CONTROL OF CALCIUM PERMEABILITY IN THE SARCOPLASMIC RETICULUM

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1. Introduction

Several reports indicate that the calcium permeability of sarcoplasmic reticulum vesicles isolated from skeletal muscle is not constant, but is influenced by the Ca^{2+} concentrations outside (Ca_o) and inside (Ca_i) the vesicles. The level of Ca_o at which oxalate-supported calcium uptake reached steady state was found by Weber et al. [1] to increase when more calcium was presented to these vesicles, findings that were interpreted to mean that the leakiness of the vesicles was increased. These investigators also reported that calcium exchange diminished at the start of the calcium uptake reaction, when Ca_i increased [1]. Hasselbach et al. [2] and Makinose [3] found a marked acceleration of calcium efflux from vesicles preloaded with ^{40}Ca when addition of ^{45}Ca to the medium outside the vesicles increased Ca_o and led to renewed calcium uptake.

The present study was undertaken to define the effects of varying Ca_o on the calcium permeability of sarcoplasmic reticulum vesicles under conditions where Ca_i was maintained at different levels by calcium oxalate or calcium phosphate precipitates.

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2. Experimental

Sarcoplasmic reticulum vesicles were prepared according to Makinose and Hasselbach [4]. Calcium uptake by 10 μg protein/ml in 0.12 M KCl, 5 mM MgATP, 40 mM histidine buffer (pH 6.8) and varying concentrations of oxalate or phosphate was measured by the Millipore Filtration method as described previously [5]. Ca_o was calculated from measurements of total calcium outside the vesicles, taking into account the binding of calcium to ATP [6]. Ca_i was calculated from the solubility product of calcium oxalate (2.0×10^{-8} M^2, 7) or calcium phosphate (7.5×10^{-6} M^2 at pH 6.8; W. Hasselbach, unpublished observations).

Calcium permeability was calculated from the equation: Calcium Efflux = Calcium Permeability × Ca_i. Calcium efflux was measured by the use of paired identical reaction mixtures, started concurrently with ^{45}Ca and ^{40}Ca. The former allowed measurement of the slow calcium uptake or calcium release at a time, 10–20 min after the start of the reaction, when calcium uptake approached steady state. Calcium influx at this time was measured by addition of carrier-free tracer ^{45}Ca (1 mCi per μmol, total added Ca < 1 nM) to the reaction mixture started with ^{40}Ca. Tracer uptake, measured at 15 sec intervals, was linear with time for 60–90 sec. after the initial 15 sec interval. Calcium influx was calculated from the rate of tracer influx, adjusted to reflect uptake of total Ca outside the vesicles. The latter was obtained from analysis of the data from the reaction started with ^{45}Ca. Calcium efflux was calculated as the sum of calcium influx *plus* calcium release, or calcium influx *minus* calcium uptake at the time of tracer addition.

3. Results and discussion

Calcium efflux rates at any level of Ca_i were found to increase with increasing Ca_o, as suggested by earlier reports [1–3]. At any level of Ca_o, calcium permeability decreased markedly as Ca_i increased, and a close correlation was found between calcium permeability and the Ca^{2+} gradient across the membrane (Ca_i/Ca_o) (fig.1). This relationship was not affected significantly when phosphate instead of oxalate was used as the calcium precipitating anion.

These findings indicate that the membranes of the sarcoplasmic reticulum contain a 'gating mechanism' that controls calcium efflux rate, and an almost 1000-fold change in calculated calcium permeability was found to accompany a 3000-fold change in the ratio Ca_i/Ca_o. The maximum calcium permeability of 100 nmol/mg min/μM Ca_i would allow a calcium efflux rate of approximately 2×10^{-7} mol/mg sec at a Ca_i concentration of 100 μM, which corresponds to a calcium efflux rate of $\sim 10^{-10}$ mol/cm^2 sec. This value is approximately 1 order of magnitude less than that of 10^{-9} mol/cm^2 sec calculated for calcium efflux from the sarcoplasmic reticulum of activated skeletal muscle [8–10], and would allow efflux of sufficient calcium to bind to 2 of the troponin–calcium binding sites of muscle ($\sim$ 0.2 μmol/g muscle) in approx. 400 msec at a Ca_i level of 100 μM. It is possible that the 'gating mechanism' observed in the present study may participate in the physiological control of calcium fluxes by the sarcoplasmic reticulum of intact muscle.

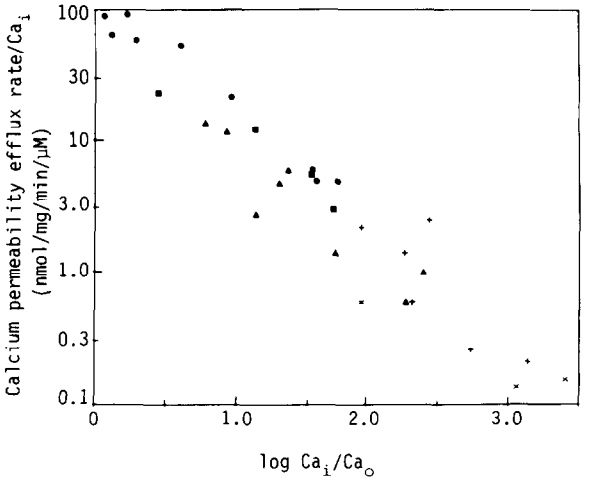

Fig.1. Relationship between calcium permeability and the Ca^{2+} gradient across the sarcoplasmic reticulum. All data obtained in 5 series of experiments are plotted. Ca_i was 4 μM (5.0 mM oxalate, •), 8 μM (2.5 mM oxalate, ▪), 20 μM (1.0 mM oxalate, ▲), 150 μM (50 mM phosphate, +) or 750 μM (10 mM phosphate, X).

References

[1] Weber, A., Herz, R. and Reiss, I. (1966) Biochem. Z. 345, 329.
[2] Hasselbach, W., Fiehn, W., Makinose, M. and Migala, A. J. (1969) in: The Molecular Basis of Membrane Function, p. 299 (D. C. Tosteson, ed.) Prentice Hall, Englewood Cliffs, N. J.
[3] Makinose, M. (1973) FEBS Lett. 37, 140.
[4] Makinose, M. and Hasselbach, W. (1965) Biochem. Z. 343, 360.
[5] Katz, A. M. and Repke, D. I. (1973) Biochim. Biophys. Acta 298, 270.
[6] Katz, A. M., Repke, D. I., Upshaw, J. E. and Polascik, M. (1970) Biochim. Biophys. Acta 205, 473.
[7] Hasselbach, W. and Makinose, M. (1963) Biochem. Z. 338, 94.
[8] Jobsis, F. F. and O'Connor, M. J. (1966) Biochem. Biophys. Res. Commun. 25, 246.
[9] Ashley, C. C. and Ridgway, E. B. (1970) J. Physiol. (London) 209, 105.
[10] Martonosi, A. (1972) in: Current Topics in Membrane and Transport, Vol. 3, p. 83 (F. Bronner and A. Kleinzeller, eds.) Academic Press, New York.