Proc. Natl. Acad. Sci. USA
Vol. 78, No. 8, pp. 4828–4832, August 1981
Biochemistry

A proton gradient controls a calcium-release channel in sarcoplasmic reticulum

(excitation–contraction coupling/skinned muscle fibers/proton ionophores/proteolipid)

VARDA SHOSHAN*†, DAVID H. MACLENNAN*‡, AND DONALD S. WOOD§

*Banting and Best Department of Medical Research, Charles H. Best Institute, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6; and §H. Houston Merritt Clinical Research Center, Department of Neurology, Columbia University, New York, New York 10032

Communicated by David E. Green, May 1, 1981

ABSTRACT **Sarcoplasmic reticulum vesicles from mammalian skeletal muscle have previously been shown to develop a proton gradient (alkaline inside) of 0.15–0.5 pH units during active Ca^{2+} uptake. We found that dissipation of this gradient by the proton ionophores gramicidin, nigericin, and carbonyl cyanide *p*-trichloromethoxyphenylhydrazone caused a rapid transient tension in skinned rabbit psoas muscle fibers. Increases, but not decreases, in medium pH of ≈0.2 units over the range from pH 6.5 to pH 7.5 also elicited transient tensions. In isolated vesicles, physiological levels of Ca^{2+} (3.3 μM), inhibited pH-induced Ca^{2+} release. Dicyclohexylcarbodiimide blocked pH- and ionophore-induced Ca^{2+} release under conditions in which it could bind to sarcoplasmic reticulum proteins but did not inhibit Ca^{2+} uptake. We propose that a proton gradient generated across sarcoplasmic reticulum membranes during Ca^{2+} uptake maintains a Ca^{2+} release channel in a closed conformation and that dissipation of this gradient permits the Ca^{2+} release channel to open. We further propose that elevated myoplasmic Ca^{2+} also causes the Ca^{2+} channel to close, permitting Ca^{2+} uptake through Ca^{2+}/Mg^{2+}-ATPase to function effectively. As the proteolipids of sarcoplasmic reticulum bind dicyclohexylcarbodiimide under conditions in which Ca^{2+} release is blocked and as they have previously been shown to have Ca^{2+} ionophoric activity, we propose that the Ca^{2+}-release channel either resides in the proteolipids or is controlled by H^+ fluxes through the proteolipids.**

The sarcoplasmic reticulum controls myoplasmic Ca^{2+} concentrations, thereby regulating the contraction–relaxation cycle of skeletal muscle (1). Calcium uptake is mediated by enzymatic reactions of Ca^{2+}/Mg^{2+}-ATPase (2). Although these reactions are reversible, permitting Ca^{2+} efflux through the ATPase, this mechanism of Ca^{2+} release is unlikely to have a major role in excitation–contraction coupling because it requires concentrations of ATP lower than physiological and because the rate of Ca^{2+} release is 1/100 to 1/10,000 that of the rate of contractile activation (3).

No other pathway for Ca^{2+} efflux has been identified, but Ca^{2+} release, not involving ATPase, can be induced in isolated sarcoplasmic reticulum vesicles and skinned fibers. For example, increasing myofilament space Ca^{2+} or changing Cl^- for propionate$^-$ elicits Ca^{2+} release and a rapid transient tension in skinned muscle fibers, (4, 5). Ca^{2+} release by either manipulation is not blocked by quercetin, which inhibits both the forward and backward reactions of Ca^{2+}/Mg^{2+}-ATPase. (6). The molecular mechanisms for either Ca^{2+}- or anion-induced release are unknown. Ca^{2+}-induced release occurs under physiological conditions in cardiac muscle preparations (7, 8). In skeletal muscle, there is evidence against this mechanism having a major role in excitation–contraction coupling (9), including a requirement in the reaction for Ca^{2+} levels higher than physiological (4). Anion-induced Ca^{2+} release has been studied as a possible model for effects of depolarization on sarcoplasmic reticulum. However, it has been difficult to determine whether depolarization or some other effect, such as osmotic change, causes Ca^{2+} release (4, 10).

Recent studies suggesting that a H^+ gradient develops during Ca^{2+} uptake have led us to assess a possible physiological role for H^+ in Ca^{2+} release from skeletal muscle sarcoplasmic reticulum. Madeira (11) reported that formation of a transmembrane H^+ gradient (alkaline inside) is an early event in Ca^{2+} transport. Chiesi and Inesi (12) found that H^+ is exchanged for Ca^{2+} during the initial Ca^{2+} uptake phase, and Meissner (13) has postulated that countermovement of H^+, K^+, and Na^+ may nullify the membrane potential formed during active Ca^{2+} uptake. The H^+ gradient formed is 0.15–0.5 pH units (11, 14). Nakamaru and Schwartz (15) found that increasing the pH of the medium by 0.5–1 unit caused Ca^{2+} release from isolated sarcoplasmic reticulum vesicles. In skinned muscle fibers, increasing the pH has also been reported to induce Ca^{2+} release. However, Endo (4) found Ca^{2+} release only from fibers that were heavily loaded with Ca^{2+}. Fabiato and Fabiato (16) suggested that, as the Ca^{2+} loading capacity of sarcoplasmic reticulum is pH sensitive, effects of pH change on Ca^{2+} release could be indirect. Possible stimulation of Ca^{2+} release by pH changes of 0.15–0.5 unit has not been studied in skinned fibers, although net Ca^2 accumulation has been shown to vary in this range (7).

In this paper, we present observations on skinned fibers and sarcoplasmic reticulum vesicles isolated from skeletal muscle suggesting that dissipation of a H^+ gradient developed during active Ca^{2+} uptake causes a rapid transient Ca^{2+} release. The speed and extent of Ca^{2+} release suggest that it may be a physiologically relevant process.

EXPERIMENTAL PROCEDURES

Materials. ATP, Tris, imidazole, carbonyl cyanide *p*-trichloromethoxyphenylhydrazone (CCCP), and gramicidin were purchased from Sigma, dicyclohexylcarbodiimide (DCCD) was from Eastman, propionic acid was from Fisher, and $^{45}CaCl_2$ was from New England Nuclear. Nigericin was a gift from Eli Lilly.

Preparation. Sarcoplasmic reticulum vesicles were prepared from rabbit psoas muscle as described by MacLennan (17) except that an additional sucrose gradient step was used in the final purification (18). Chemically skinned psoas muscle fibers were prepared as described by Wood *et al.* (19).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: DCCD, dicyclohexylcarbodiimide; CCCP, carbonyl cyanide *p*-trichloromethoxyphenylhydrazone; EGTA, ethylene glycolbis(β-aminoethyl ether)-*N*, *N*, *N'*, *N'*-tetraacetic acid.

† Present address: Department of Biology, Ben Gurion University, Beer Sheva, Israel.

‡ To whom reprint requests should be addressed.

Assays. Ca^{2+} uptake was assayed by the millipore filtration method (17). Ca^{2+} release was analyzed in the same system except that release was initiated by a rapid change in buffer. Protein was determined according to Lowry *et al.* (20). In skinned fibers, Ca^{2+} release was assayed by measuring isometric tension. Procedures and solutions for Ca^{2+} uptake were as described by Wood (21). Ca^{2+} release was initiated by adding the ionophores to 185 mM K propionate/2.5 mM Mg^{2+}/2.5 mM ATP/10 mM imidazole, pH 7.00 ± 0.02. In experiments on pH-induced release, the solution pH changes were made by adding propionic acid or KOH as needed. The concentration of free Ca^{2+} in the Ca^{2+}/ethylene glycol bis(β-aminoethyl ether)-*N,N,N*^{1},*N*^{1}-tetraacetic acid (EGTA) used for loading the sarcoplasmic reticulum was calculated with a computer program for solving the multiple equilibria equations for Ca^{2+}, Mg^{2+}, EGTA, and ATP. An apparent K_a of 2×10^6 M^{-1} at pH 7.00 was used for Ca^{2+}/EGTA (22).

Tension Measurements in Skinned Fibers. The experimental apparatus has been described (23). A single skinned fiber, 0.5- to 1.0-mm long, was placed in a temperature-controlled, vigorously stirred bath at 21°C. Each end of the fiber was secured in a micro-pinch clamp, one of which was attached to a strain gauge mounted on a micromanipulator. Fibers were stretched from 110% to 120% of slack length. Isometric tension was recorded with a mechanoelectronic transducer (Grass Instruments) using a Gould Brush recorder.

RESULTS

Induction of Ca^{2+} Release by pH Changes. Fig. 1 shows that when sarcoplasmic reticulum vesicles were permitted to carry out ATP-dependent Ca^{2+} uptake and then the pH was suddenly increased, Ca^{2+} was released from the Ca^{2+}-loaded vesicles. This confirms observations of Nakamaru and Schwartz (15). More Ca^{2+} was released when the pH was increased by 1 unit than when it was increased by 0.5 unit but the rate of Ca^{2+} release was very rapid under both conditions; $t_{1/2} < 10$ sec. EGTA more than doubled the amount of Ca^{2+} released after a pH increase but was relatively ineffective when the medium was maintained at pH 6.9.

H^+ Regulated Ca^{2+} Release in Skinned Fibers. To evaluate whether a H^+ gradient developed during active Ca^{2+} uptake is involved in physiological Ca^{2+} release, we challenged skinned

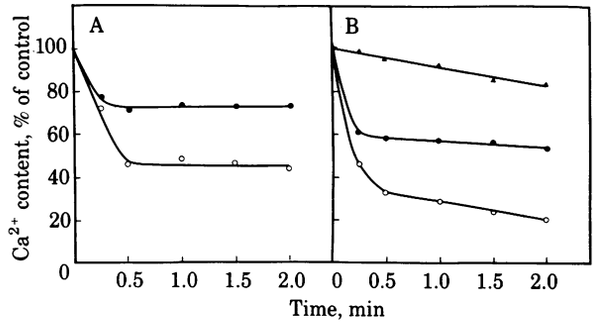

FIG. 1. Release of Ca^{2+} from sarcoplasmic reticulum vesicles by a pH increase. Ca^{2+} release was measured with Ca^{2+}-loaded vesicles. The reaction mixture for Ca^{2+} uptake was 20 mM Tris·maleate, pH 6.8/100 mM KCl/5 mM $MgCl_2$/5 mM ATP/0.1 mM EGTA/0.1 mM $CaCl_2$, (containing ^{45}Ca, 2×10^7 cpm/μmol; free Ca^{2+} = 3.3 μM) and sarcoplasmic reticulum vesicles at 140 μg/ml. After 6 min at 22°C, the pH of the medium was increased to 7.4 (*A*) or 7.9 (*B*) by the addition, respectively, of 5 or 15 μl of 1 M Tris base per ml of assay medium. At the times indicated, samples were assayed for Ca^{2+} content by millipore filtration. Additions: ▲, 1 mM EGTA: ●, Tris base; ○, 1 mM EGTA/Tris base. Control uptake activity at 6 min (100%) was 0.27 μmol of Ca^{2+}/mg of protein.

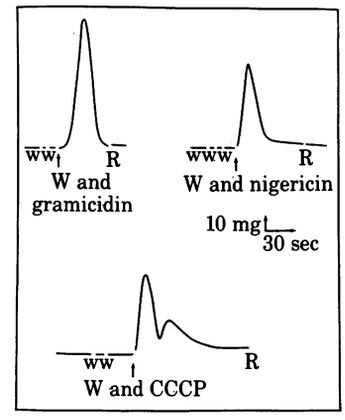

FIG. 2. Proton ionophore-induced tension in skinned fibers. In separate experiments, skinned fibers (70- or 75-μm diameter) were bathed in EGTA/Ca^{2+} (free Ca^{2+} = 0.1 μM) for 1 min to permit Ca^{2+} uptake by the sarcoplasmic reticulum. After the period for Ca^{2+}-loading, a wash solution (W) was used to rinse the fiber and remove EGTA/Ca^{2+}. The wash solution contained 0.67% ethanol, which did not elicit tension. An ethanolic (0.67%) wash solution of gramicidin (6.7μg/ml), CCCP (6.7 μM), or nigericin (6.7 μg/ml) caused tension development. R, relaxing solution containing 5 mM EGTA.

fibers with three H^+ ionophores, CCCP and gramicidin, which are H^+ carriers, and nigericin, which exchanges H^+ for K^+ (24–26). Fig. 2 shows that, at neutral pH, each ionophore elicited a rapid transient tension. The amount of ionophore required to elicit tension varied from fiber to fiber. The data shown represent the lowest effective level for CCCP and nigericin under the Ca^{2+}-loading conditions used—a 1-min exposure of the fiber to EGTA/0.1 μM free Ca^{2+}. Effects of gramicidin were observed at 1.0–10 μg/ml.

To estimate whether the H^+ gradient across the sarcoplasmic reticulum of skinned fibers was of the same magnitude (0.15–0.5 pH units) and direction (alkaline inside) as that in isolated vesicles, the effects of changes in bath pH were evaluated. Fig. 3 shows that, when the bath pH was increased from 7.00 to 7.22, a rapid transient tension was obtained. Decreases in pH of up to 0.7 units never elicited tension. These data on skinned fibers are consistent with predictions from studies of isolated vesicles that a H^+ gradient of ≈0.2 pH units (alkaline inside) is generated across the sarcoplasmic reticulum membrane and that rapidly reducing the gradient by increasing medium pH stimulates Ca^{2+} release.

In analyzing the tensional responses of skinned fibers to in-

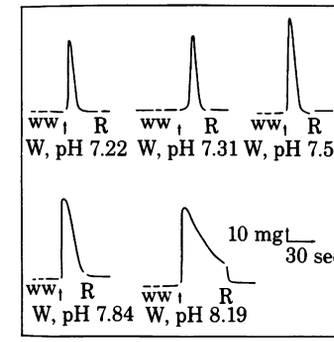

FIG. 3. pH-Induced tension in a single skinned fiber. Ca^{2+} loading of the sarcoplasmic reticulum was as described in the Legend to Fig. 2 (fiber diameter, 70 μM). Loading and rinses with wash solution (W) were at pH 7.00 ± 0.02. Tensions were elicted by adding KOH to the wash solution to the indicated pH. pH 7.10 did not elicit tension (not shown). R, relaxing solution containing 5 mM EGTA.

creases in medium pH, however, two other effects of increased pH must be considered: depression of Ca^{2+}/Mg^{2+}-ATPase activity and increased tensional response of contractile proteins to Ca^{2+} (16, 27). As shown in Fig. 3, pH changes up to pH 7.55 increased tension amplitudes and increases up to pH 8.19 caused pronounced delays in relaxation. The increased tension amplitude is consistent with effects of increased pH on contractile proteins and the delayed relaxation is consistent with decreased Ca^{2+} reuptake by the sarcoplasmic reticulum at elevated pH. It is unlikely, however, that depression of Ca^{2+} reuptake by ATPase could account for the relatively rapid tension development observed within the limits of the recording system. Half-maximal tension occurred in ≈0.6 sec at each pH. Efflux of Ca^{2+} from isolated sarcoplasmic reticulum vesicles is relatively slow when Ca^{2+} uptake ceases due to ATP depletion (3). In skinned fiber preparations from rabbit psoas muscle, there is also a relatively slow Ca^{2+} loss after substrate removal with a half-time of 5–7 min (ref. 28; unpublished observations).

To determine whether a H^+ gradient across the sarcoplasmic reticulum membrane or a specific H^+ concentration at the outer membrane surface was involved in regulation of Ca^{2+} release, we evaluated the effects of pH changes after Ca^{2+} loading at various H^+ concentrations (Fig. 4). After Ca^{2+} uptake had proceeded at pH 6.5–7.5, an abrupt increase in bath pH of ≈0.2 units always caused Ca^{2+} release and tension development. Because pH affects the K_a for Ca^{2+}/EGTA (16, 29) and also the activity of Ca^{2+}/Mg^{2+}-ATPase, it is unlikely that the Ca^{2+} load in the sarcoplasmic reticulum was the same in each case. Nonetheless, the results rule out the possibility that release is controlled by a specific H^+ concentration at the outer surface of the membrane and are consistent with the postulate that a H^+ gradient regulates Ca^{2+} release.

Effect of Ca^{2+} on pH-Induced Ca^{2+} Release. H^+ ionophore- and pH-induced Ca^{2+} release was always transient, suggesting that the H^+-regulated Ca^{2+} channels opened briefly and then closed under our experimental conditions. pH-Induced Ca^{2+} release could be transient due to redevelopment of the H^+ gradient during Ca^{2+} reuptake. Transient tensions obtained with H^+ ionophore-induced Ca^{2+} release, however, cannot be so readily explained because the presence of the ionophore should inhibit redevelopment of a H^+ gradient. We considered, therefore, that the increasing Ca^{2+} concentration in the myofilament space might also be involved in regulation of Ca^{2+} release. We could not test this point directly in skinned fibers because the need to use EGTA to control medium Ca^{2+} depressed tensional responses due to Ca^{2+} efflux from the sarcoplasmic reticulum. However, we were able to evaluate the effect of Ca^{2+} on Ca^{2+} release from isolated vesicles. As shown in Fig. 5, a physiological concentration of free Ca^{2+} (3.3 μM) in the release medium decreased by about 40% the total amount of Ca^{2+} released by an increase in pH. Fig. 1 also shows that EGTA in the medium enhanced Ca^{2+} release from isolated vesicles, presumably by decreasing the external free Ca^{2+} concentration. Ca^{2+} itself induced some Ca^{2+} release at pH 6.9, but this was only a small fraction of that released by increased pH.

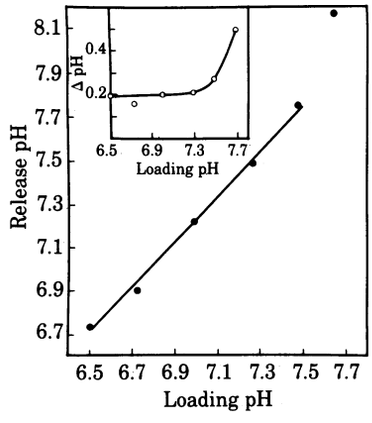

FIG. 4. Relationship between loading pH and release pH on tension development in skinned fibers. After the Ca^{2+} loading and rinse steps with wash solution at the loading pH, the fiber was challenged with wash solution at a higher pH (release pH). Data are the smallest pH increases that elicited tension at each load condition (pH steps of 0.10 ± 0.02 were tested). (*Inset*) Δ pH difference between the pH required for triggering Ca^{2+} release and the Ca^{2+} loading pH vs. pH of the loading solution.

DCCD Blocks pH- or H^+ Ionophore-Induced Ca^{2+} Release. DCCD, an effective inhibitor of H^+ translocation in several membranes (30–35), inhibits the activity of Ca^{2+}/Mg^{2+}-ATPase (36). Fig. 6*A* shows that incubation of skinned fibers with DCCD for 15 min at pH 6.5 in the presence of EGTA led to complete inhibition of gramicidin- and pH-induced Ca^{2+} release. Fig. 6*B* shows that Ca^{2+} in the incubation medium prevented the inhibition by DCCD of both gramicidin- and pH-induced Ca^{2+} release. DCCD inhibition was pH dependent and was not observed when the fibers were incubated with DCCD at pH 7.5 in the presence of EGTA. Inhibition was also time dependent, being observed at 5 min but not at 2 min. DCCD did not inhibit caffeine-induced tensions, indicating that Ca^{2+} uptake was not blocked and that contractile protein function remained under the experimental conditions used (data not shown).

DISCUSSION

Of the events leading to skeletal muscle contraction, those involved in Ca^{2+} release from the sarcoplasmic reticulum are the least understood. Our new observations, however, permit us to develop a hypothesis for the mechanism of Ca^{2+} release from the sarcoplasmic reticulum.

Control Mechanism for the Ca^{2+}-Release Channel. Previous studies have shown that H^+ are released from isolated sarcoplasmic reticulum vesicles during Ca^{2+} uptake, resulting in the formation of a transmembrane H^+ gradient (11, 12, 14, 37). This gradient could then serve as a controlling element for a Ca^{2+}-release channel.

We have shown that the H^+ ionophores gramicidin, nigericin, and CCCP trigger Ca^{2+} release from the sarcoplasmic reticulum of skinned skeletal muscle fibers (Fig. 2). Each iono-

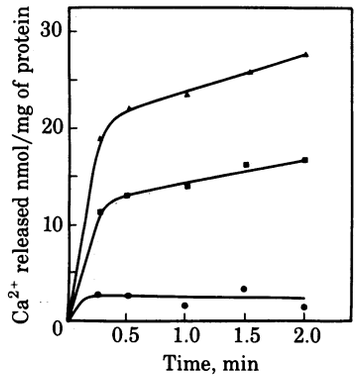

FIG. 5. Ca^{2+} Inhibition of pH-induced Ca^{2+} release from isolated sarcoplasmic reticulum vesicles. Conditions for the uptake and release of Ca^{2+} were as described in the Legend to Fig. 1. Where indicated, $^{45}CaCl_2$ at the same specific activity as used in the uptake step was added together with the Tris base. The total Ca^{2+} accumulated by the vesicles before Ca^{2+} release was induced was 50 nmol/mg of protein. Conditions: ▲, pH 7.9; ■, pH 7.9, $^{45}CaCl_2$; ●, pH 6.9, $^{45}CaCl_2$.

Correction. In the article "A proton gradient controls a calcium-release channel in sarcoplasmic reticulum" by Varda Shoshan, David H. MacLennan, and Donald S. Wood, which appeared in the August 1981 issue of *Proc. Natl. Acad. Sci. USA* (78, 4828–4832), the authors request that the following correction be noted. In the experiment reported in Fig. 5 and discussed on pages 4830 and 4831, the concentration of Ca^{2+} used to inhibit Ca^{2+} release was, in fact, 100 μM not 3.3 μM as reported. Consequently, although Ca^{2+} release was measurably reduced by Ca^{2+} in the experiments of Fig. 5, the data neither support nor preclude the possibility that physiological Ca^{2+} levels ($\leq$10 μM) can inhibit Ca^{2+} release.

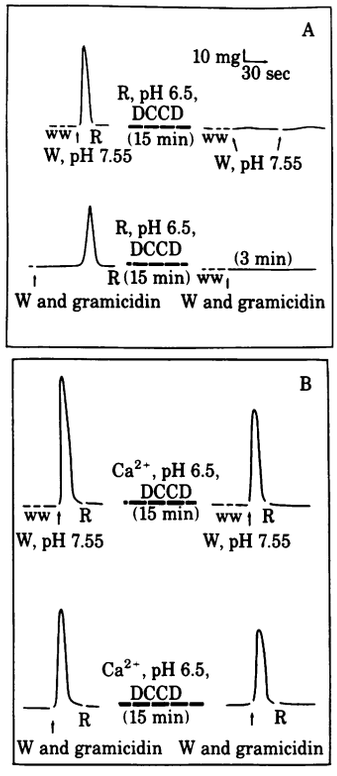

FIG. 6. DCCD inhibition of Ca^{2+} release in skinned fibers. (*A*) Effect of 15-min incubation with 50 μM DCCD on pH-and gramicidin-induced Ca^{2+} release. Incubation was at pH 6.5 in relaxing solution (R) containing 170 mM potassium propionate/2.5 mM Mg^{2+}/2.5 mM ATP/5 mM EGTA/10 mM imidizole. (*B*) The same as in *A* except that the DCCD incubation solution contained 0.1 μM free Ca^{2+}. W, wash solution.

phore can dissipate a H^+ gradient, but nigericin could also affect K^+ distribution. We interpret these results as indicating that a H^+ gradient is generated across the sarcoplasmic reticulum membrane during Ca^{2+} uptake in skinned fibers and that dissipation of this gradient triggers Ca^{2+} release. Increasing the pH of the medium by $\approx$0.2 unit in the range from pH 6.5 to pH 7.5 also triggered Ca^{2+} release while decreasing the pH never elicited Ca^{2+} release. These results indicate that a change in a pH gradient, rather than a specific H^+ concentration at the outer membrane surface, is the critical variable triggering Ca^{2+} release. Furthermore, the magnitude of the pH increase leading to Ca^{2+} release and tension was in the same range as the H^+ gradients measured across the membranes of isolated vesicles during active Ca^{2+} uptake (11, 12, 14, 37). The possibility that Ca^{2+} release induced by changes in medium pH is due primarily to depression of Ca^{2+}-uptake activity is unlikely. Blocking Ca^{2+}/Mg^{2+}-ATPase activity by removing substrate leads to a relatively slow Ca^{2+} loss with a half-time of minutes. However, in our experiments, rates of tension development were rapid; half-maximal tensions developed in <0.6 sec, the limit of resolution of the recording system.

Proton ionophores always elicited a transient tension. Analysis of pH-induced Ca^{2+} release in isolated vesicles showed that a free Ca^{2+} concentration of 3.3 μM decreased the amount of Ca^{2+} released. Analysis of the relationship between free Ca^{2+} concentration and tension in skinned rabbit psoas fibers indicates that 3.3 μM Ca^{2+} corresponds to $\approx$90% of the maximum isometric tension output (ref. 38; unpublished observations). Thus, Ca^{2+} levels corresponding to peak tension can inhibit Ca^{2+} release from H^+-regulated Ca^{2+} channels. These observations suggest that myoplasmic Ca^{2+} could control the Ca^{2+}-release channel after dissipation of the H^+ gradient. Closing of the H^+-regulated Ca^{2+} channel by Ca^{2+} would permit the pump to remove Ca^{2+} and account for relaxation in the presence of ionophore. The lower limit of Ca^{2+} effects on the channel was not determined, but our data on pH- and ionophore-induced release in skinned fibers suggest that the H^+ gradient developed during Ca^{2+} uptake eventually replaces Ca^{2+} in maintaining a closed channel. We emphasize that our results do not preclude other effects of Ca^{2+} on the Ca^{2+} permeability of sarcoplasmic reticulum. Moreover, we do not know whether Ca^{2+} in the myofilament space could act directly to close the Ca^{2+} release channel or indirectly, for example, by activation of a regulatory enzyme. To our knowledge, however, this is the first indication that Ca^{2+}, at physiological levels, may shut off a rapid Ca^{2+}-release process in skeletal muscle.

Identification of the Ca^{2+}-Release Channel. The composition of the Ca^{2+}-release channel is unknown. The sarcoplasmic reticulum contains several intrinsic proteins, a glycoprotein (18), Ca^{2+}/Mg^{2+}-ATPase (17), and one or two proteolipids (39–41). There is no evidence to suggest that the glycoprotein is involved in Ca^{2+} release. The reversal of Ca^{2+} uptake, as noted above, seems unlikely to be a physiological pathway for Ca^{2+} release, although the possibility remains that a Ca^{2+}-release channel could reside in one or more transmembrane passages of the ATPase molecule not involved in formation of the Ca^{2+} uptake channel.

The proteolipid is a possible candidate as a Ca^{2+}-release channel. Racker and Eytan (40) and Knowles *et al.* (41) have shown that proteolipid can promote Ca^{2+} movements in reconstituted systems, and they have interpreted their data as suggesting that the proteolipid is involved in Ca^{2+} uptake. However, we have shown that removal of 96% of the proteolipid from ATPase does not affect its ability to transport Ca^{2+} in a reconstituted system (42). We have also found that DCCD, an inhibitor of Ca^{2+} uptake (36), binds both to ATPase and to proteolipid with a molar stoichiometry of $\approx$1:1. (unpublished studies). However, DCCD inhibits proteolipid-depleted ATPase very effectively, indicating that it is binding to the ATPase that results in ATPase inhibition. As it is unlikely that Ca^{2+} is released through the Ca^{2+}-uptake channel (6), we propose that DCCD inhibition of pH- and ionophore-induced Ca^{2+} release (Fig. 6*A*) is exerted through its binding to the proteolipid. In other proteolipids, DCCD has been found to bind to carboxyl groups of aspartic or glutamic acid within relatively hydrophobic amino acid sequences (43).

Proteolipid-mediated cation transport in reconstituted systems was not specific for Ca^{2+} (41). This would not rule out a role for the proteolipid in Ca^{2+} release, however, as the driving force for Ca^{2+} efflux is the steep Ca^{2+} concentration gradient across the sarcoplasmic reticulum membrane. Also, the need for charge neutrality requires that Ca^{2+} efflux must be accompanied either by comigration of anions or by countermovement for cations. Possibly, the proteolipid serves as a common pathway for these charge movements during Ca^{2+} release.

Proton Gradient Dissipation in Excitation–Contraction Coupling. Our hypothesis concerning the involvement of H^+ gradients in the control of Ca^{2+} release raises the important question of how the pH gradient could be formed and dissipated in excitation–contraction coupling. Studies with isolated vesicles show that the required H^+ gradient can be formed during the initial phases of Ca^{2+} uptake (12). At present, mechanisms for dissipation of this gradient are speculative. An increase in myoplasmic pH of $\approx$0.2 pH units during excitation, thereby neutralizing the H^+ gradient across the sarcoplasmic reticulum membrane and stimulating release, or the opening of a H^+ channel in the sarcoplasmic reticulum membrane, permitting dis-

* Correction - Proc. Natl. Acad. Sci. V79(6) March 1982

sipation of the H^+ gradient by inward H^+ diffusion along its concentration gradient, are two possible mechanisms for triggering Ca^{2+} release. Meissner and Young (44) recently found evidence for a H^+ pathway distinct from the K^+/Na^+ pathway in isolated vesicles. Whether the H^+ pathway is regulated is unknown. Clearly, investigation is needed of H^+ gradient formation and dissipation in muscle cells or compartments during the excitation–contraction cycle *in vivo*.

We thank Dr. Beverly Britt for the use of facilities in her laboratory to carry out a preliminary experiment. The expert technical assistance of Ms. Geraldine Plynton, Columbia University, is gratefully acknowledged. This research was supported by Grant MT-3399 from the Medical Research Council of Canada and by a grant from the Muscular Dystrophy Association of Canada to D.H.M. D.S.W. was supported by a senior investigatorship from the New York Heart Association of America, by Grant NS 11766 from the National Institutes of Health, and by the Muscular Dystrophy Association of America. V.S. was a Postdoctoral Fellow of the Muscular Dystrophy Association of Canada.

1. Ebashi, S., Endo, M. & Ohtsuki, I. (1969) *Q. Rev. Biophys.* **2,** 351–384.
2. de Meis, L. & Vianna, A. L. (1979) *Annu. Rev. Biochem.* **48,** 275–292.
3. Martonosi, A. (1972) in *Current Topics In Membranes and Transport*, eds. Bronner, F. & Kleinzeller, A. (Academic, New York), Vol. 3, pp. 83–197.
4. Endo, M. (1977) *Physiol. Rev.* **57,** 71–108.
5. Fabiato, A. & Fabiato, F. (1979) *Annu. Rev. Physiol.* **41,** 473–484.
6. Shoshan, V., Campbell, K. P., MacLennan, D. H., Frodis, W. & Britt, B. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4435–4438.
7. Fabiato, A. & Fabiato, F. (1975) *J. Physiol. (London)* **249,** 469–495.
8. Fabiato, A. & Fabiato, F. (1978) *Ann. N.Y. Acad. Sci.* **307,** 491–522.
9. Fabiato, A. & Fabiato, F. (1977) *Circ. Res.* **40,** 119–129.
10. Meissner, G. & McKinley, D. (1976) *J. Membr. Biol.* **30,** 79–98.
11. Madeira, V. M. C. (1980) *Arch. Biochem. Biophys.* **200,** 319–325.
12. Chiesi, M. & Inesi, G. (1980) *Biochemistry*, **19,** 2912–2918.
13. Meissner, G. (1981) *J. Biol. Chem.* **256,** 636–643.
14. Nomura, K. & Nakamaru, Y. (1976) *J. Biochem.* **80,** 1393–1399.
15. Nakamaru, Y. & Schwartz, A. (1970) *Biochem. Biophys. Res. Commun.* **41,** 830–836.
16. Fabiato, A. & Fabiato, F. (1978) *J. Physiol. (London)* **276,** 233–255.
17. MacLennan, D. H. (1970) *J. Biol. Chem.* **245,** 4508–4518.
18. Campbell, K. P. & MacLennan, D. H. (1981) *J. Biol. Chem.* **256,** in press.
19. Wood, D. S., Zollman, J., Reuben, J. P. & Brandt, P. W. (1975) *Science* **187,** 1075–1076.
20. Lowry, O. H. Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
21. Wood, D. S. (1978) *Expt'l. Neurol.* **58,** 218–230.
22. Orentlicher, M., Reuben, J. P., Grundfest, H. & Brandt, P. W (1974) *J. Gen. Physiol.* **63,** 168–186.
23. Reuben, J. P., Wood, D. S. & Eastwood, A. B. (1977) in *Pathogenesis of the Human Muscular Dystrophys.* eds. Rowland, L. P. (Excerpta Medica, Amsterdam), pp. 259–269.
24. Pressman, B. C. (1976) *Annu. Rev. Biochem.* **45,** 501–530.
25. Hopfer, U., Lehninger, A. L. & Thompson, T. E. (1968) *Proc. Natl. Acad. Sci. USA* **59,** 484–490.
26. Degani, H. & Shavit, N. (1972) *Arch. Biochem. Biophys.* **152,** 339–346.
27. Shigekawa, M., Finegan, J.-A. M. & Katz, A. M. (1976) *J. Biol. Chem.* **251,** 6894–6900.
28. Wood, D. S., Kahn, D. A., Selinger, S. & Reuben, J. P. (1977) *Biophys. J.* **17,** 201 (abstr.)
29. Fabiato, A. & Fabiato, F. (1979) *J. Physiol. (Paris)* **75,** 463–505.
30. Racker, E. & Horstman, L. L. (1967) *J. Biol. Chem.* **242,** 2547–2551.
31. Patel, L., Schuldiner, S. & Kaback, H. R. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3387–3391.
32. McCarty, R. E. & Racker, E. (1967) *J. Biol. Chem.* **242,** 3435–3439.
33. Nelson, N., Eytan, E., Nostani, B., Sigrist, H., Sigrist-Nelson, K. & Gitler, C. (1978) *Proc. Natl. Acad. Sci. USA* **74,** 2375–2378.
34. Fillingame, R. H. (1976) *J. Biol. Chem.* **251,** 6630–6637.
35. Celis, H. (1980) *Biochem. Biophys. Res. Commun.* **92,** 26–31.
36. Pick, U. & Racker, E. (1979) *Biochemistry* **18,** 109–113.
37. Kodama, T., Kurebayashi, N. & Ogawa, Y. (1980) *J. Biochem.* **88,** 1259–1265.
38. Brandt, P. W., Cox, R. N. & Kawai, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4717–4720.
39. MacLennan, D. H., Yip, C. C., Iles, G. H. & Seeman, P. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **37,** 469–478.
40. Racker, E. & Eytan, E. (1975) *J. Biol. Chem.* **250,** 7533–7534.
41. Knowles, A., Zimniak, P., Alfonzo, M., Zimniak, A. & Racker, E. (1980) *J. Membr. Biol.* **55,** 233–239.
42. MacLennan, D. H., Reithmeier, R. A. F., Shoshan V., Campbell K. P. & Lebel, D. (1980) *Ann. N.Y. Acad. Sci.* **358,** 138–148.
43. Sebald, W., Hoppe, H. & Wachter, E. (1979) in *Function and Molecular Aspects of Biomembrane Transport.* ed. Quagliariello, E. (Elsevier/North-Holland, Amsterdam) 63–74.
44. Meissner, G. & Young, R. C. (1980) *J. Biol. Chem.* **255,** 6814–6819.