A STUDY OF CALCIUM BINDING AND UPTAKE BY ISOLATED CARDIAC SARCOPLASMIC RETICULUM: THE USE OF A NEW IONOPHORE (X537A)[1]

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SUMMARY

Calcium "binding" (absence of oxalate) and "uptake" (presence of oxalate) were studied in isolated cardiac sarcoplasmic reticulum. X537A (< 5 μg/mg protein) inhibits binding and uptake similarly; the same concentrations induce a highly significant augmented calcium release from binding sites only in the absence of oxalate. At higher concentrations, "uptake" is virtually eliminated while "binding" (I_{50} = 16 μg/mg) is less inhibited suggesting an additional action of X537A on a step unique to uptake. Data suggest that "binding" and "transport" of calcium may be different but they may initially share sites.

INTRODUCTION

ATP dependent calcium accumulation by sarcoplasmic reticulum (SR) has been studied in the presence of oxalate or phosphate ("uptake") and in their absence ("binding"). The relationship between "uptake" and "binding" has not been well delineated. It has been presumed that they both are part of the same process although some data suggest they may be somewhat different (1,2).

Studies in this laboratory have detailed specific binding and release phases in a cycle initiated by ATP ("binding" = absence of oxalate)(3,4,5). Under specific conditions (5) some sites bound calcium while others appeared to release calcium. The data suggested (5) that the cycle occurs on a saturable number of independent binding sites on the SR membrane.

In the present study we attempt to relate this binding-release cycle to

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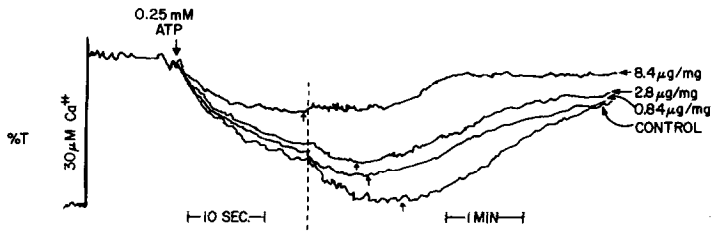


Figure 1: Effect of X537A on calcium binding and relase: Reaction measured at 30°C in 3 ml containing 0.2 mM murexide, 40 mM Tris maleate (pH 6.8), 10 mM $MgCl_2$, 0.1 M KCl, 40 μM $CaCl_2$, 0.25 mM ATP, 0.8 mg/ml CRS and X537A as shown; control contained equivalent ethanol to experimental. Arrows point to the time of release (T_R) of the various curves. Dotted line indicates beginning of different chart speed.

a calcium transport process resulting in observed uptake of calcium into the SR vesicle. The studies utilize a new antiobotic ionophore, X537A (Hoffman-LaRoche®), having broad range cation selectivity (6).

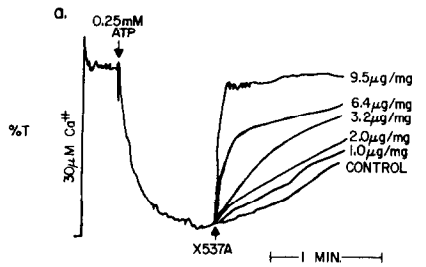
METHODS

The method of isolation of SR (cardiac relaxing system,"CRS") from dog hearts is similar to that previously described (1). Calcium accumulation was measured by both the spectrophotometric (using murexide) and millipore methods (using $^{45}Ca^{++}$) (1). ATPase activities were measured by phosphate liberation (7) and by enzymatic spectrophotometric method (8,9).

RESULTS

Calcium Binding - (Fig. 1). X537A, when added prior to initiation of the reaction by ATP, inhibited the rate of calcium binding (I_{50} = 20-25 μg/mg or 40-50 nmoles/mg) and peak calcium binding (I_{50} = 8-16 μg/mg or 16-32 nmoles/mg). The time of release (T_R) (arrows Fig. 1) was shortened (50% shortened at 3 μg/mg); the rate of release as a function of binding ($^{R}R/_{B}$) (5) was increased with a similar concentration response to T_R.

Ionophore-Induced Release - When X537A was injected after ATP-induced total binding was reached,it induced a rapid release (Fig. 2a). The concentration response was non-saturable (Fig. 2b).



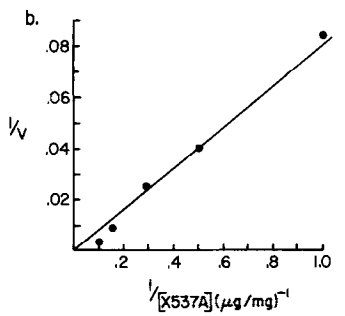


Fig. 2.

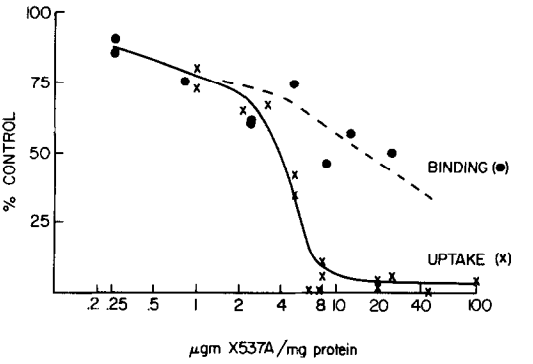


Fig. 3.

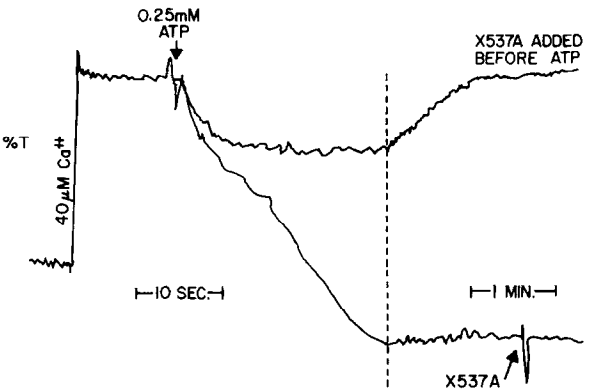


Fig. 4.

Figure 2a: Rapid induced release effected by X537A injected at peak binding: Reaction as in Figure 1; ionophore in amount shown.

Figure 2b: Reciprocal plot of data in Figure 2a.

Figure 3: Log concentration-response of X537A for calcium binding and uptake: Results from both millipore and spectrophotometric experiments in protein concentrations from 0.1 - 0.8 mg/ml; reaction as in Figure 2 (plus 5 mM oxalate for uptake measurements).

Figure 4: Dissociation of calcium binding and uptake: Reaction as in Figure 1 except 60 μM $CaCl_2$ and 5 mM oxalate were added. Upper trace contained X537A, 8.2 μgm/mg protein, from beginning; lower trace served as control and X537A was injected as shown in the same concentration after all calcium was accumulated.

Calcium Uptake - Calcium uptake was markedly inhibited (>90%) by 6 μg/mg; binding at this concentration was still greater than 50% of normal (Fig. 3). This partial dissociation of the two processes is shown directly in Fig. 4. The control tracing describes a typical reaction course in the presence of

oxalate; there was an initial "binding burst" which normally occurs prior to the onset of the linear uptake process. When all calcium was removed no spontaneous release occurred; and injection of X537A resulted in no release (in contrast to binding experiments, c.f. Fig. 2). When X537A (8.2 μg/mg) was present from the beginning, however, a reduced calcium binding burst occurred and spontaneous release followed despite the presence of oxalate; i.e., uptake into the"oxalate compartment" appeared to be completely blocked. Similar results were obtained with inorganic phosphate substituted for oxalate (data not shown).

The initial "binding burst" shown in Fig. 4 was present only upon the first addition of ATP. If all of the calcium or all of the ATP was exhausted, uptake ceased; however, when these components were added back, uptake continued without the presence of the initial binding burst (Fig. 5) implying that the binding sites which "primed" the initial uptake remained occupied and were not vacated either spontaneously or by addition of X537A (Fig. 4).

ATPase (Table 1) - Ca^{++} stimulated ATPase was not affected by any concentration of X537A (Table 1). The "basic" ATPase was also not significantly inhibited. A semi-purified Na^{+},K^{+}-ATPase from dog heart that was 97% ouabain-inhibitable (10) was not affected by X537A (Table 1) nor was the interaction of the enzyme with ouabain affected (data not shown).

DISCUSSION

The ionophore X537A is a polycyclic monovalent anion with six oxygen atoms (11,12). It is thought to bind the divalent cation, barium, by "sandwiching" it between two molecules with all oxygen atoms internal and a hydrophobic external surface. Circular dichroism studies indicate that the structure of X537A-Ca^{++},-Sr^{++}, and -Mg^{++} complexes are analogous (S.R. Alpha, B.C. Pressman- personal communication). It forms lipid soluble complexes with alkali and alkaline earth cations.

The original thesis of oxalate or phosphate action (13) assumed that the anion permeated the vesicle and precipitated the calcium after the ion was

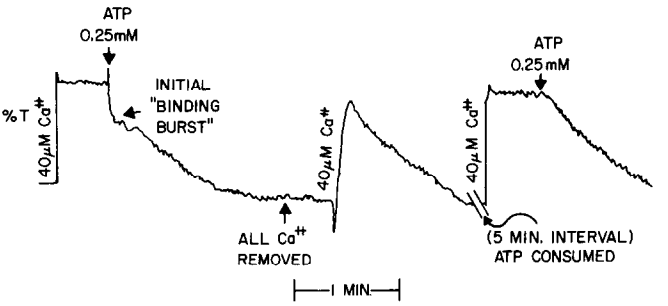


Figure 5: The presence of initial binding "burst" during calcium uptake process: Reaction as in Figure 4 except CRS protein 0.4 mg/ml and 50 μM $CaCl_2$. Note that binding "burst" occurs only with first addition of ATP. (See text).

Table 1

The Effect of X537A on Various ATPase Preparations

	μmoles/mg/hour		
	Na^+,K^+ ATPase	Basic CRS ATPase	Ca^{++} Stimulated ATPase
Control	18.8	16.4	12.4
X537A 4 μg/mg	--	15.9	13.0
X537A 16 μg/mg	18.5	16.5	12.0
X537A 300 μg/mg	18.25	15.9	13.0

Legend: Reactions carried out at 37°C; 25-35 μg/ml enzyme protein; assay = phosphate liberation (7) and enzyme linked (8,9) methods. CRS ATPase - 1 mM Tris ATP, 10 mM $MgCl_2$, 0.1 m KCl, 40 μM $CaCl_2$, 40 mM Tris Maleate (pH 6.8). The tubes used for "basic ATPase" contained 0.5 mM EGTA. Na^+,K^+-ATPase - 2.5 mM Tris ATP, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, 10 mM KCl. CRS = cardiac relaxing system; SR.

transported and accumulated inside the vesicle. According to this view, in the absence of these anions, calcium still entered the vesicle in a similar manner but reached equilibrium with passive efflux. Recent work from our laboratory (5) suggests that the accumulation in the absence of oxalate may represent calcium binding to SR to an ATP-induced membrane "conformation". Calcium binding then induces a "conformation" which results in spontaneous calcium release. The high activation energy (5) of the release phase suggests that it is not a simple passive back-diffusion of calcium.

Evidence for differences in mechanism of "binding" and "uptake" include:

(a) the induced release by X537A after peak binding does not occur (at any concentration studied) in the presence of oxalate or phosphate. In fact, the calcium bound in the initial binding "burst" at the onset of uptake was not released, (b) difference in concentration-response to X537A: At lower concentrations of the ionophore the response of both processes is similar suggesting the possibility that a common variable is affected. At higher concentrations a site or function peculiar to the uptake process may be affected. The first of these processes may well be related to increased release since the range of 1-6 μg/mg spans the range of effects on the release parameters. The second may prevent bound calcium from contacting the intravesicular space since despite adequate binding (50% of normal) no uptake occurs and release actually ensues (Fig. 4). It is important that neither of these effects related directly to calcium-stimulated ATPase which was unaffected by X537A. Basic ATPase and an unrelated membrane transport ATPase were also unaffected. It is attractive to postulate that the binding sites may be associated with the contraction relaxation process; these sites may be superficial and easily release bound calcium. The data suggest (but do not prove) that binding and uptake may be different; but may share an initial binding step.

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ADDENDUM

After this manuscript was completed a preliminary report by Scarpa appeared (FEBS Letters 22: 273, 1972) showing X537A induced calcium release in skeletal muscle microsomes. The interpretation of this datum was significantly different from ours possibly resulting from the absence of the data from the experiments shown in Figures 1,3,4 and 5 of this study.

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