus, 210 nmol of Ca^{2+}/ml of muscle in a few milliseconds (Endo, 1977). Calcium release is a graded function of surface membrane depolarization (Constantin & Taylor, 1973), and the release process in the sarcoplasmic reticulum does not seem to be an all-or-none process (Hodgkin & Horowicz, 1960). It is generally accepted that the depolarization of the transverse tubular system of skeletal muscle initiates the release of calcium from the terminal cisternae of the sarcoplasmic reticulum (Endo, 1977; Ebashi & Endo, 1968; Sandow, 1970; Fuchs, 1974). In recent years there have been several mechanisms proposed for the link between the depolarization of the walls of the T-tubule and the release of Ca^{2+} from the sarcoplasmic reticulum (Endo, 1977; Ebashi & Endo, 1968), but it still remains one of the least understood processes in muscle contraction. The skeletal muscle membranes directly involved in excitation-contraction coupling are the transverse tubular membrane and the junctional sarcoplasmic reticulum membrane (Franzini-Armstrong, 1975). There has

* *Present address:* Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario M5G 1L6, Canada.

** *Present address:* Department of Biological Chemistry, University of Maryland, 660 West Redwood Street, Baltimore, Maryland 21201.

0022-2631/80/0054-0073 $01.60

been some recent progress in understanding excitation-contraction coupling in terms of electrophysiological properties of the transverse tubular membrane, and in chloride-induced (or 'depolarization' induced) release from the sarcoplasmic reticulum in skinned skeletal muscle fibers. Fluorescence changes of Nile blue A (Bezanilla & Horowicz, 1975) and optical changes (Baylor & Oetliker, 1975) during muscle activation may suggest that depolarization of the SR is responsible for Ca^{2+} release, although they could be parallel phenomena or even the result of Ca^{2+} release. The most convincing evidence that depolarization of the sarcoplasmic reticulum causes release of Ca^{2+} ions comes from the work of Nakajima and Endo (1973) and Endo and Blinks (1973). After loading the sarcoplasmic reticulum in a skinned fiber, the bathing medium was changed from sulfate to chloride which resulted in the release of Ca^{2+}. Stephenson and Poldolsky (1977) in their studies on chloride induced Ca^{2+} release have shown that the chloride stimulus varies with the concentration gradient of chloride across the internal membranes. Their results support the hypothesis that chloride-induced Ca^{2+} is due to a membrane depolarization. Kasai and Miyamoto (1976*a, b*) demonstrated that the replacement of methanesulfonate with chloride caused a rapid release of Ca^{2+} from isolated sarcoplasmic reticulum vesicles. Inesi and Malan (1976) have reported that Ca^{2+} can be released from isolated sarcoplasmic reticulum vesicles when potassium is exchanged for a less permeable cation. Meissner and McKinley (1976) have studied both anionic and cationic induced Ca^{2+} release of passively loaded SR[1] vesicles. They concluded that part or all of the ion-induced changes in the sarcoplasmic reticulum membrane permeability may be due to a massive influx of salt and water into the passively loaded vesicles, thereby causing osmotic swelling and increased membrane permeability.

The purpose of our investigation was fourfold: (1) the study of chloride-induced Ca^{2+} release from LSR and HSR which had actively accumulated Ca^{2+}, (2) the effects of osmotic buffering on the release of Ca^{2+} from LSR and HSR, (3) the effect of Na dantrolene on the release of Ca^{2+} by chloride-induced Ca^{2+} release from LSR and HSR, (4) correlation of the results on skinned fibers with the results on LSR and HSR. The data indicate the presence of a chloride-induced, Na dantrolene inhibited, Ca^{2+} release from the terminal cisternae vesicles. A preliminary report of this work has been presented (Campbell & Shamoo, 1977).

[1] *Abbreviations:* SR, sarcoplasmic reticulum vesicles; ATPase, adenosine triphosphatase; SDS, sodium dodecyl sulfate; EGTA, ethylene glycol bis (B-aminoethyl ether) N', N'-tetraacetic acid; LSR, light SR; ISR, intermediate SR; HSR, heavy SR; KMS, potassium methanesulfonate.

Materials and Methods

Ultra pure sucrose was obtained from Schwarz/Mann. Calcium-45 was obtained from Amersham/Searle. 3H-water and Omniflour were obtained from New England Nuclear. HEPES (N-2-hydroxethylipiperazine-N'-2-ethanesulfonic acid) and PIPES (piperazine-N-N'bis-2 ethane sulfonic acid) were obtained from Calbiochem. Millipore filters HAWP 025 (0.45 μm) and GSWP 025 (0.22 μm) were obtained from Millipore. All other reagents used were of analytical grade. All solutions were made with deionized distilled water.

Isolation of Light and Heavy Sarcoplasmic Reticulum Vesicles

Briefly, approximately 1,000 g of white rabbit leg muscle was homogenized in a Waring blender in 0.25 M sucrose for 30 sec. The homogenate was centrifuged twice to remove cell debris and mitochondria and was centrifuged a third time to sediment the SR vesicles. The SR vesicles were placed on a linear sucrose gradient, 26–45% (wt/wt), and centrifuged at $100{,}000 \times g$ for 16 h. Fractions containing 30–32.5% sucrose (LSR), 33.5–38% sucrose (Intermediate SR, ISR) and 38.5–42% sucrose (HSR) were diluted with 1.0 M KCl, 1 mM HEPES (pH 7.4). After 2 h incubation in 0.6 M KCl at 0 °C the purified SR vesicles were pelleted, homogenized in 0.25 M sucrose, 1 mM HEPES (pH 7.4), and stored in liquid nitrogen. This procedure is similar to Meissner (1975); for the detailed procedure *see* Campbell, Franzini-Armstrong, and Shamoo (1980).[2]

Assays

Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Calcium Uptake

$^{45}Ca^{2+}$ uptake was measured by the Millipore filtration method of Martonosi and Feretos (1964) using 0.45 μm filters. ATP-dependent Ca^{2+} uptake was measured at room temperature at a protein concentration of 0.2 mg/ml in a medium containing 0.1 M KCl, 5 mM $MgCl_2$, 0.05 mM $CaCl_2$ (9,000 cpm/nmol), 10 mM HEPES (pH 7.1) and 5 mM ATP (Meissner & Fleischer, 1971). The reaction mixture except ATP was incubated at room temperature for 30 min. Uptake was initiated by the addition of ATP and terminated after 8 min by passing the solution through a 0.45 μm filter. This pore size had already been shown to be suitable for Ca^{2+} uptake studies (Martonosi & Ferretos, 1964). The amount of accumulated Ca^{2+} is determined by measuring the $^{45}Ca^{2+}$ content of the complete medium and of the Millipore filtrate by scintillation counting. Ca^{2+} binding to the vesicles and filter was measured in the same manner, except ATP was omitted from the reaction mixture. Ca^{2+} uptake was calculated as the difference in ATP-dependent Ca^{2+} accumulation and Ca^{2+} binding. Calcium uptake is given as nmole of Ca^{2+} accumulated per mg of protein.

[2] Campbell, K.P., Franzini-Armstrong, C., and Shamoo, A.E. 1980. Further characterization of light and sarcoplasmic reticulum vesicles. Identification of the "SR. Feet" with heavy sarcoplasmic reticulum vesicles. *(submitted)*

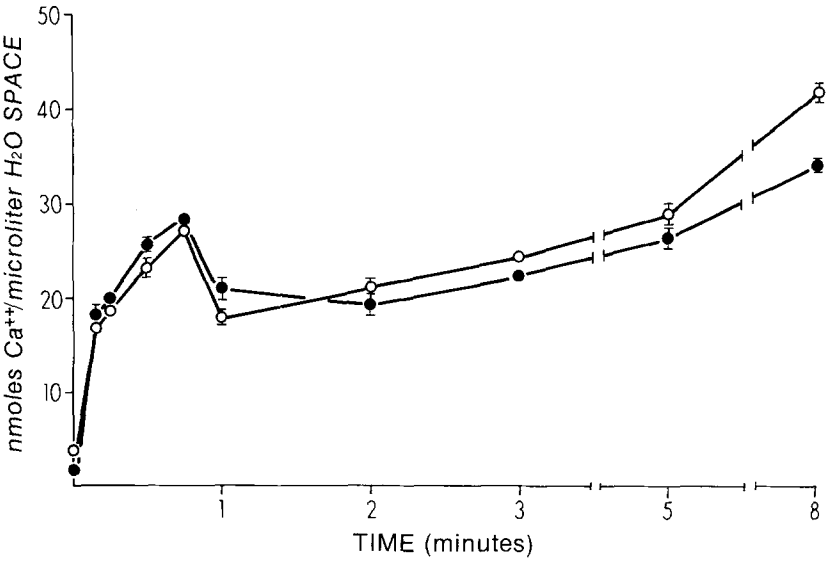

Fig. 1. Ca^{2+} uptake for LSR and HSR *vs.* time. The ordinate is nmol Ca^{2+} µl of water space, which was obtained by dividing the uptake value (nmol Ca^{2+}/mg of protein) by the vesicle volume (µl of H_2O/mg of protein for each type of SR vesicle). Ca^{2+} uptake was measured by the millipore filtration method (*see* Materials and Methods). The initial fast uptake of Ca^{2+} is followed by a fast release of 30–40% of the accumulated Ca^{2+}, which is followed by a slow phase of Ca^{2+} accumulation. The closed circles represent LSR and the open circles represent HSR. Vesicle volumes have been previously determined (Campbell et al., 1980)

Chloride-Induced Calcium Release

Sarcoplasmic reticulum vesicles (2 mg/ml) were preincubated in 150 mM K methanesulfonate (MS), 5 mM Mg sulfate, 10 mM HEPES (pH 6.8) and 50 µM $^{45}CaCl_2$ for 30–60 min at room temperature. Calcium uptake was initiated by the addition of 5 mM Na ATP. At set intervals, aliquots (1.0 ml) were taken and filtered through a Millipore filter (0.45 µm). The filtered vesicles were immediately washed with KMS buffer without calcium to measure uptake or KCl buffer without calcium to measure release. $^{45}Ca^{2+}$ remaining on the filter was measured, after drying the filters, by soaking the dried filters in 10 ml of scintillation fluid and counting in a liquid scintillation counter. An alternate method to study release involved dilution of the vesicles at set times, filtering an aliquot of diluted vesicles and, counting the filtrates for $^{45}Ca^{2+}$. Values given for Ca^{2+} uptake and release are the mean ± SEM for at least 3 determinations. Values of Ca^{2+} uptake and release are also given as nmol Ca^{2+}/mg ATPase or membrane protein which was calculated from the percent of 100,000-dalton ATPase in LSR and HSR measured from scans of SDS gels of LSR and HSR (Campbell et al., 1980).

The effect of sucrose on the chloride induced calcium release from sarcoplasmic reticulum vesicles as studied by the addition of solid sucrose to the potassium methanesulfonate and potassium chloride buffers. Ca^{2+} uptake and release was carried out as described with sucrose in all the solutions. The effects of Na dantrolene on the chloride induced Ca^{2+} release by the addition of Na dantrolene to the potassium methanesulfonate and potassium chloride buffers at a final concentration of 20 µM. Ca^{2+} uptake and release was carried out as described with Na dantrolene in all the solutions. The maximum concentration of Na dantrolene in salt solutions is 20 µM.

The specificity of Ca^{2+} release from light and heavy SR vesicles was measured by passively loading the vesicles with 10 mM $^{22}Na^+$ (1 µCi/ml) or 10 mM ^{14}C-sucrose (8 µCi/ml). The light and heavy sarcoplasmic reticulum vesicles were then incubated on ice for 36 h to equilibrate the sucrose or sodium. Calcium uptake and release were carried out as described, except cold calcium replaced $^{45}Ca^{2+}$. The amount of $^{22}Na^+$ or ^{14}C-sucrose retained in the vesicles was measured in the same way as $^{45}Ca^{2+}$ was measured.

Results

Calcium uptake for LSR and HSR is given in Fig. 1. The time course of Ca^{2+} uptake was biphasic for both LSR and HSR. It had an initial rapid phase of accumulation followed by a fast release of Ca^{2+}, followed by a slow phase of Ca^{2+} accumulation. The cause of the calcium release is unknown. It is possible that the second phase of accumulation was due to the formation of inorganic phosphate by the hydrolysis of ATP, which offers a sink for calcium during the accumulation by SR vesicles. At the peak of the initial phase of Ca^{2+} accumulation, the concentration of Ca^{2+} within the vesicles was almost 30 mM.

Chloride-Induced Ca^{2+} Release

Table 1 shows the change in Ca^{2+} retained by ISR when methanesulfonate was exchanged for chloride

Table 1. Effect of changing the ionic environment on calcium release from intermediate SR vesicles [a]

	nmol Ca^{2+}/mg	nmol Ca^{2+} released	% Ca^{2+} released
KMS to KMS	83.8 ± 0.8		
KMS to KCl	61.6 ± 0.5	22.7	27.0
KCl to KCl	84.4 ± 2.8		
KCl to KMS	82.8 ± 0.9	1.6	1.9

[a] The values given are the mean ± SEM for 3 determinations and are corrected for control values measured in the absence of ATP. Ca^{2+} retained by the vesicles was measured according to the Methods, using ISR which had accumulated Ca^{2+} for 8 min

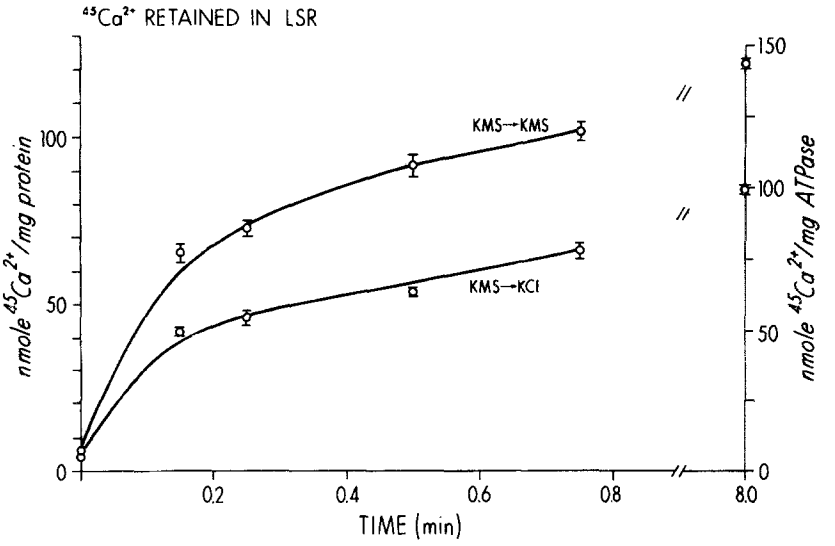

Fig. 2. The amount of Ca^{2+} retained in LSR *vs.* time of accumulation for Ca^{2+} uptake (KMS→KMS) and after chloride-induced Ca^{2+} release (KMS→KCl). The left-hand axis is nmol Ca^{2+}/mg protein and the right-hand axis is nmol Ca^{2+}/mg of ATPase in LSR. The amount of ATPase in the LSR was obtained from scans of SDS-gels (*see* Materials and Methods). Note the 8 min time points

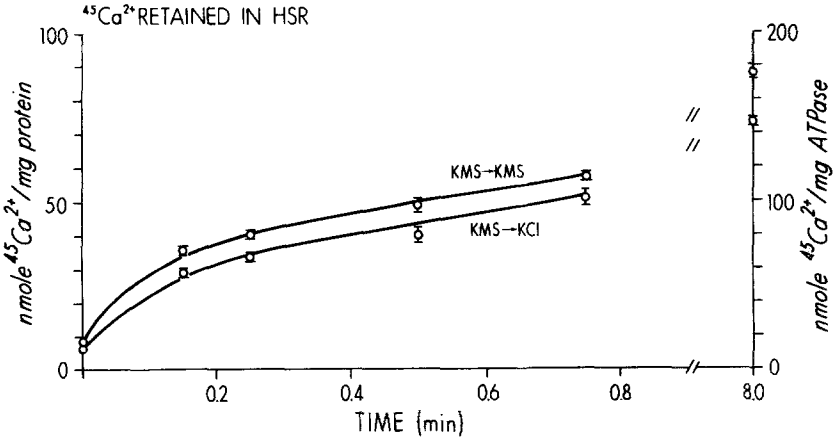

Fig. 3. The amount of Ca^{2+} retained in HSR *vs.* time of accumulation for Ca^{2+} uptake (KMS→KMS) and after chloride-induced Ca^{2+} release (KMS→KCl). The left-hand axis is nmol Ca^{2+}/mg protein and the right-hand axis is nmol Ca^{2+}/mg of ATPase in HSR. The amount of ATPase in the HSR was obtained from scans of SDS-gels (*see* Materials and Methods). Note the 8 min points

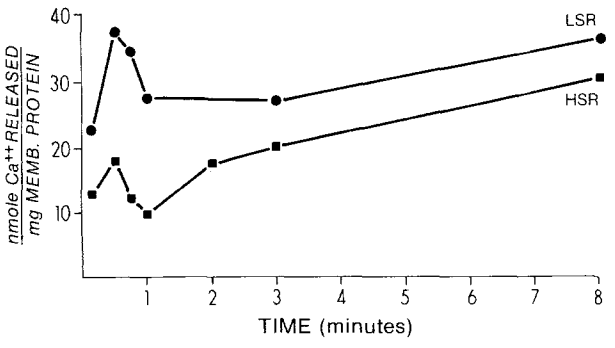

Fig. 4. The amount of Ca^{2+} released per mg of membrane protein (ATPase) by chloride-induced release *vs.* time of Ca^{2+} accumulation for LSR and HSR. The time course for release is similar to the time course for Ca^{2+} uptake in LSR and HSR (*see* Fig. 1). The amount of Ca^{2+} release was calculated from the difference between the amount of Ca^{2+} accumulated and the amount of Ca^{2+} retained after chloride-induced release

and when chloride was exchanged for methanesulfonate. The amount of Ca^{2+} accumulated for 8 min by the vesicles was independent of the monovalent anion in the medium. The amount of Ca^{2+} released by the methanesulfonate to chloride change is 22.7 nmol/mg which was 27% of the accumulated Ca^{2+}. The amount of Ca^{2+} released by the chloride to methane-sulfonate change was 1.6 nmol/mg, which was within the error on the measurement of Ca^{2+} uptake. It should be noted that including 0.5 mM EGTA in the washing solution caused an increase in the amount of Ca^{2+} release by chloride-induced release from 44% to 57% of the accumulated Ca^{2+}.

Figures 2 and 3 show the results of changing the ionic conditions for LSR and HSR, respectively. The amount of Ca^{2+} retained in the vesicles was plotted against time of Ca^{2+} accumulation for the methanesulfonate to methanesulfonate change and for meth-

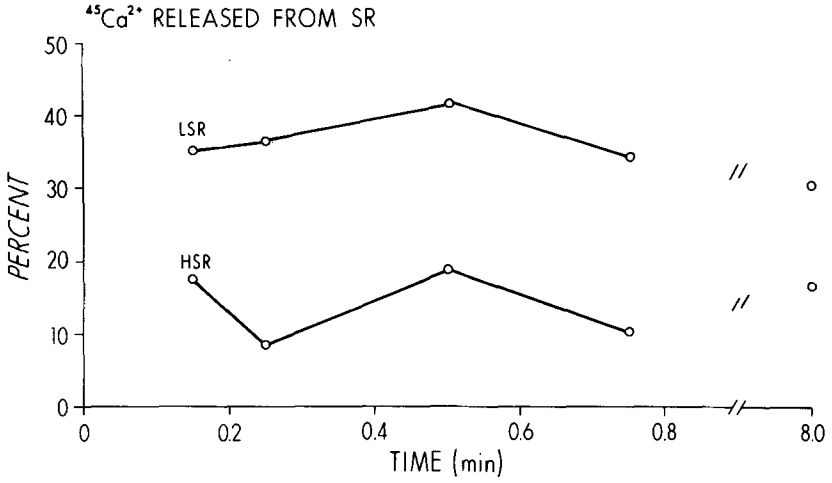

Fig. 5. The percent of Ca^{2+} released by chloride-induced release versus time of Ca^{2+} accumulation for LSR and HSR. The percent of Ca^{2+} release is relatively constant for LSR and HSR from 0 to 8 min. The percent of Ca^{2+} release was calculated from the amount of Ca^{2+} released and the total amount of Ca^{2+} taken up at a given time

anesulfonate to chloride change. The left axis is nmol Ca^{2+}/mg protein and the right-hand axis is nmol Ca^{2+}/mg of ATPase. This was calculated by dividing the nmol Ca^{2+}/mg of protein by the percentage of ATPase protein in the vesicles. The difference between the potassium methanesulfonate to potassium methanesulfonate and potassium methanesulfonate to potassium chloride curves was the amount of Ca^{2+} released. In the absence of ATP there was practically no difference in the Ca^{2+} retained by the vesicles under uptake or release conditions. At early times the amount of Ca^{2+} released by the LSR was much larger than that released by the HSR but after 8 min both types of vesicles release approximately the same amount of Ca^{2+} per mg of membrane protein or ATPase (30–35 nmol Ca^{2+}/mg of ATPase or membrane protein). Figure 4 shows the amount of Ca^{2+} released per mg of membrane protein *vs.* the time of Ca^{2+} accumulation. Both the LSR and HSR had a biphasic response, which is similar to the uptake curves.

Figure 5 shows the percent of accumulated Ca^{2+} released by LSR and HSR *vs.* the time of Ca^{2+} accumulation. The percent of accumulated Ca^{2+} released by LSR was practically double that released by the HSR. The percentage of Ca^{2+} released from LSR and HSR was relatively independent of the time of Ca^{2+} accumulation or the amount of Ca^{2+} accumulated.

Effect of Sucrose on Ca^{2+} Release

Table 2 shows the effect of sucrose on the accumulation and release of Ca^{2+} from ISR. Sucrose (100 mM) caused a 66% increase in the amount of Ca^{2+} accumulated. The amount of chloride-induced Ca^{2+} release went from 25 to 17.2 nmol/mg and the percent of Ca^{2+} released went from 35 to 14.5 in the presence of sucrose. The effect of 200 mM sucrose on the percentage of Ca^{2+} release from LSR was to reduce substantially the Ca^{2+} release (32 to 15%), whereas the release from the HSR seems to be unaffected by the presence of sucrose (17 to 15%). Therefore, it seems that osmotic swelling does not take place in HSR upon chloride-induced Ca^{2+} release.

Table 2. Effect of sucrose on chloride induced calcium release from intermediate SR vesicles[a]

	nmol Ca^{2+}/mg	nmol Ca^{2+} released	% Ca^{2+} released
5 mM sucrose			
KMS to KMS	71.6±1.0		
KMS to KCl	46.6±0.3	25.0	35.0
100 mM sucrose			
KMS to KMS	118.4±1.6		
KMS to KCl	101.2±1.0	17.2	14.5

[a] Values given are the mean±SEM for at least 3 determinations and are corrected for control values measured in the absence of ATP. Ca^{2+} uptake (KMS to KMS) and release (KMS to KCl) were measured according to the Methods, using ISR which had accumulated Ca^{2+} for 8 min.

$^{22}Na^+$ and ^{14}C-Sucrose Release

Table 3 shows the results of chloride-induced release on the amount of $^{22}Na^+$ or ^{14}C-sucrose retained by the LSR and HSR. The control values obtained for the amount of $^{22}Na^+$ or ^{14}C-sucrose retained by LSR and HSR were converted into Na^+ space/mg or sucrose space/mg by dividing by 10 mM, which was the concentration at which the vesicles were equilibrated. The Na space was 4.1 and 1.3 µl/mg for LSR and HSR, respectively. The sucrose space was 3.5 and 1.9 µl/mg for the LSR and HSR, respectively. These values are in agreement with those previously obtained for the internal water space of the LSR and HSR, 3.2 and 2.4 µl/mg (Campbell et al., 1980).

Table 3. $^{22}Na^+$ and ^{14}C-sucrose retained by light and heavy SR vesicles[a]

	LSR (nmol)	HSR (nmol)
$^{22}Na^+$		
KMS to KMS	41.0±1.0	13.0±0.1
KMS to KCl	26.0±0.7	10.0±0.3
Amount released	15	3
^{15}C-sucrose		
KMS to KMS	35.2±4.6	19.8±2.1
KMS to KCl	27.5±1.6	17.0±1.2
Amount released	7.7	2.8

[a] Values given are the mean±SEM for at least 3 determinations. LSR and HSR were passively loaded with 10 mM $^{22}Na^+$ or 10 mM ^{14}C-sucrose for 36 h at 0 °C. Ca^{2+} uptake and release were carried out using nonradioactive Ca^{2+}, and the amount of ^{22}Na or ^{14}C-sucrose retained in the vesicles was measured in the same way ^{45}Ca was measured (*see* Materials and Methods).

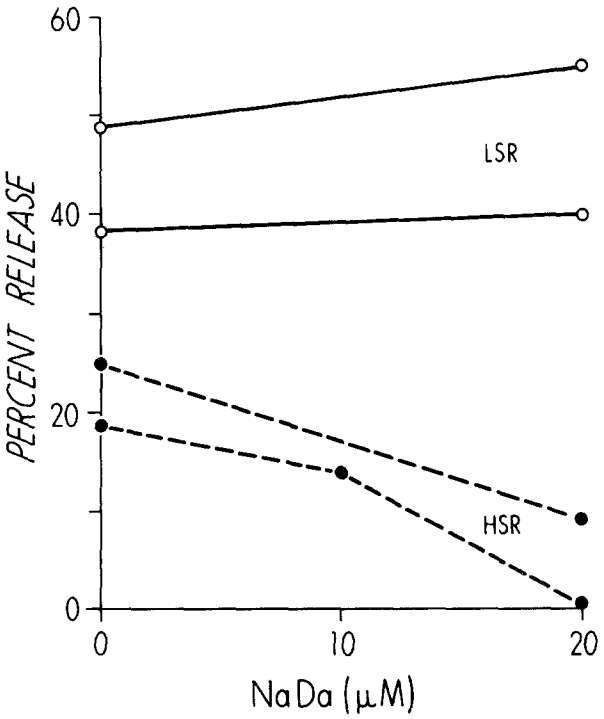

Fig. 6. Effect of Na dantrolene on the percent of chloride-induced Ca^{2+} release from LSR and HSR. Ca^{2+} uptake and release were carried out according to the experimental procedures with a given concentration of Na dantrolene in all solutions, and the SR vesicles were preincubated with the drug for 30 min at room temperature. Experiments for two preparations of LSR (solid line) and HSR (dashed line) are shown. Ca^{2+} release was measured after 8 min of Ca^{2+} accumulation

The amount of $^{22}Na^+$ released by the change from methanesulfonate to chloride was five times greater from the LSR than from the HSR. There was no significant difference in the amount of ^{14}C-sucrose released from LSR or HSR due to the large errors in the measurements of ^{14}C-sucrose retained by the SR vesicles.

Effects of Na Dantrolene on Ca^{2+} Uptake and Release

The binding of calcium to LSR and HSR was unaffected by the presence of 20 μM Na dantrolene. Ca^{2+} uptake by LSR was the same in the presence or absence of Na dantrolene. Na dantrolene did cause a 20% increase in the Ca^{2+} accumulated by the HSR. The increase in Ca^{2+} uptake was in the amount of Ca^{2+} accumulated, not in the rate of Ca^{2+} accumulation. We have previously shown that Na dantrolene decreased the passive efflux of Ca^{2+} from HSR while it did not affect LSR (Campbell et al., 1980).

Figure 6 shows the affect of Na dantrolene on the chloride-induced Ca^{2+} release from LSR and HSR. The drug did not affect the percent or amount of Ca^{2+} released by LSR, whereas the release of Ca^{2+} from HSR was reduced by approximately 50%. The figure shows the effect of the drug on two preparations of LSR and HSR each.

Discussion

Light and heavy sarcoplasmic reticulum vesicles have been shown to be derived from the longitudinal and terminal regions of the sarcoplasmic reticulum, respectively (Meissner, 1975; Campbell et al., 1980). They are both able to accumulate Ca^{2+} in the presence of ATP, which makes them ideally suitable to study the mechanism of Ca^{2+} release. It is not certain that the terminal cisternae is the sole source of Ca^{2+} during release, but it is clear from the morphological studies that the depolarization of the transverse tubular system initiates calcium release through a mechanism involving the junctional membrane of the terminal cisternae. Therefore, in the study of calcium release, it is interesting to compare the LSR and HSR because of their different origin in the sarcoplasmic reticulum.

We have found that actively accumulated Ca^{2+} could be released from both LSR and HSR by changing the anion outside the vesicles from methanesulfonate to chloride. The chloride-induced release of Ca^{2+} from LSR was inhibited by osmotic buffering (external sucrose), showed a large release of ^{22}Na, and was not affected by Na dantrolene, whereas the chloride-induced release of Ca^{2+} from HSR was not affected by osmotic buffering (external sucrose), showed a small release of ^{22}Na and was inhibited by Na dantrolene. These results indicate that the Ca^{2+} release from LSR and HSR is possibly caused by two different mechanisms (osmotic swelling or depolarization).

The release of Ca^{2+} induced by the change of the external anion from methanesulfonate to chloride could be caused by osmotic swelling or depolariza-

tion. Komentani and Kasai (1978) have shown that chloride was approximately 50 times more permeable to the SR membrane than methanesulfonate or potassium was. Thus, after the solution change, chloride diffuses into the vesicle faster than methanesulfonate diffuses out of the vesicles. This produces a net positive charge on the outside of the vesicle, which is called depolarization. Although an actual membrane potential change in the SR vesicles has not been measured upon chloride depolarization, there are some indications that one does take place. Fabiato and Fabiato (1977) have shown an increase of light absorption in a merocyanine dye by the replacement of potassium propronate with Tris chloride in the bathing fluid of a skinned muscle fiber. McKinley and Meissner (1978) have shown that a change in the ionic environment of isolated SR vesicles from potassium gluconate to Tris chloride causes a rapid decrease in fluorescence for di-C5-(3). Yet, there is some evidence against the existence of a chloride gradient across the membrane of the sarcoplasmic reticulum of the intact skeletal muscle (Somlyo, Shuman & Somlyo, 1977).

Osmotic swelling of the SR vesicles upon chloride-induced Ca^{2+} release has been shown by Meissner and McKinley (1976) to be due to an influx of potassium and chloride into the vesicles, thereby increasing the internal osmolarity and causing an increase membrane permeability, which is responsible for Ca^{2+} release. Meissner and McKinley (1976) showed that, when ionic composition of the solutions was such to cause a depolarization but no osmotic effects, Ca^{2+} release was not observed in passively loaded SR vesicles. Our experiments have dealt with chloride-induced release using actively located LSR and HSR vesicles in the presence of ATP. Our results agree with Meissner and McKinley (1976) in the case of LSR, but in the case of HSR, chloride-induced Ca^{2+} release seems not to be caused by osmotic swelling.

The chloride-induced release of Ca^{2+} from LSR, which was inhibited by osmotic buffering (external sucrose), showed a larger release of $^{22}Na^{+}$, and was not affected by Na dantrolene was probably due to osmotic swelling. The morphology of the LSR (Campbell et al., 1980) shows that the LSR consists of various shaped vesicles of different sizes with no internal protein, which indicates why the LSR are probably very prone to osmotic swelling.

An estimate of the specificity of calcium release from LSR and HSR can be calculated by dividing the amount of Ca^{2+} released/internal Ca^{2+} concentration by the amount of $^{22}Na^{+}$ release/internal $^{22}Na^{+}$ concentration. The results of these calculations gave a Ca^{2+}/Na^{+} ratio of 0.6 and 2.5 for the LSR and HSR, respectively. The release of Ca^{2+} from HSR was more specific for Ca^{2+} than the release from LSR. The chloride-induced release of Ca^{2+} from HSR which was not affected by osmotic buffering, more specific for Ca^{2+} than Na^{+} and inhibited by Na dantrolene, was probably caused by a chloride depolarization of HSR. Osmotic swelling seems less likely the cause of Ca^{2+} release in HSR due to the inability of sucrose to inhibit the release, the specificity of the release, and the inhibition effect of Na dantrolene. Also, the morphology of the HSR, which are rounded vesicles of uniform size filled with electron dense material (calsequestrin, *see* Campbell et al., 1980) suggests that the HSR would be more resistant to osmotic swelling than the LSR. An estimate of the concentration of calsequestrin within the HSR [knowing the amount of calsequestrin and the water volumes (Campbell et al., 1980)] ranges from 100–200 mg/ml. This high concentration of protein within the vesicles possibly offers the HSR some resistance to osmotic swelling due to Donnan effects.

The ability of Na dantrolene to inhibit the release of Ca^{2+} from the HSR is very interesting because Na dantrolene is a known inhibitor of calcium release (Desmedt & Hainaut, 1977). Na dantrolene also decreased the Ca^{2+} permeability of the HSR membrane since it increased the Ca^{2+} accumulated by the HSR. We have previously shown that Na dantrolene could decrease the passive Ca^{2+} permeability of HSR but not LSR (Campbell et al., 1980). These results support the work of Desmedt and Hainaut (1977) on barnacle muscle fibers injected with aequorin. They have shown that dantrolene inhibits rather selectively the Ca^{2+} release mechanism for the sarcoplasmic reticulum. In addition, the resting Ca^{2+} efflux from the sarcoplasmic reticulum is decreased by dantrolene which is consistent with the increased uptake by HSR.

The fact that the percent of Ca^{2+} released from both LSR and HSR was a constant was consistent with the skinned fiber results of Thorens and Endo (1975). They showed that the amount of Ca^{2+} released is a constant fraction of the amount of Ca^{2+} within the sarcoplasmic reticulum immediately before chloride-induced Ca^{2+} release. The inhibition of the release of Ca^{2+} from the LSR by sucrose is also in agreement with the work of Thorens and Endo (1975), although skinned fibers can be inhibited by a lower concentration of sucrose. It is known that in a resting muscle fiber the Ca^{2+} is localized in the terminal cisternae, but it seems probable in skinned muscle fibers, after loading the SR with buffered Ca^{2+} solutions, that both the longitudinal and terminal cisternae are filled with Ca^{2+}. In addition, Na dantrolene does not seem to affect the release of Ca^{2+} in skinned muscle fibers (Endo, 1977), but it does affect the release of Ca^{2+} from the HSR. Therefore, the ability of sucrose to inhibit the release of Ca^{2+} from LSR and the ability of Na dantrolene

to inhibit the release of Ca^{2+} from HSR suggests that in skinned fiber experiments most of the calcium released by chloride originates from the longitudinal sarcoplasmic reticulum. The fact that chloride-induced release can function under conditions of low Ca^{2+} uptake supports the hypothesis that the terminal cisternae can release Ca^{2+} upon chloride depolarization.

Finally, it is important to point out that the chloride-induced release experiments were all performed on SR vesicles in which calcium was taken up using ATP. This is important for two reasons: (1) endo and Kitazawa (1976) have shown that the amount of calcium released by the SR from chloride depolarization was less in the absence of ATP than in its presence; (2) the response of the SR to chloride depolarization will probably be different according to whether the SR has been passively loaded or actively loaded with Ca^{2+}, due to the possible electrogenicity of the Ca^{2+} pump (Zimniak & Racker, 1978).

The results of these experiments suggest that the HSR, which are derived from the terminal cisternae, are able to release Ca^{2+} upon chloride depolarization, that this release is more specific in HSR than in LSR, and that the HSR release is inhibited by Na dantrolene. Future experiments using ionic depolarization of HSR and inhibitors of Ca^{2+} release seem to be a promising approach to the study of Ca^{2+} release.

This paper is based on work performed under contract with the U.S. Department of Energy at the University of Rochester, Department of Radiation Biology and Biophysics, and has been assigned Report No. UR-3490-1639. This paper was also supported by a Grant # R01 18892 from the N.I.H., Program Project ES-10248 from NIEHS, and by a grant from MDA. Kevin Campbell was Elon Huntington Hooker Graduate Fellow. This work was submitted (by K.P.C.) to the Graduate School of the University of Rochester in partial fulfillment of the requirements for a Ph.D. degree. Adil E. Shamoo is an Established Investigator of the American Heart Association.

References

Baylor, S.M., Oetliker, H. 1975. Birefringence experiments on isolated skeletal muscle fibres suggest a possible signal from the sarcoplasmic reticulum. *Nature (London)* **253**:97

Bezanilla, F., Horowicz, P. 1975. Fluorescence intensity changes associated with contractile activation in frog muscle stained with Nile Blue A. *J. Physiol. (London)* **246**:709

Campbell, K.P., Shamoo, A.E. 1977. Depolarization induced calcium release from light and heavy sarcoplasmic reticulum vesicles. *Biophysical J.* **17**:200a

Constantin, L.L., Taylor, S.R. 1973. Graded activation in frog muscle fibres. *J. Gen. Physiol.* **61**:424

Desmedt, J.E., Hainaut, K. 1977. Inhibition of the intracellular release of calcium by Dantrolene in barnacle giant muscle fibres. *J. Physiol. (London)* **265**:565

Ebashi, S., Endo, M. 1968. Calcium ions and muscle contraction. *Prog. Biophys. Mol. Biol.* **18**:123

Endo, M. 1977. Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.* **57**:1.71

Endo, M., Blinks, J.R. 1973. Inconstant association of aequorin luninescence with tension during calcium release in skinned muscle fibres. *Nature (London) New Biol.* **246**:218

Endo, M., Kitazawa, T. 1976. The effect of ATP on the calcium release mechanisms in the sarcoplasmic reticulum of skinned skeletal muscle. *Proc. Jpn. Acad.* **52**:595

Fabiato, A., Fabiato, F. 1977. Variations of the membrane potential of the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscle detected with a potential-sensitive dye. *J. Gen. Physiol.* **70**:6a

Franzini-Armstrong, C. 1975. Membrane particles and transmission at the triad. *Fed. Proc.* **34**:1382

Fuchs, F. 1974. Striated Muscle. *A. Rev. Physiol.* **36**:461

Hodgkin, A.L., Horowicz, P. 1960. The effect of nitrate and other anions on the mechanical response of single muscle fibres. *J. Physiol. (London)* **153**:404

Inesi, G., Malan, N. 1976. Mechanisms of calcium release in sarcoplasmic reticulum. *Life Sci.* **18**:773

Kasai, M., Miyamoto, H. 1976*a*. Depolarization-induced calcium release from sarcoplasmic reticulum fragments. I. Release of calcium taken up upon using ATP. *J. Biochem.* (Tokyo) **79**:5.1053

Kasai, M., Miyamoto, H. 1976*b*. Depolarization-induced calcium release from sarcoplasmic reticulum fragments. II. Release of calcium incorporated without ATP. *J. Biochem. (Tokyo)* **79**:5.1067

Kometani, T., Kasai, M. 1978. Ionic permeability of sarcoplasmic reticulum vesicles measured by light scattering method. *J. Membrane Biol.* **41**:295

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**:265

Martonosi, A., Ferreros, R. 1964. Sarcoplasmic reticulum I. The uptake of Ca^{2+} by sarcoplasmic reticulum fragment. *J. Biol. Chem.* **239**:648

Meissner, G. 1975. Isolation and characterization of two types of sarcoplasmic reticulum vesicles. *Biochim. Biophys. Acta* **389**:51

Meissner, G., Fleischer, S. 1971. Characterization of sarcoplasmic reticulum from skeletal muscle. *Biochim. Biophys. Acta* **241**:356

Meissner, G., McKinley, D. 1976. Permeability of sarcoplasmic reticulum membrane. The effect of changed ionic environments on Ca^{2+} release. *J. Memrane Biol.* **30**:1.79

Nakajima, Y., Endo, M. 1973. Release of calcium induced by depolarization of the sarcoplasmic reticulum membrane. *Nature New Biol.* **246**:216

Sandow, A. 1970. Skeletal Muscle. *A Rev.* **17**:87

Somlyo, A.V., Shuman, H., Somlyo, A.P. 1977. Composition of sarcoplasmic reticulum *in situ* by electron probe X-ray microanalysis. *Nature (London)* **268**:566

Stephenson, E.W., Poldolsky, R.J. 1977. Influence of magnesium on chloride-induced calcium release in skinned musle fibers. *J. Gen. Physiol.* **69**:17

Thorens, S., Endo, M. 1975. Calcium-induced calcium release and depolarization-induced calcium release: Their physiological significance. *Proc. Jpn. Acad.* **51**:473

Zimniak, P., Racker, E. 1978. Electrogenicity of Ca^{2+} transport catalyzed by the Ca^{2+}ATPase from sarcoplasmic reticulum. *J. Biol. Chem.* **253**:4631

Received 1 October 1979; revised 4 December 1979