CALCIUM METABOLISM IN CARDIAC MICROSOMES INCUBATED WITH LANTHANUM ION[1]

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Summary

Lanthanum ion, which has been shown to reverse the polarity of artificial phospholipid membranes and destroy their permeselectivity for calcium ion, did not alter the calcium accumulation or exchange in dog cardiac microsomes. The activity of "calcium-stimulated ATPase" was likewise unaffected. These results suggest that in contrast to artificial membrane systems, microsomal transport of calcium is dependent on a specific and selective site rather than simple electrostatic attraction.

In a recent paper, von Breeman (1) described an artificial membrane system which exhibited selective cation permeability. The membrane system had great affinity for calcium ion when compared with potassium. It was demonstrated that lanthanum ion at a concentration of 10^{-6}M was capable of destroying the permeselectivity and reversing the polarity of the membrane in the presence of a 5 to 50 micromolar calcium ion gradient. The present report deals with the effect of lanthanum ion on calcium transport in dog cardiac microsomes which actively accumulate calcium.

METHODS

Dog cardiac microsomes were prepared as previously described (2). Except where noted, $^{45}Ca^{++}$ accumulation was measured after incubating the microsomes in basic medium (5mM MgATP, 10mM histidine, 0.12M KCl, pH 7.0). The calcium

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concentration was 2.5×10^{-5} for all experiments except the ATPase experiments which were performed in 10^{-4}M Ca^{++}. Calcium exchange was measured by first incubating the microsomes with nonradioactive calcium for 5 minutes and then adding a trace of label without appreciably diluting the media as previously described (3). All radioactive calcium accumulation experiments were terminated by extrusion through a Millipore filter (0.45 micron pore diameter), and accumulation was calculated by taking the difference between the filtered and unfiltered samples. Inorganic phosphorus from ATPase activity was measured in the basic medium in a volume of 10 ml with 0.1 mg per ml microsomal protein by the method of Post and Sen (4). The additional ATPase activity stimulated by the addition of calcium was measured by running each reaction in the presence and absence of calcium. All experiments were performed at 25° C. In all studies, lanthanum ion was incubated with the microsomes for 20 minutes before the addition of calcium since the effect of La^{+++} on phospholipid membranes required this time (1). Calcium displacement by lanthanum ion was measured by incubating microsomes in basic medium for 5 minutes and measuring $^{45}Ca^{++}$ accumulation, after which a small volume of concentrated lanthanum ion was added to bring its concentration to 10^{-5}M. Samples were then filtered at various time intervals.

RESULTS AND DISCUSSION

The results of steady state accumulation of calcium ion are shown at various time intervals in Table I. In both the presence and absence of ATP, La^{+++} at 10^{-5}M or 10^{-6}M did not alter the steady state accumulation. La^{+++} lacked effect in either the presence or absence of ATP and had no effect when preincubated with microsomes for a greater (60 minutes) or lesser (5 minutes) time. Reduction of Mg^{++} or K^{+} concentration reduced Ca^{++} accumulation as previously reported (3, 5, 6) but failed to reveal any La^{+++} inhibition of Ca^{++} accumulation (Table II) either in the presence or absence of ATP.

Table I. Calcium Accumulation of Stated Intervals in the Presence and Absence of Lanthanum Ion and ATP

	30 sec	1 min	2 min	3 min
Control + ATP	11.8	12.9	13.4	13.2
10^{-5}M La^{+++} + ATP	11.4	13.6	13.4	13.4
10^{-6}M La^{+++} + ATP	11.2	12.9	13.5	13.5
Control s ATP	5.1	5.9	6.4	6.2
10^{-5}M La^{+++} s ATP	4.7	5.7	5.9	6.1
10^{-6}M La^{+++} s ATP	4.8	5.9	5.9	6.0

Microsomes were incubated in 3 cc volumes at 25° C, pH 7.0, 12M KCl, 10mM histidine buffer, 5mM Mg^{++} and 2.5×10^{-5}M $^{45}Ca^{++}$. Reaction was stopped by extrusion through a Millipore filter. ATP when present was 5mM, calcium accumulation values in muMoles/mg microsomal protein (Biuret).

In an attempt to determine whether the exit of calcium ion from microsomes might be impaired by La^{+++}, $^{45}Ca^{++}$ exchange for previously accumulated calcium was measured. The data in Figure 1 demonstrated rapid exchange of calcium as previously demonstrated (3), and also that La^{+++} did not alter this exchange in either the presence or absence of ATP. Although present methods prevent the evaluation of initial rate of exchange, the time to reach equilibrium was not different in the presence of La^{++}, and the total calcium exchange virtually identical. As in steady state studies, no La^{+++} inhibition could be demonstrated in low Mg^{++} or K^{+} concentration.

ATPase experiments described in Figure 2 further demonstrated that the lack of La^{+++} effect on microsomal calcium metabolism. In the presence or absence of La^{+++} the rates of basic and calcium-stimulated ATPase are virtually identical.

Table II. Calcium Accumulation in the Presence and Absence of La^{+++} at Varied Mg^{++} and K^+ concentrations (n = 6)

	Control	La^{+++} 10^{-5}M
5mM ATP		
5mM Mg^{++}, .12MK^+	10.0 ± 0.8	10.3 ± 1.1
0 Mg^{++}, .12MK^+	6.8 ± 1.3	6.8 ± 1.5
0 Mg^{++}, .01MK^+	7.0 ± 1.1	6.6 ± 1.5
5mM Mg^{++}, .01MK^+	8.6 ± 0.5	8.4 ± 0.5
OATP		
5mM Mg^{++}, .12MK^+	4.8 ± 0.5	4.9 ± 0.8
0 Mg^{++}, .12MK^+	4.8 ± 0.6	4.6 ± 1.0
0 Mg^{++}, .01MK^+	4.9 ± 1.1	4.9 ± 1.2
5mM Mg^{++}, .01MK^+	5.4 ± 0.3	5.7 ± 0.6

Microsomes were incubated in 5mM histidine buffer, pH 7.0 at 25° C with constituents as described above and 2.5×10^{-5}M $^{45}CaCl_2$ for 5 minutes at 25°C. Calcium accumulation values in muMoles/mg protein (Biuret).

Recent work in the literature has emphasized the role of nucleophilic acid phosphate groups in the binding and transport of calcium ion in membranes. Work with artificial phospholipid membranes has demonstrated a marked affinity of such membranes for calcium in relation to other monovalent and divalent cations in both monolayer (7) and bilayer (1) preparations. Experiments with cardiac and skeletal muscle microsomes similarly demonstrated a selection of calcium ion in preference to sodium, potassium, magnesium and zinc (5, 6). In addition, it has been demonstrated that removal of the

acid phosphate groups from microsomes by phosphlipase C destroys their calcium accumulating ability (8) and that strong bases may inhibit calcium binding to lipid monolayers, presumably by decreasing the charge density in the membrane (9).

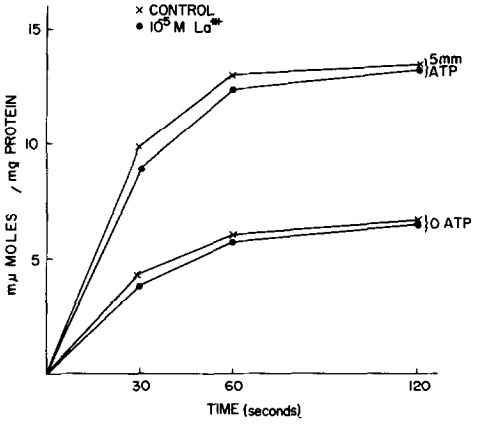

Figure 1. Ca^{++} exchange in the presence and absence of 10^{-5}M La^{+++} and 5mM ATP. Incubation 3 ml basic media and 2.5 x 10^{-5}M $CaCl_2$ for 5 minutes at 25° C. At minutes 2.5 x 10^{-5}M $^{45}CaCl_2$ (. 075 ml) is added and samples filtered at 30, 60, and 120 seconds to measure $^{45}Ca^{++}$ accumulated in exchange for nonradioactive Ca^{++}.

The work of von Breeman (1) demonstrates the production of an artificial bilayer membrane with selective cation permeability and transmembrane potential. In his investigation, he noted that the trivalent cation, La^{+++} was capable of reversibly destroying the permeselectivity of the membrane for calcium ion and reversing the membrane polarity. This was done presumably by chelate binding of the trivalent cation to nucleophilic sites on the membrane, thus imparting the remaining positive charge to these sites and reversing their polarity. These findings stimulated our experiments to determine if simple electrostatic binding was the mechanism of calcium accumulation in microsomal membranes as well. The results demonstrate that, at concentrations up to 10 times those used by von Breeman, La^{+++} did not affect calcium

binding, exchange, or influence the rate of calcium stimulated ATPase. In addition, La^{+++} did not displace previously accumulated calcium. This failure to influence metabolism of calcium was found in both the presence and absence of ATP and at varied cation concentrations.

The work of Carvalho and Leo (6) has previously suggested an active accumulation of calcium in exchange for other cations enhanced in the presence of ATP and less marked in its absence. The present report further suggests that the calcium transport site is highly selective. The finding that La^{+++} did not influence calcium uptake or exchange (i.e., transport in or out of the microsome vesicle) demonstrates that in membrane sites in the presence or absence of ATP is not a simple electrostatic attraction. Thus, although acid phosphates may be important for calcium accumulation by biologic membrane systems they apparently are part of a specific complex selective for calcium.

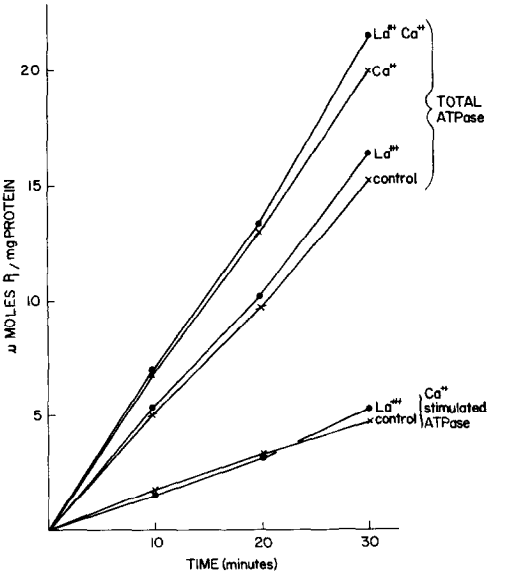

Figure 2. Phosphate release from ATP in presence and absence of La^{+++} (10^{-5}M) and Ca^{++}(10^{-4}M). Incubation in basic media at 25° C. Ca^{++} - stimulated ATPase curve derived from subtracting total ATPase in absence of Ca^{++} from ATPase in presence of Ca^{++}.

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