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# The bulk of $Ca^{2+}$ released to the myoplasm is free in the sarcoplasmic reticulum and does not unbind from calsequestrin

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Calsequestrin (CS) is the major $Ca^{2+}$ binding protein contained in the lumen of sarcoplasmic reticulum (SR) $Ca^{2+}$ binding properties and tissue concentration of CS of frog skeletal muscle were measured. At equilibrium, maximal $Ca^{2+}$ binding capacity of purified CS was about 1.2 μmol $Ca^{2+}$/mg protein. Apparent $K_d$s for $Ca^{2+}$ were around 50 μM in the absence of salts, around 0.9 mM in the presence of 100 mM KCl, and around 1.1 mM under 'physiological' conditions. Quantitation of CS in homogenates was accomplished by three methods (Stains-all staining, immunoblotting and $^{45}Ca$ ligand overlay). Frog muscle contained about 0.5 mg of CS/g wet weight, that is 6.1 mM CS inside the SR. At rest the in situ free $[Ca^{2+}]$ of SR was calculated to be 3.6 mM, and, thus, CS is largely saturated with $Ca^{2+}$. Moreover, computer simulations of $Ca^{2+}$ release indicated that about 75% of $Ca^{2+}$ released during a twitch is free in the SR and does not unbind from CS.

Skeletal muscle, $Ca^{2+}$, Calsequestrin, $Ca^{2+}$ release, Sarcoplasmic reticulum

## 1. INTRODUCTION

The SR, the membrane-bound $Ca^{2+}$ store of skeletal muscle, controls the contraction/relaxation cycle by raising and lowering the myoplasmic free $[Ca^{2+}]$, and consists of two continuous yet distinct regions, the longitudinal, or non-junctional SR and the junctional SR, i.e. the area of terminal cisternae (TC) directly facing the transverse tubules [1]. Skeletal muscle contraction is elicited by the release of calcium from TC [2]. After $Ca^{2+}$ pump-mediated active uptake, $Ca^{2+}$ is stored in the TC lumen where it is believed to be bound to CS. The role of CS as a $Ca^{2+}$ storage site was first proposed by MacLennan and Wong [3].

The kinetics of $Ca^{2+}$ release from TC in vivo are determined by the gating properties of the $Ca^{2+}$ release channels and the $Ca^{2+}$ gradient across the SR membrane, which is established by the $Ca^{2+}$ pump and thought to depend mainly upon the affinity and capacity of CS for $Ca^{2+}$, and probably, rate constants of $Ca^{2+}$ unbinding from CS [4,5].

In this study, we have measured the $Ca^{2+}$ binding properties and tissue concentration of frog skeletal muscle CS. We have also calculated the free $[Ca^{2+}]$ in the lumen of SR, and determined, by computer simulations, that a large proportion of the $Ca^{2+}$ released during a twitch is free in the SR and does not unbind from CS.

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## 2. EXPERIMENTAL

### 2.1 *Homogenates of frog skeletal muscle*

Frog (*Rana catesbiana*) fast-twitch skeletal muscles were homogenized with 10 vols of 0.3 M sucrose, 5 mM imidazole, pH 7.4, and 100 μM phenylmethylsulfonyl fluoride using a Brinkmann homogenizer for 60 s at setting 10. Homogenates were supplemented with 0.2% (w/v) sodium dodecyl sulphate (SDS) and centrifuged at 2000 × *g* for 10 min to remove debris and nuclei. The yield of homogenate supernatants was about 100 mg of protein/g wet weight.

### 2.2 *Isolation of TC fractions and calsequestrin*

TC fractions were purified as described [6], in the presence of 100 μM phenylmethylsulfonyl fluoride. CS was extracted from TC [6] and purified by phenyl-Sepharose chromatography [7]. Fractions eluted with 10 mM $CaCl_2$ were analyzed by SDS-polyacrylamide gel electrophoresis, dialyzed against 5 mM Tris Cl, pH 7.5, and stored at −80°C.

### 2.3 *$^{45}Ca$ binding measurements by equilibrium dialysis*

$Ca^{2+}$ binding to purified CS was measured by equilibrium dialysis at 4°C. Three ionic conditions were used and the composition of the assay media is detailed in Table I. Samples (0.2–0.25 mg of protein in 0.5 ml) were dialyzed against 100 ml of the appropriate buffer for 48 h. Total $^{45}CaCl_2$ concentration varied between 1 μM and 5 mM. $CaCl_2$ standard solution (0.1 M) was from Orion Research Inc. (Cambridge, MA). Apparent dissociation constants ($K_d$) and maximal binding capacity ($B_{max}$) were calculated by Scatchard plot.

### 2.4 *Anti-(CS) polyclonal antibodies*

Anti-(frog CS) ascites was raised in SLJ mice (Jackson Laboratories, Bar Harbor, ME) by intraperitoneal injections of purified CS (15 μg) and complete Freund's adjuvant, as described by Tung [8]. The IgG fraction was purified from mouse ascites by Na-sulphate precipitation [8]. Specificity of anti-(frog CS) IgG was tested by immunoblot (not shown).

### 2.5 *Quantitation of CS in muscle homogenates*

The CS content of muscle homogenates was determined using three independent procedures.



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Table I

$Ca^{2+}$ binding properties of frog skeletal muscle CS

| | $B_{max}$ (nmol $Ca^{2+}$/mg) | $K_d$ (mM) |
|---|---|---|
| 5 mM Tris-Cl, pH 7.5 | 1231.3 ± 96.2[a] | 0.044 ± 0.005 |
| 5 mM Tris-Cl, pH 7.5, 100 mM KCl | 1206.3 ± 100.2 | 0.905 ± 0.09 |
| 150 mM K, 16.6 mM Na, 5 mM Mg, 10 mM Cl, 1.4 mM $P_i$, buffered to pH 7.5 with Hepes[a] | 1181.7 ± 110.3 | 1.126 ± 0.10[c] |

Determinations were carried out in duplicate on 3 different CS preparations. Data are expressed as mean ± SD. 'Physiological' ion concentrations (third entry) are derived from Godt and Maughan [14] and were assumed to mimic intraluminal concentrations.

[a] K was added as KOH, Na as 15.2 mM NaOH and 1.4 mM $NaH_2PO_4$ and Mg as 5 mM $MgCl_2$.

[b] $B_{max}$ of frog CS is 30% higher than that of rabbit CS (cf. [3]).

[c] The intrinsic dissociation constant for K ($K_K$) calculated from the first two entries is 5.08 mM and similar to that which can be calculated from Table III of MacLennan and Wong [3], i.e. 4.78 mM. The intrinsic dissociation constant for $Mg^{2+}$ ($K_{Mg}$) can be calculated from the same Table III of MacLennan and Wong [3] and is around 0.13 mM. If $K^+$ and $Mg^{2+}$ were to compete with $Ca^{2+}$ for the same $Ca^{2+}$ binding sites, the apparent $K_d$ for $Ca^{2+}$ should be much higher than 1.1 mM. As previously suggested by Ikemoto et al. [12], our data imply that there is no simple competition between $K^+$, $Mg^{2+}$ and $Ca^{2+}$.

(i) After SDS polyacrylamide gel electrophoresis and Stains-all staining [7], the absorbance of each frog CS band was measured at 535 nm [10] in a Gilford 250 spectrophotometer. The CS content was measured with reference to calibration curves obtained with 0.4–4 μg of purified calsequestrin [6,9].

(ii) The CS content was also measured by immunoblot. After reaction with the specific antibody and the alkaline phosphatase-conjugated anti-IgG, the CS band was identified by staining with 5 bromo-4-chloro-3 indolyl phosphate and Nitro blue tetrazolium [6]. The staining reaction was calibrated with reference to 0.6–3 μg of purified CS. Images of immunoblots were obtained with a Hamamatsu videocamera and digitized. Densitometry of CS bands and peak area determination were carried out using, respectively, 'JAVA' and 'Analyze' software (Jandel, Inc.). A similar procedure for quantitation of CS in rabbit and human muscle extracts has been described [11].

(iii) The CS content was also measured by $^{45}Ca$ ligand overlay. After SDS-slab gel electrophoresis and transfer to nitrocellulose paper, blots were incubated for 20 min at room temperature in 5 mM imidazole, pH 7.4, 60 mM KCl, 5 mM $MgSO_4$, 10 μM $^{45}CaCl_2$, rinsed in 100 ml of 30% ice-cold ethanol and exposed to Kodak X-Omat films [6]. The CS content of muscle homogenates was measured with reference to calibration curves obtained with 0.6–3 μg of purified CS. Densitometric scans of autoradiograph were carried out as described in section (ii).

## 3. RESULTS AND DISCUSSION

### 3.1. *$Ca^{2+}$ binding properties of frog skeletal muscle CS*

$K_d$ and $B_{max}$ values of skeletal muscle CS were determined by equilibrium dialysis using three different assay conditions (Table I). In the absence of KCl, in the presence of 100 mM KCl and under 'physiological' conditions, CS displayed a $B_{max}$ of about 1200 nmol $Ca^{2+}$/mg of protein. Given an apparent $M_r$ of 47 kDa [10], CS can bind up to 56 mol $Ca^{2+}$/mol.

$K_d$ was increased from about 0.05 mM to 0.9 mM by 100 mM KCl [3] (Table I). Under 'physiological' conditions, $K_d$ values were slightly higher (about 1.1 mM). This might reflect the presence of $Mg^{2+}$ at millimolar concentrations [3,12,13], as well as higher potassium concentrations [3] (see legend to Table I). The ionic conditions deemed as 'physiological' are based on literature estimates ([14] and references therein) and must be taken cautiously, particularly since these are estimates for myoplasm and not the SR lumen. However, electron probe analysis [15,16] indicated that there is no membrane potential across the SR and thus any permeant ion should have the same concentration within the SR as within the myoplasm.

Table II

CS content of frog skeletal muscle

| Method | CS content (mg CS/g wet weight) |
|---|---|
| Stains-all | 0.50 |
| Immunoblot | 0.57 |
| $^{45}Ca$ ligand overlay | 0.54 |

Data are average of measurements carried out on two different homogenate preparations, using 3 different procedures (see section 2 for details). Data varied by less than 10%.

### 3.2. *Quantitation of CS in frog skeletal muscle homogenates*

The tissue concentration of CS was estimated by three procedures (Table II), which are based on calibration curves obtained with purified CS. CS was identified by either metachromatic blue staining with Stains-all [7,9], specific polyclonal antibodies, or $^{45}Ca$ binding [6].

The tissue content of CS was found to be about 0.5 mg/g wet weight. Allowing for 10–15% loss of CS during homogenization (not shown), experimental values constitute a reasonable lower limit for CS content.

### 3.3. *Estimated concentration of CS in isolated TC and in situ*

Quantitation of CS in homogenates of frog skeletal muscle made possible calculations of CS concentration in situ. Knowing the molecular weight of CS, the content of CS (μg protein/g wet weight) and assuming that 1 g wet weight is equivalent to 1 ml fiber volume, the concentration of frog CS was estimated to be about 12 μmol/liter fiber (Table III). The concentration of $Ca^{2+}$ binding sites on CS was obtained from the number of $Ca^{2+}$ binding sites/mol (Table III). The

Table III

Estimated concentrations of CS and $Ca^{2+}$ binding sites and calculated concentrations of total and free $Ca^{2+}$ in vitro and in situ

| | Isolated TC | Muscle fiber | In situ SR |
|---|---|---|---|
| CS (mM) | 2.53[a] | 0.012[b] | 0.109[c] |
| $Ca^{2+}$ binding sites (mM) | 141.60[a] | 0.67[b] | 6.09[c] |
| Total $Ca^{2+}$ (mM) | n.d. | 0.91[d] | 8.27[e] |
| Free $Ca^{2+}$ (mM) | n.d. | - | 3.60[f] (2.33) [2.95] |

[a] The concentration of CS in isolated TC is estimated knowing that CS accounts for 48% of TC protein [6] and assuming that 1 mg protein is equal to 4 μl [9,26]. The concentration of $Ca^{2+}$ binding sites on CS is obtained from the number of binding sites per mol, i.e. 56

[b] The concentration of CS is calculated from the highest tissue content values of Table II assuming that 1 g whole muscle corresponds to 1 ml fiber volume

[c] CS concentration in SR is calculated by dividing CS concentration in muscle fiber by the fractional volume of the SR (0.11, which is the average between 0.13 measured by Peachey [18] and 0.09 measured by Mobley and Eisenberg [19]). Although CS is present only in the lumen of TC, determinations of CS concentration are based on the reliable assumption that the lumen of SR is continuous between the longitudinal and TC portions. The assumption is also made that the volume occupied by CS and other luminal proteins is negligible

[d] Total $Ca^{2+}$ concentration is derived from Schneider et al. [17] by converting their value of 1.58 mmol $Ca^{2+}$/liter of myoplasmic $H_2O$ to 0.91 mmol $Ca^{2+}$/liter fiber. The conversion factor is 0.58 [4]

[e] Total $Ca^{2+}$ concentration is calculated by dividing SR $Ca^{2+}$ concentration (0.91 mM) by the fractional volume of SR (0.11)

[f] Calculations are performed assuming that CS is the only $Ca^{2+}$ binding protein and $K_d$ is 1.1 mM. Bracketed value represents estimates of free $[Ca^{2+}]$ in the presence of additional $Ca^{2+}$ binding sites, square bracketed value is obtained if CS concentration is increased by 20%

maximal $Ca^{2+}$ binding capacity of SR, if due only to CS, was calculated to be 6.1 mM in frog muscle (Table III) since the fractional volume of frog SR is 0.11 [18,19].

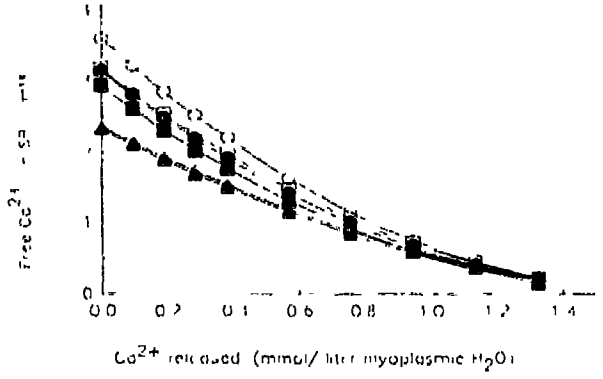
It is important to note that the in situ concentration of CS was at least 20-fold lower than that of isolated TC (Table III). This likely reflects the SR fragmentation caused by tissue homogenization, i.e. membrane vesicles derived from the TC portion of the SR enclose small volumes filled with CS molecules. The difference in CS concentration may influence the comparison of results obtained in isolated TC and muscle fibers (see below).

### 3.4. *What is the free $Ca^{2+}$ concentration of SR in situ?*

We sought to calculate the SR intraluminal free $[Ca^{2+}]$, under resting conditions, on the basis of the present results (apparent $K_d$ of CS for $Ca^{2+}$ under 'physiological' conditions (Table I) and CS concentration (Table III)) and of two literature data which are available only for frog skeletal muscle fibers: the fractional volume of SR (0.11 in [18,19]) and the total $Ca^{2+}$ content of SR [15–17]. Spectrophotometric estimates of total $Ca^{2+}$ content in SR ([17]: 0.91 mmol/liter fiber) are close to those derived from electron probe X-ray microanalysis of frozen muscle sections [15].

If the SR lumen is a freely diffusible compartment, and if CS is the only $Ca^{2+}$ binding protein, our calculations indicate that the free $[Ca^{2+}]$, under resting conditions, is 3.6 mM (Table III). Free $Ca^{2+}$ in the SR was estimated in the mM range by Weber [20a] and Maylie et al. [20] later suggested a value of 2.6 mM based on optical measurements of the $Ca^{2+}$ indicator tetramethylmurexide sequestered within the SR of cut frog muscle fibers.

CS, however, is not the only low affinity $Ca^{2+}$ binding protein within the SR lumen. There are also sarcalumenin [21] and calreticulin [22], although in minute amounts compared to CS, and low affinity $Ca^{2+}$ binding sites on the luminal side of the $Ca^{2+}$-pump molecule [23]. Both $n$ and $K_d$ of $Ca^{2+}$-pump low affinity $Ca^{2+}$ binding sites are controversial and range from 1.4 mol/mol and 1.3 mM ($\gamma$-sites of [24]) to 3–12 mol/mol and 7 mM [25]. Moreover, Miyamoto and Kasai [26] have described several classes of $Ca^{2+}$ binding sites and, among them, luminal $\beta_3$ sites having high capacity and low affinity ($K_d$ of 38 mM). If such additional $Ca^{2+}$ binding sites are separately taken into account and added to those of CS, intraluminal free $[Ca^{2+}]$ could decrease to as low as 2.3 mM (Fig. 1, and Table III).




$Ca^{2+}$ released (mmol/ liter myoplasmic $H_2O$)

Fig. 1. In situ free $[Ca^{2+}]$ as a function of released $Ca^{2+}$. Computer simulation of changes of free $[Ca^{2+}]$ in frog SR was carried out using the MathCad version 2.0 software. Abscissa values were obtained as follows: $\Delta[Ca] \times 0.11 / 0.58$, where 0.11 is the SR fractional volume and 0.58 is the factor for converting $g$ whole muscle to $g$ myoplasmic $H_2O$ [4]. Six different situations are simulated (circles) CS is the only $Ca^{2+}$ binding protein and the $Ca^{2+}$ buffering capacity is 6.09 mM (see Table III), (filled circles) CS plus low affinity $Ca^{2+}$ binding sites on the $Ca^{2+}$ pump ($\gamma$-sites of [24], $n$ = 1.4, $K_d$ = 1.3 mM). For our calculations, the concentration of $Ca^{2+}$ pump in SR (0.6 mM) is derived from Baylor et al. [7], (squares) CS plus 3 low affinity $Ca^{2+}$ binding sites on the $Ca^{2+}$ pump ($n$ = 3, $K_d$ = 7 mM, [25]), (filled triangles) CS plus 12 low affinity $Ca^{2+}$ binding sites on the $Ca^{2+}$ pump ($n$ = 12, $K_d$ = 7 mM; [25]); (triangles) CS plus $\beta_3$ luminal low affinity $Ca^{2+}$ binding sites (144 nmol/mg SR protein converted to 30 mM, $K_d$ = 38 mM; [26]), (filled squares) 1.2 × CS. CS concentration was increased by 20% because some CS was lost during homogenization.

Additional factors, which might affect our calculations, have also been considered: underestimates of CS content and presence of other cations which bind to CS. A 20% increase of CS content would decrease the free $[Ca^{2+}]$ by 25% (Fig. 1 and Table III). Cations such as $K^+$ and $Mg^{2+}$ interfere in vitro with $Ca^{2+}$ binding to CS [3,12,13]. Calculated free $[Ca^{2+}]$ would increase by 20% if both 5 mM $Mg^{2+}$ and 130 mM $K^+$ (intraluminal concentrations; [15]) were to compete with $Ca^{2+}$ for the same binding sites. No simple competition between $K^+$, $Mg^{2+}$ and $Ca^{2+}$ was reported by Ikemoto et al. [12]. In fact, given intrinsic $K_d$s for $K^+$ and $Mg^{2+}$ of about 5 and 0.13 mM, respectively, the calculated free $[Ca^{2+}]$ does not increase, as expected (Table IV). However, given the intrinsic $K_K$ and $K_{Mg}$, we estimate that virtually all $Ca^{2+}$-free CS binding sites are saturated with either $K^+$ or $Mg^{2+}$ (about 1 mM of both Mg-CS and K-CS). In this respect, Somlyo et al. [15] have reported that the $K^+$ content of TC is larger than that of both longitudinal SR and adjacent cytoplasm, and suggested that "excess $K^+$ in the TC is bound to CS".

If the highest estimate for free (intraluminal) $[Ca^{2+}]_i$ were to be correct (3.6 mM), the $[Ca^{2+}]_i/[Ca^{2+}]_o$ ratio would be $7.2 \times 10^4$, given free (myoplasmic) $[Ca^{2+}]_o$ equal to 50 nM [27]. This value is greater than the experimental ratios of $2 \times 10^3$–$2 \times 10^4$ achieved by the $Ca^{2+}$ pump of isolated SR vesicles [28] but near the theoretical limits [29].

### 3.5. *CS saturation and $Ca^{2+}$ release in situ*

Fig. 1 shows a computer simulation of the change of free $[Ca^{2+}]$ within frog SR as a function of released $Ca^{2+}$. For a single twitch, i.e. release of 0.2 mmol $Ca^{2+}$/liter of myoplasmic $H_2O$ [4,17], the bulk of released $Ca^{2+}$ (around 75%) would be accounted for by free (unbound) $Ca^{2+}$, should CS be the only $Ca^{2+}$ binding protein (Table IV). Further computer simulations were carried out either assuming additional $Ca^{2+}$ binding sites or increasing the measured CS content by 20%. Under such circumstances and during a single twitch, the amount of $Ca^{2+}$ released from CS would not increase substantially (Table IV).

The relationship between $Ca^{2+}$ released from SR and % saturation of CS is non-linear (Fig. 2). For a single twitch, the degree of CS saturation would decrease from either 76% to 72% in the presence of CS only, from 68% to 64% in the presence of CS and $\beta_3$ $Ca^{2+}$ binding sites, or from 72% to 68% if CS concentration is increased by 20%. The presence of either $\beta_3$ $Ca^{2+}$ binding sites [26] or a large number of low affinity $Ca^{2+}$ binding sites on the $Ca^{2+}$ pump [25] would provide substantial, additional buffering capacity and decrease the percentage of free $Ca^{2+}$ being released (Table III). Under all circumstances, however, at least 50% of released $Ca^{2+}$ would be accounted for by free $Ca^{2+}$ (Table IV). If the network formed by CS within the TC lumen does not constitute a diffusional barrier for $Ca^{2+}$ transport and accumulation, one of the implications of this study is that a large proportion of the released $Ca^{2+}$ is free in the SR and does not unbind from CS during a twitch.

Ikemoto et al. [30] have reported that most of the intravesicular $Ca^{2+}$ is bound to CS in isolated TC fractions; this is not unlikely due to the high CS concentration of isolated TC (see Table III). However, the observation that a $Ca^{2+}$-dependent conformational change of CS precedes $Ca^{2+}$ release in vitro [30] does not seem to have any physiological relevance, based on our data and calculations. If 75% of released $Ca^{2+}$ is not bound to CS (Table IV), a $Ca^{2+}$-dependent conformational change of CS may follow but not precede the opening of $Ca^{2+}$ release channels.

Table IV

Contribution of free and CS-bound $Ca^{2+}$ to $Ca^{2+}$ released during a twitch

| | % $Ca^{2+}$ bound to CS | % $Ca^{2+}$ bound to other binding sites | % free $Ca^{2+}$ |
|---|---|---|---|
| [CS][a] | 26 4 | – | 73 6 |
| [CS] + $\gamma$-sites | 29 2 | 4 3 | 66 5 |
| [CS] + 5 low affinity $Ca^{2+}$ binding sites | 27 6 | 8 3 | 64 1 |
| [CS] + 12 low affinity $Ca^{2+}$ binding sites | 28.9 | 26 9 | 44 2 |
| [CS] + $\beta_3$ sites | 27 0 | 30 3 | 42 7 |
| 1.2 × [CS] | 36.8 | – | 63 2 |
| [CS] + $K^+$ + $Mg^{2+}$[b] | 24 2 | – | 75 8 |

Calculations of free and bound $Ca^{2+}$ are performed using the MathCad version 2.0 software. During a twitch, about 0 2 mmol $Ca^{2+}$/liter of myoplasmic $H_2O$ is released to the myoplasm [4,17] Properties ($K_d$, $B_{max}$) and concentrations of additional $Ca^{2+}$ binding sites are detailed in the legend to Fig 1

[a] [CS], or concentration of $Ca^{2+}$ binding sites of frog CS, is 6.09 mM and is derived from Table III

[b] Computer simulation was performed using 130 mM and 5 mM as the total $K^+$ and $Mg^{2+}$ concentrations and 0 044 mM, 5 mM and 0 13 mM (Table I) as the intrinsic $K_{Ca}$, $K_K$ and $K_{Mg}$, respectively

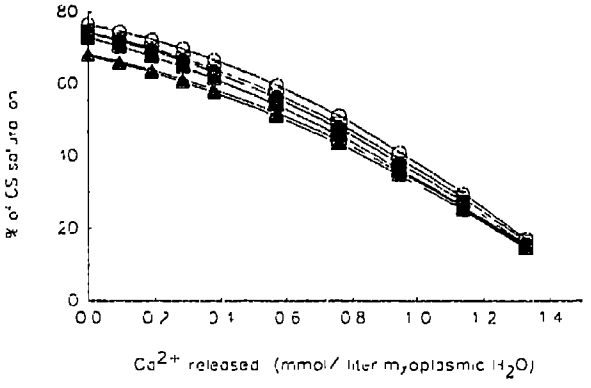



Fig 2 Saturation of frog CS as a function of released $Ca^{2+}$ Computer simulations, calculations and symbols are as in Fig 1

During a tetanus, about 60% of stored $Ca^{2+}$ (1 mmol $Ca^{2+}$/liter myoplasmic $H_2O$) is released [16], and the calculated free [$Ca^{2+}$] would drop below 1 mM (Fig. 1). Either additional $Ca^{2+}$ binding sites or higher CS concentration do not make any significant difference. The degree of CS saturation would decrease, however, to around 40% (Fig. 2) and a sizeable amount (45%) of released $Ca^{2+}$ would come off CS.

The calculated free [$Ca^{2+}$] of SR in situ depends in part upon specified assumptions, and further experimentation is required to directly measure free [$Ca^{2+}$]. Present and future data are relevant to the understanding of the $Ca^{2+}$ release mechanism in view of the recent suggestion that intraluminal $Ca^{2+}$ controls $Ca^{2+}$ release channels [31].

## REFERENCES


[1] Campbell, K.P. (1986) in: Sarcoplasmic Reticulum in Muscle Physiology, vol. 1 (Entman, M.L. and Van Winckle, W B. eds) pp. 65–99, CRC Press, Boca Raton, FL
[2] Fleischer, S. and Inui, M (1989) Annu Rev Biophys. Chem 18, 333–364.
[3] MacLennan, D.H. and Wong, P.T.S. (1971) Proc Natl Acad. Sci. USA 68, 1231–1235.
[4] Baylor, S M., Chandler, W K. and Marshall, M.W (1983) J. Physiol (Lond ) 344, 625–666
[5] Melzer, W., Rios, E. and Schneider, M.F. (1987) Biophys J 51, 843–863.
[6] Volpe, P , Bravin, M., Zorzato, F and Margreth, A (1988) J. Biol Chem 263, 9901–9907
[7] Cala, S E and Jones, L R (1983) J Biol Chem 258, 11932–11936
[8] Tung, A.S (1983) Methods Enzymol 93, 12–24
[9] Williams, R.W. and Beeler, T.J. (1986) J Biol Chem 261, 12408–12413
[10] Salvatori, S., Bravin, M. and Margreth, A (1988) in Calcium and Calcium Binding Proteins (Gerday, Ch , Gilles, R and Bolis, L. eds) Springer-Verlag, pp. 228–235, Berlin-Heidelberg.
[11] Klitgaard, H., Aureni, S. and Damiani, E (1989) Acta Physiol. Scand. 137, 23–31.
[12] Ikemoto, N., Nagy, B., Bhatnagar, G.M. and Gergely, J. (1974) J. Biol Chem 249, 2357–2365.
[13] Mitchell, R.D., Simmerman, H.K.B. and Jones, L.R. (1988) J. Biol Chem. 263, 1376–1381.
[14] Godt, R.E. and Maughan, D W. (1988) Am. J. Physiol. 254, C591–C604.
[15] Somlyo, A.V , Gonzalez-Serratos, H , Shuman, H., McLellan, G and Somlyo, A.P. (1981) J Cell Biol. 90, 577–594
[16] Somlyo, A V., McLellan, G , Gonzalez-Serratos, H. and Somlyo, A P (1985) J. Biol. Chem. 260, 6801–6807.
[17] Schneider, M F , Simon, B J. and Szucs, G. (1987) J. Physiol (Lond.) 392, 167–192.
[18] Peachey, L.D. (1965) J Cell Biol 25, 209–231
[19] Mobley, B.A. and Eisenberg, B R. (1975) J. Gen Physiol. 66, 31–45.
[20a] Weber, A. (1971) J. Gen. Physiol. 57, 50–63.
[20] Maylie, J., Irving, M., Leung Sizto, N., Boyarsky, G. and Chandler, W.K (1987) J. Gen. Physiol 89, 145–176.
[21] Leberer, E , Charuk, J.H.M., Green, N M. and MacLennan, D.H. (1989) Proc. Natl. Acad Sci USA 86, 6047–6051
[22] Fliegel, L., Burns, K., MacLennan, D.H., Reithmeier, R A.F. and Michalak, M (1989) J Biol. Chem. 264, 21522–21528.
[23] MacLennan, D H , Brandl, C.J., Korczak, B and Green, N M (1985) Nature (Lond ) 316, 696–700.
[24] Ikemoto, N. (1974) J. Biol. Chem. 249, 649–651.
[25] Hasselbach, W and Koenig, V. (1980) Z Naturforsch. 35c, 1012–1018
[26] Miyamoto, H and Kasai, M (1979) J Biochem. 85, 765–773
[27] Blatter, L A. and Lee, N K M (1989) Biophys J 55, 490a
[28] Dixon, D., Corbett, A and Haynes, D.H. (1982) J Bioenerg Biomembr. 14, 87–96
[29] Hasselbach, W. and Oetliker, H (1983) Annu Rev Physiol 45, 325–339.
[30] Ikemoto, N , Ronjat, M , Meszaros, L.G and Koshita, M. (1989) Biochemistry 28, 6764–6771.
[31] Valdivia, C , Valdivia, H , Vilven, J. and Coronado, R (1989) Biophys J 55, 88a