

# Closure of a Rapidly Exchanging Calcium Compartment in Rat Cardiac Myocytes by Lanthanum

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Enzymatically isolated myocytes from adult rats were used to measure  $^{45}\text{Ca}$ -uptake from salt media of reduced  $\text{Ca}^{2+}$ -content (0.1 mM) with normal (4 mM) or elevated (20 mM) potassium concentrations.  $\text{Ca}^{2+}$ -uptake was interrupted by filtration followed by rapid chasing the filter with salt solutions containing no  $\text{Ca}^{2+}$ , 2 mM  $\text{Ca}^{2+}$  or 2 mM  $\text{La}^{3+}$ .

Rate and extent of  $^{45}\text{Ca}$ -uptake of resting cells were found to be 3-fold enhanced when chasing was performed with  $\text{La}^{3+}$ -containing media. In contrast  $\text{La}^{3+}$  does not affect  $\text{Ca}^{2+}$ -exchange of depolarized cells, the fluxes of which approximate rates sufficiently high for contraction activation and that are sensitive to  $\text{Ca}^{2+}$ -channel blockers. The effect of  $\text{La}^{3+}$  on resting cells suggests the existence of a small rapidly exchanging  $\text{La}^{3+}$ -sensitive  $\text{Ca}^{2+}$ -compartment located adjacent to the plasma membrane. This compartment is thought to be either closed or has become  $\text{La}^{3+}$ -insensitive in activated cells. The subsarcolemmal cisternae of the SR, the so-called "peripheral couplings" are most likely the morphological substrate of this compartment.

## Introduction

Cardiac muscle activity of all species strictly depends on the presence of calcium ions in the extracellular fluid which is in contrast to skeletal muscle. Contraction ceases in the absence of calcium ions or after the addition of low concentrations of lanthanum ions [1]. Langer has demonstrated that beating myocytes are quiescent within a few seconds when flushed with calcium-free solutions [2, 3], whereby the latency depends on the source of the preparation. It has been suggested that the calcium ions which are directly involved in contraction activation are released into the myoplasm from binding sites at the surface of the plasma membrane [3, 4]. The rapid interruption of contraction by lanthanum was assumed to result from the displacement of calcium ions from their binding sites [5]. In a more recent study these sites were assigned not to the external but to the internal leaflet of the plasma membrane [3]. In contrast Wendt-Gallitelli and Isenberg [6] suggested that lanthanum blocks contractility not by displacing superficially bound calcium but by blocking the calcium-inward current. It is shown in this report that lanthanum irrespective of its calcium displacing ef-

fect blocks the exchange of calcium between the extracellular fluid and a small intracellular calcium-storing compartment located close to the plasma membrane.

## Materials and Methods

### Reagents

Analytical grade reagents were used in all experiments. Chemicals were obtained from Sigma (München), Serva (Heidelberg) and Merck (Darmstadt).  $^{45}\text{Ca}$  was purchased from Amersham-Buchler (Frankfurt a. M.).

### Isolation of single rat heart cells

Three months old male rats weighing 250–300 g were used. The isolation was performed, using a Langendorff-perfusion system, according to the procedure previously published [7]. The pellets obtained after the perfusion, containing single heart cells, were used for the uptake experiments. Cell viability was determined by light microscopy as well as Trypan-Blue staining. The number of cells was determined prior to the first and immediately after the last filtration of each experiment.

### $^{45}\text{Ca}$ uptake

The pelleted cells were suspended in incubation media. Cell density in these suspensions was ap-

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proximately  $2 \times 10^5 \times \text{ml}^{-1}$ . Two different uptake and washing solutions respectively were used [8]. Control uptake medium composed of 130 mM NaCl, 4.75 mM KCl, 1.2 mM  $\text{MgCl}_2$  depolarizing uptake medium containing 78 mM  $\text{Na}_2$  succinate, 20 mM KCl, 1.2 mM  $\text{MgCl}_2$ . Each solution additionally contained 2 mM histidine buffer at pH 7.2, 25 mM HEPES buffer and 10 mM glucose as well as 5 mg/ml essentially-fatty acid-free bovine albumin. All suspensions were supplemented with 0.1 mM  $\text{CaCl}_2$  and incubated for 20 min at room temperature.  $P_h$  was adjusted to 7.4 and  $^{45}\text{Ca}$  uptake was started by addition of  $^{45}\text{Ca}$  and terminated by filtration using Schleicher & Schüll membrane filters (BA 85, PG 0.45  $\mu\text{m}$ , diameter 25 mm). Calcium remaining in the enclosed volume of the filters after the washing step was determined using cell-free solutions and found to be negligibly small.

Filters were washed and flushed twice with 2 ml of washing solution. The washing solutions had the same composition as the incubating media, except that they did contain neither HEPES, glucose nor albumin. These solutions were either used without further additions or supplemented with 2 mM  $\text{La}(\text{NO}_3)_3$ . In some experiments 2 mM  $\text{CaCl}_2$  were added. The accumulated radioactivity was registered in a Packard scintillation counter. Accumulated calcium was calculated using the specific activity in the medium and expressed as nmol calcium per  $10^5$  cells.

## Results and Discussion

The results of calcium uptake and release measurements performed with isolated cells or cell organelles depend on the method applied for separating the respective preparation from the incubating medium [9]. Separation by filtration or centrifugation furnishes quantities which do not only include the amount of ions that has passed the membrane but also calcium-bound to it. These methods further imply corrections to account for the ions included in the interparticular space or in the filter material. Therefore it is difficult to ascertain small quantities of uptake or binding. In most cases they are small differences of large values. On the other hand, the application of washing procedures to remove the ions from the extraparticular space does not only remove unbound ions but also ions bound to the surface membrane as well as ions lo-

cated in rapidly exchangeable compartments near the surface. Hence the time course of calcium uptake of isolated myocytes obtained by terminating the uptake reaction by filtration and subsequent washing yields minimal values for the exchange rate and the size of the compartment.

The amount of  $^{45}\text{Ca}$  which is lost is difficult to evaluate. It is certain that the duration of the washing procedure is sufficiently long (5–10 sec) to completely displace calcium from external surface structures.  $^{45}\text{Ca}$  exchange experiments carried

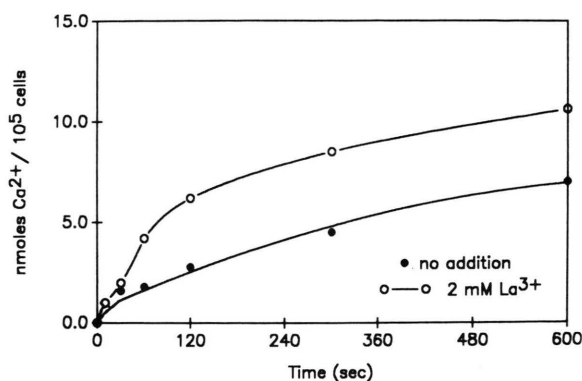


Fig. 1a. Ca-uptake by isolated resting cardiac myocytes. Myocytes were incubated in control medium (see Materials and Methods for further details) at 20 °C.  $^{45}\text{Ca}$  uptake was started by the addition of tracer amounts of  $^{45}\text{Ca}$  and terminated by filtration at times given on the abscissa. Subsequently the filters were washed for 5–10 sec with reaction medium containing no labeled calcium (●) or medium supplemented with 2 mM  $\text{La}^{3+}$  (○). The radioactivity remaining on the filter was measured and Ca-uptake/ $10^5$  cells was calculated using the specific activity of medium calcium and the respective number of cells in the medium.

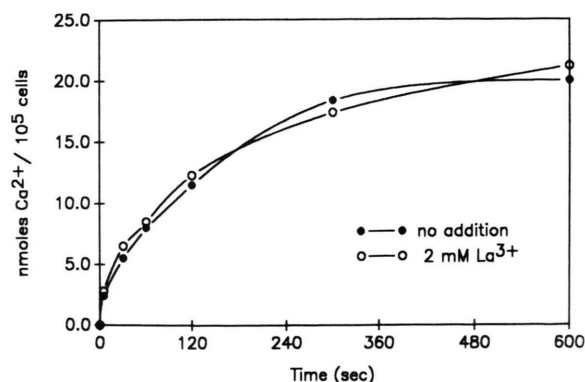


Fig. 1b. Ca-uptake by depolarized cardiac myocytes. The applied procedure is described in Fig. 1a.

out with SR membranes furnished rate constants in the order of  $1 \text{ s}^{-1}$  [10]. As shown in the figure the rate of exchange and possibly also the size of the calcium-exchanging compartment of polarized cells become considerably enhanced when the washing solution is supplemented with 2 mM  $\text{La}(\text{NO}_3)_3$  (Fig. 1a). Washing solutions supplemented with 2 mM  $\text{CaCl}_2$  did not reveal any significant difference in the exchange rate compared to the calcium-free washing media. In contrast the calcium exchange of depolarized cells, the membrane potential of which was reduced to  $-40 \text{ mV}$ , is not affected by the composition of the washing solution (Fig. 1b).

The observed apparent enhancement of the exchange of the polarized preparation indicates that lanthanum does prevent the loss of a fraction of about  $7 \text{ nmol calcium}/10^5 \text{ cells}$  during the washing procedure. Since the total amount that can be exchanged by the myocytes amounts to  $25 \text{ nmol calcium}/10^5 \text{ cells}$ , the retarded fractions corresponds to approximately 25% of the total exchangeable calcium.

The morphological substrate of the lanthanum sensitive compartment is not certainly known. If the involved calcium-binding sites of the lanthanum-sensitive compartment were arranged as single files in the plasma membrane like the calcium-binding sites of the calcium transport ATPase of the SR [11], the occupation of the outermost site with lanthanum could possibly retard the exchange of calcium-bound to sites located more deeply in the file or in the channel. One should obtain similar effects when calcium instead of lanthanum containing washing solutions were used. When lanthanum in the washing medium was replaced by cold calcium, calcium exchange was not enhanced. We are therefore inclined to assume that lanthanum closes a channel-like structure which connects a small intracellular compartment with the extracellular medium. This mode of action of lanthanum is in line with the observation of Wendt-Gallitelli and Isenberg [6], that lanthanum initially displaces only a small fraction of calcium ions superficially bound to the plasma membrane. It is suggestive to assume that this compartment might be related to the subsarcolemmal cysternae of the SR, the so-called peripheral couplings [8].

In the depolarized cells this compartment seems to have vanished. As shown in the figure the depo-

larized cell exchanges calcium much more rapidly than the resting cell (Fig. 1b). Yet neither the rate nor the extent of the calcium exchange is affected by lanthanum. The site where lanthanum acts have either disappeared or have become lanthanum insensitive. Thus the compartment cannot be closed and would be emptied during the washing procedure with or without lanthanum containing washing solutions. Alternatively the compartment might permanently be closed. In this case one has to assume that this compartment is not directly involved in the enhanced calcium influx of the depolarized cells.

The observed flux rate of  $0.06 \text{ nmol calcium}/10^5 \text{ cells}$  in  $1 \text{ s}$  which corresponds to  $20 \text{ nmol/ml}$  in  $100 \text{ ms}$ , is sufficiently high to account for contraction activation in media of physiological calcium content. For this estimate we took into account that the concentration of calcium in the uptake medium was 20 times lower than in physiological extracellular medium and assumed that calcium uptake was linearly related to the concentration of calcium. The absolute rates of calcium uptake varied considerably within a range of  $10 \pm 5$  (mean  $\pm \text{SD}$ ,  $n = 5$ )  $\text{nmol}$  in the first minute. Yet the relative enhancement of calcium uptake as seen with lanthanum washing as compared to lanthanum-free washing medium was highly reproducible. Evaluation of five experiments furnished calcium fluxes that became 2-fold enhanced during the first two minutes. The respective internal compartments

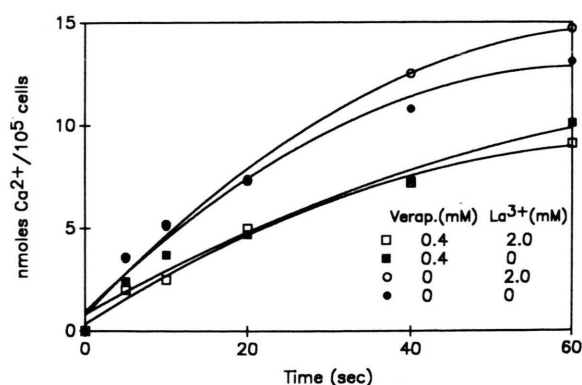


Fig. 2. Comparison of  $\text{Ca}$ -uptake by depolarized myocytes exposed to  $0.4 \text{ mM}$  verapamil. Control assay represents depolarized cells without addition of verapamil. The same method was applied as given in Fig. 1a.

are mainly filled by calcium that enters the cell *via* its calcium channels. Calcium-channel blockers such as verapamil and nifedipine which are effective only in the depolarized cell severely suppress calcium influx [12, 13] even after the  $\text{Na}^{2+}/\text{Ca}^{2+}$ -ex-

changer has been blocked by substituting sodium against Tris ions in the incubating medium. Neither in the presence nor in the absence of calcium-channel blockers does lanthanum affect the exchange rate (Fig. 2).

- [1] G. R. Mines, *J. Physiol. (London)* **40**, 327–346 (1980).
- [2] G. A. Langer, J. S. Frank, T. L. Rich, and F. B. Orner, *Am. J. Physiol.* **252** (Heart Circ. Physiol. **21**), H 314–H 324 (1987).
- [3] G. A. Langer and T. L. Rich, *Am. J. Physiol.* **250** (Heart Circ. Physiol. **19**), H 247–H 254 (1986).
- [4] K. D. Philipson, D. M. Bers, and A. Y. Nishimoto, *J. Mol. Cell Cardiol.* **12**, 1159–1173 (1980).
- [5] W. G. Sanborn and G. A. Langer, *J. Gen. Physiol.* **56**, 192–217 (1970).
- [6] M. F. Wendt-Gallitelli and G. Isenberg, *Pflügers Arch.* **405**, 310–322 (1985).
- [7] J. Eckel and H. Reinauer, *Biochim. Biophys. Acta* **629**, 510–521 (1980).
- [8] P. A. Anderson, A. Manring, J. R. Sommer, and E. A. Johnson, *J. Molec. Cell Cardiol.* **8**, 123–134 (1976).
- [9] M. Chiesi and G. Inesi, *J. Biol. Chem.* **254**, 10370–10377 (1979).
- [10] F. Fernandez-Belda, *Arch. Biochem. Biophys.* **267**, 770–775 (1988).
- [11] G. Inesi, *J. Biol. Chem.* **262**, 16338–16342 (1987).
- [12] K. S. Lee and R. W. Tsien, *Nature* **302**, 790–794 (1983).
- [13] H. Reuter, *Ann. Rev. Physiol.* **46**, 473–484 (1984).